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EDITORIAL

It is our pleasure to come up with the 38th volume of Nepalese Veterinary Journal (NVJ). The current issue of NVJ includes a range of original research and review articles, short communications and case studies on several aspects of animal husbandry including health, breeding, nutrition, and management of livestock, pets and wildlife, and public health. We believe that this information will be useful to the concerned stakeholders such as veterinarians, animal scientists, public health workers, farmers and policy makers.

Livestock sector of Nepal faced several challenges in the past year. There were unusually high number of highly pathogenic avian influenza (HPAI) outbreaks in 2022 in more than 16 districts of Nepal resulting in culling of more than 700,000 birds. In year 2023 also, several outbreaks of HPAI have been detected warranting for continuous effort to be done in the sustainable management of HPAI outbreaks. A new deadly pig disease, African Swine Fever (ASF) entered for the first time in Nepal in 2022 devastating commercial and backyard pig farming in dozens of districts of Nepal. As there is no treatment or vaccine available for ASF, this disease is particularly worrying for pig farmers of Nepal. Focus should be placed in strengthening the biosecurity measures and good husbandry practices in pig farms of Nepal to prevent the entry of ASF. Lumpy Skin Disease (LSD), a cattle and buffalo disease, which entered Nepal in 2020 from the eastern part of Nepal took an epidemic form during early 2023 and reached almost all districts of Nepal causing huge economic losses to the dairy farmers. However, the exact losses being caused by these new infectious diseases, LSD and ASF, have not been yet estimated. The Government of Nepal has made a policy decision to allow vaccination against LSD and commercial LSD vaccine is already available in Nepal which is expected to contribute to reduce the burden of LSD outbreaks in the future. The emergence and reemergence of several infectious livestock diseases call for the need of an “Infectious Animal Disease Control Act” for the timely control and containment of infectious animal diseases to minimize economic losses and promote public health by reducing the spillover events.

More broadly, given the high burden of infectious diseases, there is a need to strengthen the animal health systems at all three tiers of government. Given the new federal structure of Nepal, veterinary services at province and local level is particularly weak in terms of both infrastructure and human resources. The upgrading of livestock services unit at the local level to the veterinary hospitals equipped with modern veterinary facilities and qualified human resources can be pivotal to uplift the livestock industry of Nepal. This will ultimately help to boost the local economy and farmers' income which can bring the chain effect to the national economy. Further, the unit cost of production of livestock and its product in Nepal is higher compared to our neighboring countries. Breeding, feeding and health management of livestock and fish need to be enhanced through effective policies

and investments in the public-private partnership model to increase production and productivity and to make cost of production competitive.

Besides, the ongoing COVID-19 pandemic has clearly highlighted the importance of one health approach especially for diseases that occur at the human-animal-environment interface. This is a high time for Nepal to implement the “One Health Strategy 2019” which was endorsed by the Government of Nepal. As Nepal has already prioritized 10 zoonotic diseases following a one health approach, preparedness activities against these diseases following a one health approach can be immediately embarked upon.

Nepal Veterinary Association (NVA) is a scientific professional organization of Nepalese veterinarians aimed at strengthening the technical capabilities of member veterinarians and safeguarding the veterinary profession in Nepal. NVA publishes the Nepalese Veterinary Journal (NVJ), an online accessible scientific journal indexed in Nepal Journals Online (NepJOL). The publication aims to share scientific knowledge and technology among members and stakeholders at home and abroad. We welcome original scientific research and review articles, short communications, case studies and perspectives in the area of veterinary science, animal science, food safety, public health and other cross-cutting issues. The guidelines for authors can be accessed through <https://www.nepjol.info/index.php/nvj/about>.

We would like to express our sincere thanks to the authors and reviewers for their hard work and valuable contribution to the veterinary profession. We would also like to acknowledge Ms. Pramina Shrestha for her secretarial service. We are also indebted to the executive committee of NVA for their support and encouragement.

We apologize for errors, if any, despite our effort to minimize them. We welcome constructive suggestion from our readers.

Editor-in-Chief
Surendra Karki, PhD

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Safety and Immunogenicity of ND Vaccines Used in Nepal

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ABSTRACT

Viral infections, including Newcastle disease (ND), constitute a major health problem in the rapidly growing poultry industry of Nepal. Despite regular vaccination in the commercial farms, ND virus (NDV) outbreaks are frequently reported raising concern over the safety and immunogenicity of live-attenuated vaccines being used. This study was performed to compare the safety and immunogenicity of four commercially available ND vaccines in Nepal after administration at recommended (1X) and higher (10X) dose. There was no safety concern associated with the use of lentogenic live-attenuated ND vaccines even at higher dose. A varying degree of antibody response was observed at recommended and higher doses with the thermostable I-2 vaccine outperforming other groups. A higher dose did not improve antibody response except for the F1 vaccine. To prevent widespread outbreaks in future, regular molecular surveillance to identify the circulating strains of NDV together with the periodic evaluation of immunogenicity and protective efficacy of commercial vaccines is necessary in Nepal.

Keywords: I-2 vaccine, Newcastle disease virus, Poultry, Vaccine safety

INTRODUCTION

Poultry farming is rapidly growing in Nepal [1]. As per the latest livestock statistics, the standing population of fowl is around 83 million which is over 200% greater compared to the population 10 years ago [2]. In Nepal, poultry industry has over a billion-dollar

investment, provides employment to over 150,000 people, and contributes around 4% to the national gross domestic product (GDP) and 8% to the national agricultural GDP (AGDP) [3]. Poultry farming, including the backyard poultry, is an important aspect of income generation in rural areas while chicken meat and eggs serve as the major source of protein in Nepalese kitchen [3].

Viral infections, including avian influenza and Newcastle disease (ND) are the major health problems in the poultry industry of Nepal [1,4,5]. ND is also known as Ranikhet and is caused by Newcastle disease virus (NDV), aka avian paramyxovirus serotype 1, which is a single-stranded RNA virus with non-segmented genome [6]. Based on the pathogenicity in chickens, NDV is classified into very virulent (velogenic), moderately virulent (mesogenic), and avirulent (lentogenic) strains [7]. Clinical signs of NDV infection in chickens vary according to the virus pathotypes. This can range from asymptomatic enteric infection caused by lentogenic NDV to velogenic neurotropic ND characterized by severe neurological and respiratory signs [8]. The World Organization for Animal Health (WOAH) has listed ND as a List A disease for its ability to spread rapidly even beyond the national borders and cause significant socioeconomic and public health consequences [9].

Vaccination against ND is commonly practiced in the commercial farms while its use is limited in the backyard poultry of Nepal [10]. ND vaccination strategy is conventional and mostly utilizes the live-attenuated F, LaSota, I-2 or R2B strains administered through intraocular route, in drinking water, or in muscles [3,11]. First vaccination at 5-7 days is followed by periodic boosts in laying hens [11]. Despite the use of vaccines, ND outbreaks are frequent in Nepal. There were 90 reported outbreaks of ND in Nepal in 2018, that affected over 74,000 chickens and resulted in the deaths of over 7,000 birds [3]. In 2021, there were several outbreaks of ND in the poultry farms of Nepal and outbreaks were reported even in the vaccinated flocks [12]. Despite many years of ND vaccination practice in Nepal, comparative immunogenicity studies of commercial vaccines are very limited. Further, ND outbreaks in the vaccinated farms raised concern over safety of the live-attenuated vaccines. Hence, this study was performed to determine the safety and immunogenicity of four (LaSota, B1, F and I-2) commercially available and commonly used ND vaccines in Nepal after administering at recommended (1X) or higher (10X) doses.

MATERIALS AND METHODS

Study Design, Vaccination and Sample Collection

This study was carried out at the Veterinary Standards and Drug Regulatory Laboratory (VSDRL) Budhanilkantha, Kathmandu from July to September 2021. VSDRL is the regulatory body for the approval of veterinary vaccines in Nepal. Approval for this research was obtained from VSDRL on 24th June 2021 (letter number: 2077-78/49).

Day old-layer chicken (n=97) of (Brown Nick, H&N International) were purchased from a commercial hatchery and raised with ad libitum feed and water in the research facility of VSDRL. They were raised in a group of 10 in a partitioned chamber. ND vaccines of the lentogenic strains; viz. LaSota (Pestikal LaSota SPF, Genera Croatia), B1 (Himmvac Newcastle B1, KBNP, INC.), F (RaniVax Plus Vet Initial, Incepta Vaccine Ltd.), and I2 (Jovac NDV I2, Jordan Bio Industries Center); were purchased from commercial sources and cold chain (2-80C) was maintained as suggested by the producer until vaccination was carried out. Fifty animals were used for recommended dose (1X) vaccine study while remaining 47 were used for high-dose (10X) vaccine study. In regular dose vaccine study, birds (n=10/group) were immunized with live-attenuated vaccines of lentogenic strains (LaSota, B1, F, or I-2) through supraconjunctival route at 10th day using the dose recommended by the producer. In high dose (10X) vaccine study, birds (n=7-10/group) were immunized with the same lentogenic strains (LaSota, B1, F, or I-2), but with a dose 10 times greater than the recommended. For that, the vaccine vials were diluted as per the producer's instruction; recommended dose (1X) birds were given single dose (1 drop) while the high dose (10X) group were given 10 drops. In both regular and high dose studies, 10 birds were used as unvaccinated control.

To evaluate safety of the vaccines and doses, birds were monitored once daily during the time of offering feed to animals for 21-days post-vaccination for any ND-related enenteric (i.e., greenish and watery diarrhea), respiratory (viz., sneezing, gasping, nasal discharge, and coughing), and neurological (viz., depression, muscle tremors, droopy wings, circling, and paralysis) signs and mortality [13].

To measure antibody response, blood was collected from the wing vein and transferred in a clot activator tube. Blood tubes were centrifuged at 4000 rpm for 5 minutes, serum samples were collected and stored at -80°C.

Measurement of antibody response

For the detection of the antibody titers in the serum samples, indirect Enzyme-linked Immunosorbent Assay (ELISA) was performed using the ID screen Newcastle disease indirect ELISA kit (IDvet, Grabels, France). Two different dilutions of serum were used to test antibody levels. This kit has been used previously to determine antibody titers against NDV [14]. Titers above 641 were considered as having positive level as recommended by the company.

Data Analysis

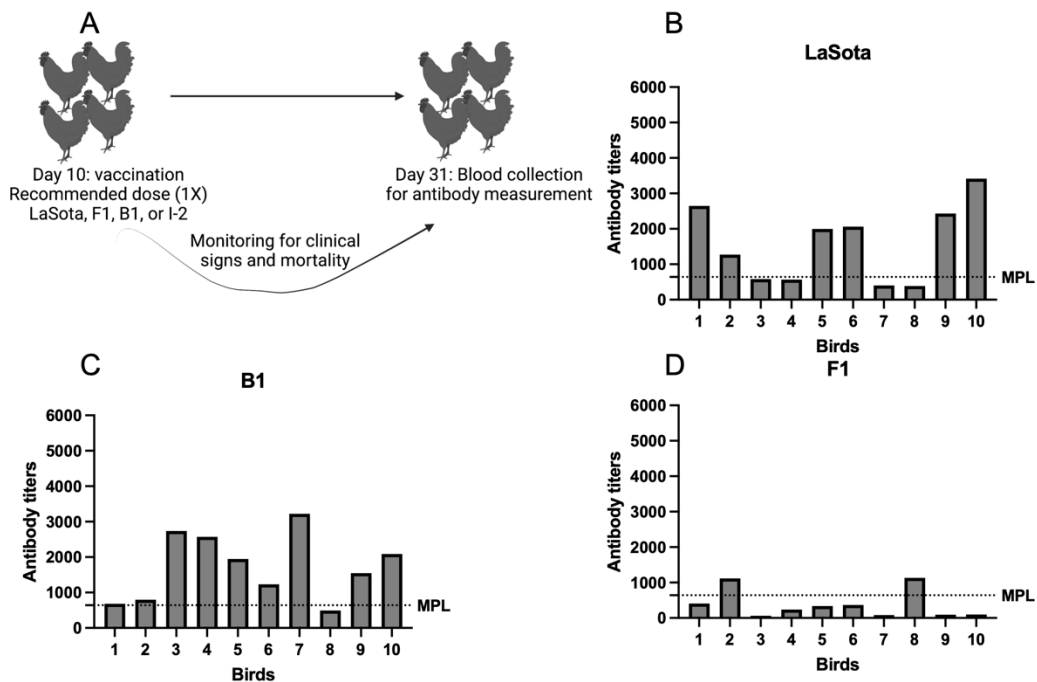
Data were analyzed using GraphPad Prism software version 9.4.1. (GraphPad, CA, USA). Antibody levels among vaccine groups after recommended or higher dose vaccination were compared using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Antibody levels in same vaccine group after recommended

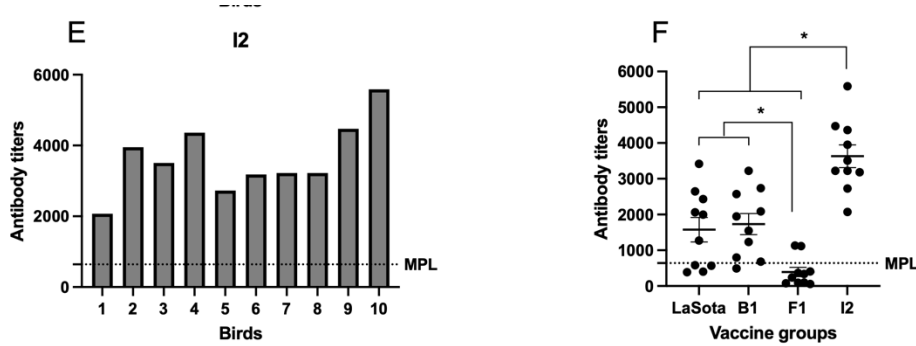
and higher dose vaccinations were compared using unpaired T-test. Similarly, changes in frequencies of positive (having antibodies above minimum protective level, MPL) after higher dose vaccination in same vaccine group was compared using Chi-square test. Data were considered statistically significant at $p < 0.05$.

RESULTS

To test the safety of commercial ND vaccines, all the birds vaccinated at recommended or higher dose were monitored daily for 21 days post vaccination. Birds were monitored for mortality and development of any clinical signs that leads to the suspicion of ND. We did not observe any mortality during the experiment. Similarly, none of the birds showed any clinical signs that lead to the suspicion of ND even at 10-times higher doses than recommended.

After vaccination with recommended dose (1X) (Figure 1A), 60% (6/10) birds in LaSota (Figure 1B), 90% (9/10) in B1 (Figure 1C), 20% (2/10) in F1 (Figure 1D), and 100% (10/10) birds in I-2 (Figure 1E) vaccine group had antibodies above the minimum protective level (MPL). While comparing antibody levels across the vaccine groups after recommended dose vaccination, I-2 vaccine group showed significantly greater antibody titers than LaSota, B1 and F1 vaccine groups (Figure 1F). Antibody levels between LaSota and B1 groups were comparable. The F1 vaccine group had the lowest antibody response which was significantly lesser than all other three vaccine groups (Figure 1F).

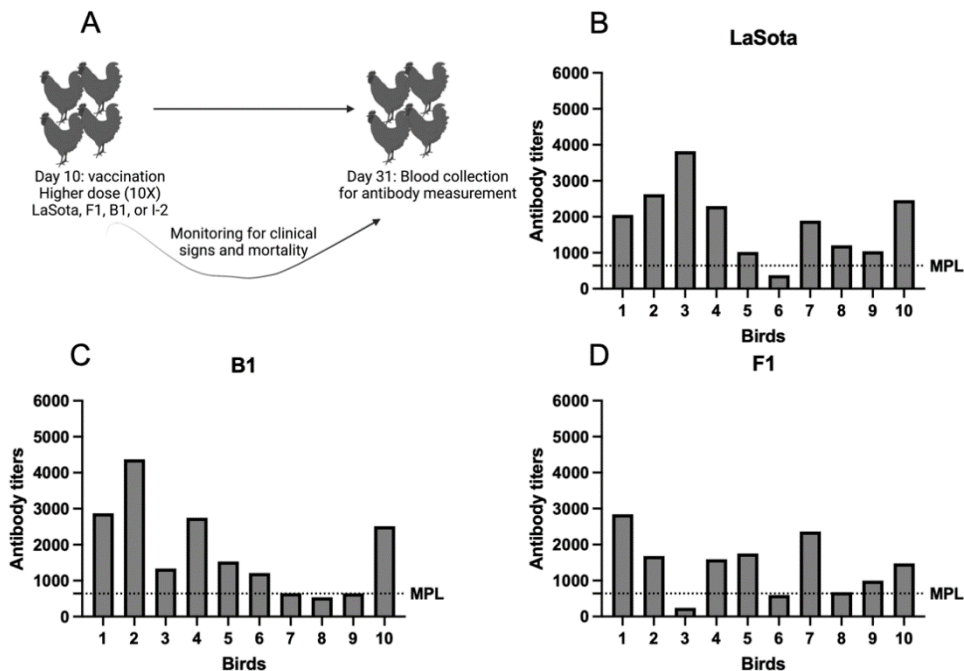




Figure

1: Antibody response after (A) recommended dose (1X) vaccination with (B) LaSota, (C) B1, (D) F1, and (E) I-2 vaccines. (F) Antibody levels among vaccine groups were compared using one-way ANOVA followed by Tukey's multiple comparisons test. MPL refers to the minimum protective level and asterisk (*) refers to significant differences between the indicated groups at $p < 0.05$.

After vaccination with higher dose (10X) (**Figure 2A**), 90% (9/10) birds in LaSota (**Figure 2B**), 90% (9/10) in B1 (**Figure 2C**), 80% (8/10) in F1 (**Figure 2D**), and 100% (7/7) birds in I-2 (**Figure 2E**) vaccine group had antibodies above the minimum protective level. The I-2 vaccine group induced antibody levels significantly greater than other three vaccine groups even after the higher dose vaccination (**Figure 2F**). At higher dose, antibody levels among LaSota, B1, and F1 groups were statistically similar (**Figure 2F**).



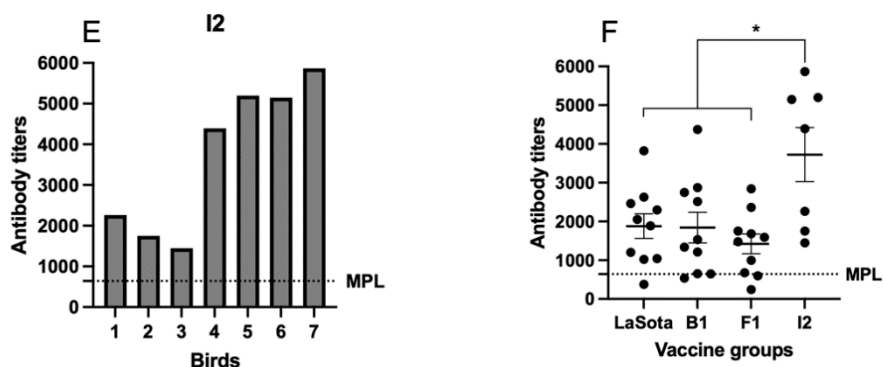


Figure 2: Antibody response after higher (10X) dose vaccination (A) with (B) LaSota, (C) B1, (D) F1, and (E) I-2 vaccines. (F) Antibody levels among vaccine groups were compared using one-way ANOVA followed by Tukey's multiple comparisons test. MPL refers to minimum protective level and asterisk (*) refers to significant differences between the indicated groups at $p < 0.05$.

Since there was no safety concern after recommended or higher dose vaccination, we next wanted to understand if higher dose vaccination could induce greater antibody responses than recommended dose. Higher dose vaccination did not increase antibody titers in LaSota (**Figure 3A**), B1 (**Figure 3B**) and I-2 (**Figure 3D**) vaccine groups. However, a higher dose vaccination induced significantly higher level of antibody response in F1 vaccine group (**Figure 3C**). Subsequently, we wanted to understand if higher dose vaccination increased the frequencies of birds that produced antibodies above the minimum protective level or not. Though higher dose vaccination with LaSota increased the frequency of birds with antibodies above MPL from 60% to 90%, it was not statistically significant (**Figure 4A**). Difference was not observed in B1 vaccine group (**Figure 4B**). However, in F1 vaccine group the frequency of birds with antibodies above MPL changed from 20% during recommended dose vaccination to 80% after higher dose vaccination and it was significantly improved (**Figure 4C**).

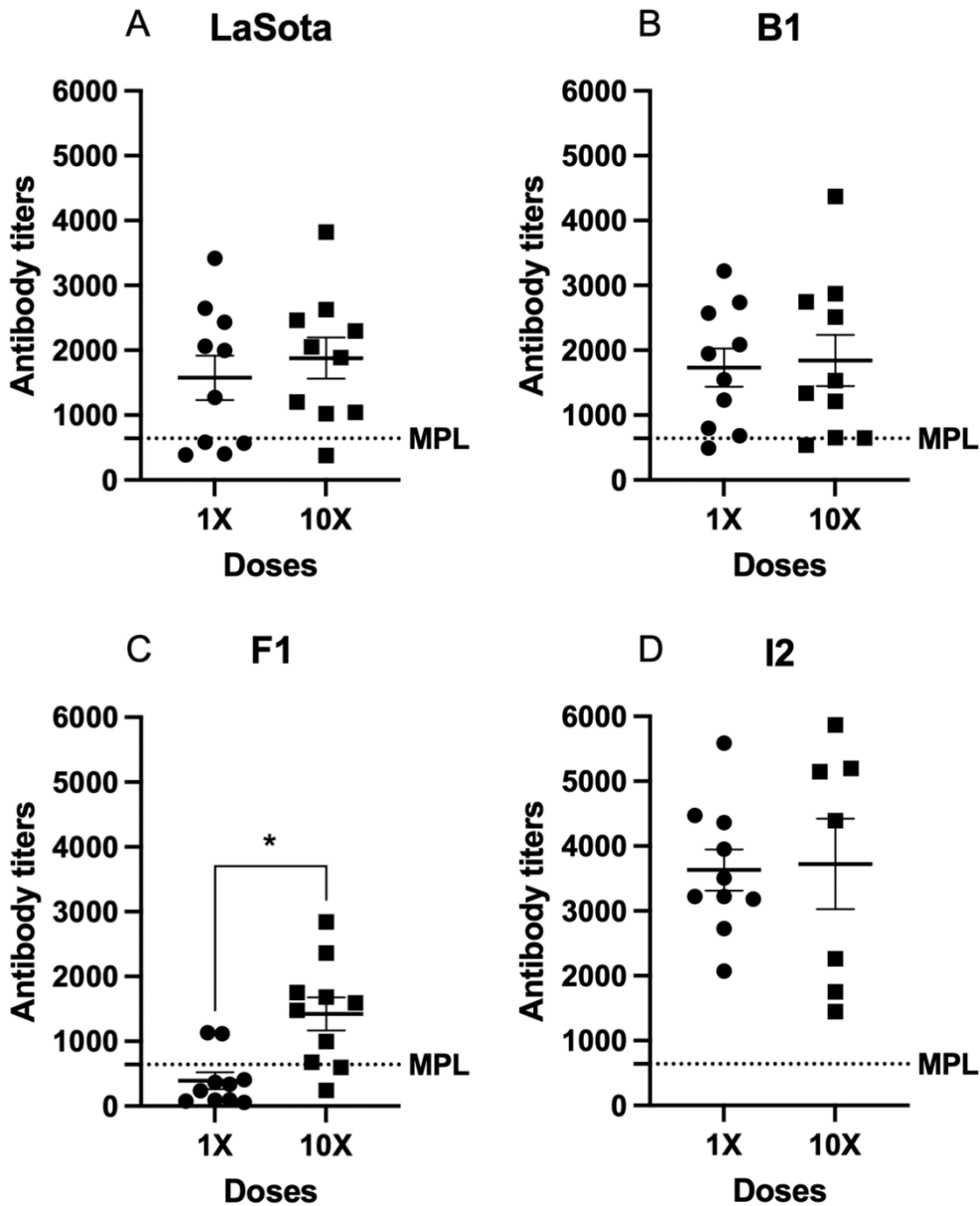


Figure 3: Antibody response after recommended (1X) and higher (10X) dose vaccination with (A) LaSota, (B) B1, (C) F1, and (D) I-2 vaccines were compared using unpaired T-test. Asterisk (*) refers to significant differences between the vaccine doses at $p < 0.05$.

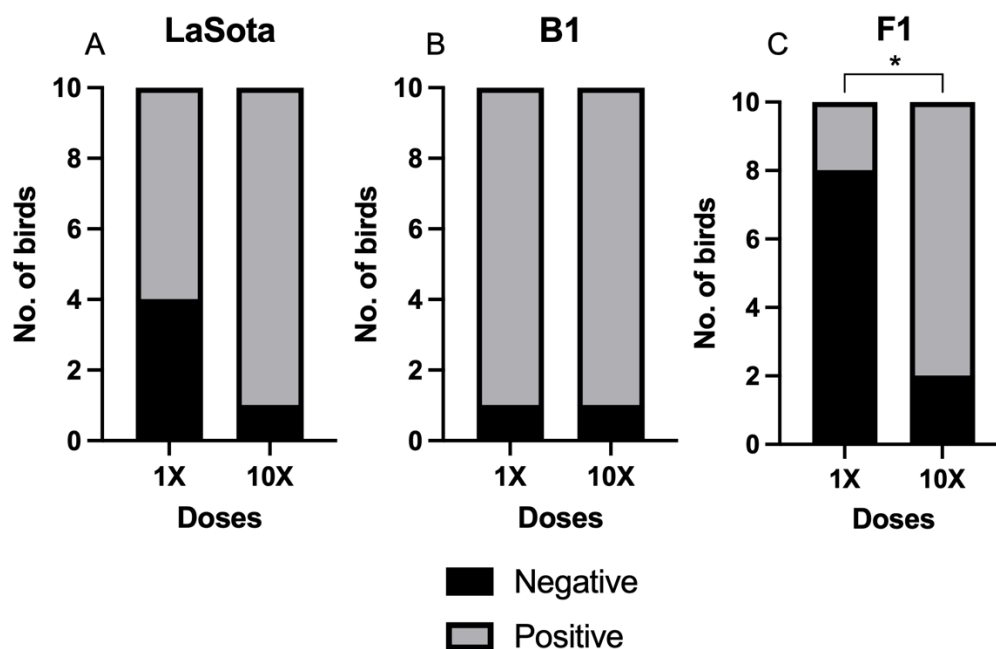


Figure 4: Frequencies of birds having antibodies above minimum protective levels after recommended (1X) versus higher (10X) dose vaccination with (A) LaSota, (B) B1, and (C) F1, vaccines were compared using Chi-square test. Asterisk (*) refers to significant differences between the vaccine doses at $p < 0.05$.

DISCUSSION

Vaccination together with stringent biosecurity measures are practiced worldwide to prevent NDV infection in the farm [15]. Most of the commercial poultry farms in Nepal also practice regular vaccination against NDV [10]. Despite vaccination, ND outbreaks are common each year leading to a huge economic loss [1,3]. Use of live-attenuated vaccines even raises concern over their possibility to cause outbreaks specially if there is wrong dosing of vaccines. In this study, for the first time, we compared four different lentogenic strain ND vaccines commercially used in Nepal for their safety and immunogenicity. Single dose immunization of chickens with recommended as well as 10-times higher dose of LaSota, B1, F1, and I-2 vaccines did not cause any clinical signs that can be assigned to ND or mortality. This showed that vaccines using lentogenic strains are safe and unlikely to cause disease outbreaks in the farms even when used at higher doses. Thus, recent outbreaks of NDV in Nepal, including those in the vaccinated farms, is unlikely to be linked to the use of live-attenuated vaccines. Rather, a recent study showed that NDV genotype VIIc (GVIIc) was the probable causative agent for the widespread outbreak of ND in 2021 in Nepal [10]. NDV GVIIc represents a velogenic strain with severe clinical outcomes [16,17]. Studies in other countries have shown that vaccination

with lentogenic strain, like LaSota, provides poor protection against virus strains of NDV GVII and viruses of this genotype still circulate in the vaccinated farms [16,18,19]. Thus, it is likely that the vaccines being used in Nepal are unable to induce antibody responses cross-protective against the circulating NDV strains leading to many outbreaks in the farms. Only a more rigorous virus challenge study can confirm this which was beyond the scope of this study.

Birds immunized with I-2 vaccine produced significantly greater antibodies than other vaccine groups. The better performance of this vaccine, in part, may be associated with its thermostability. I-2-based NDV vaccines are stable at room temperature for several days [10,20]. Though due care was given to maintain cold chain at our end, it is uncertain whether there was any breakage in the proper cold chain maintenance at any stage of the vaccine storage and transportation chain. This is important as live-attenuated NDV vaccines deteriorate quickly within few hours if cold chain is not maintained [21].

An earlier study from Nepal showed that LaSota strain vaccination in village chickens is immunogenic and efficacious against NDV infection [21]. A recent study also showed that LaSota vaccination was safe and induced a protective level of antibodies in 98% of vaccinated birds at 28 days post-vaccination [22]. Field verification study of ND I-2 vaccine also showed that it can induce protective levels of antibodies in 50-100% of birds at different study locations [23]. Moreover, ND I-2 vaccine was shown to induce protective levels of antibodies up to 90 days post vaccination in village chickens [24]. A recent study showed that thermostable I-2 NDV vaccine in tablet formulation is effective against NDV, including a virulent 2021 outbreak strain with 85% efficacy [10]. These findings, together with ours, highlight the importance of continuous use of NDV vaccination in the farms. However, whether antibodies produced by these vaccines are cross-protective against endemic and emerging NDV strains circulating in the country also need to be tested. Vaccines against ND, including F, LaSota, R2B and I-2, are produced in Nepal [3]. In-country vaccine production is not sufficient and hence ND vaccines are also imported from different countries [1]. A total of about 184 million doses of ND vaccines were imported in Nepal in the fiscal year 2020/21 [25]. Thus, quality assessment of vaccines produced within the country and imported from elsewhere in a regular manner is important to make sure they provide the expected level of protection in the field against the circulating strains.

CONCLUSION

The NDV vaccines being used in Nepal appear safe and produce a varying degree of antibody responses. However, they may not be protective enough against NDV strains circulating in the farms. Regular molecular surveillance to identify the circulating strains of NDV in Nepal and periodic evaluation of immunogenicity and protective efficacy of

commercial vaccines against the circulating strains of NDV is important to prevent its widespread outbreaks in the future.

Author Contributions: Conceptualization, S.M., N.U., and P.S.; methodology, S.M., N.U. and P.S.; software, S.D; validation, S.M. and S.D.; formal analysis, S.D.; investigation, S.M., N.U., P.S and S.S; resources, N.U., P.S., S.S., and S.S.; data curation, S.D.; writing—original draft preparation, S.M., S.D.; writing—review and editing, S.M., N.U. and S.D. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be available upon reasonable request.

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REFERENCES

- [1] Poudel U, Dahal U, Upadhyaya N, Chaudhari S, Dhakal S. Livestock and Poultry Production in Nepal and Current Status of Vaccine Development. *Vaccines* 2020, 8, 1–9..
- [2] Animal Genetic Resources and Economic Analysis Division Hariharbhawan, Lalitpur Nepal 2076. Available online: http://dls.gov.np/downloadfile/Livestock%20Statistics%202076_77_1646045136.pdf (accessed on 27 August 2022).
- [3] Poudel U, Dahal U, Dhakal S. Review of Poultry Production and Poultry Vaccine Manufacture in Nepal. *Global Journal of Agricultural and Allied Sciences* 2021, 3-1, 1-7.
- [4] Subedi D, Phuyal P, Bhandari S, Kandel M, Shah S, Rawal G, Karki S, Dhakal S. Risk Factors Associated with Avian Influenza Subtype H9 Outbreaks in Poultry Farms of Central Lowland Nepal. *Infectious Disease Reports* 2022, 14, 525-536
- [5] Gompo TR, Shah BR, Karki S, Koirala P, Maharjan M, Bhatt DD. Risk factors associated with Avian Influenza subtype H9 outbreaks in poultry farms in Kathmandu valley, Nepal. *PLoS One* 2020, 15(4).
- [6] Dimitrov KM, Abolnik C, Afonso CL, Albina E, Bahl J, Berg M, Briand FX, Brown IH, Choi KS, Chvala I, Diel DG, Durr PA, Ferreira HL, Fusaro A, Gil P, Goujgoulova GV, Grund C, Hicks JT, Joannis TM, Torchetti MK, Kolosov S, Lambrecht B, Lewis NS, Liu H, Liu H, McCullough S, Miller PJ, Monne I, Muller CP, Munir M, Reischak D, Sabra M, Samal SK, Servan de Almeida R, Shittu I, Snoeck CJ, Suarez DL, Van Borm S, Wang Z, Wong FYK.. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infect Genet Evol* 2019, 74.
- [7] Brown C, King DJ, Seal BS. Pathogenesis of Newcastle disease in chickens experimentally infected with viruses of different virulence. *Vet Pathol* 1999;36:125–32. <https://doi.org/10.1354/VP.36-2-125>.
- [8] Brown C, King DJ, Seal BS. Pathogenesis of Newcastle disease in chickens experimentally

- infected with viruses of different virulence. *Vet Pathol* 1999, 36, 125–132.
- [9] Old Classification of Diseases Notifiable to the OIE – List A - WOAHP - World Organisation for Animal Health. Available online: <https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/old-classification-of-diseases-notifiable-to-the-oie-list-a/> (accessed on 27 August 2022).
- [10] Napit R, Poudel A, Pradhan SM, Manandhar P, Ghaju S, Sharma AN, Joshi J, Tha S, Dhital K, Rajbhandari U, Basnet A, Rajbhandari RM, Schwind JS, Karmacharya D. Newcastle disease burden in Nepal and efficacy of Tablet I-2 vaccine in 1 commercial and backyard poultry production 2018.
- [11] Poudel U, Dahal U. Vaccination: A key way to prevent Newcastle disease in poultry of Nepal *International Journal of Veterinary Sciences and Animal Husbandry* 2020, 5-1, 104-107.
- [12] Newcastle disease kills 1,200 chickens in Ilam. Available online: <https://english.onlinekhabar.com/newcastle-disease-kills-1200-chickens-in-ilam.html> (accessed on 27 August 2022).
- [13] Miller PJ, Decanini EL, Afonso CL. Newcastle disease: Evolution of genotypes and the related diagnostic challenges. *Infect Genet Evol* 2010, 10, 26–35.
- [14] Ferreira HL, Miller PJ, Suarez DL. Protection against Different Genotypes of Newcastle Disease Viruses (NDV) Afforded by an Adenovirus-Vectored Fusion Protein and Live NDV Vaccines in Chickens. *Vaccines* 2021, 9, 1–18.
- [15] Mayers J, Mansfield KL, Brown IH. The role of vaccination in risk mitigation and control of Newcastle disease in poultry. *Vaccine* 2017, 35, 5974–80.
- [16] Yu L, Wang Z, Jiang Y, Chang L, Kwang J. Characterization of Newly Emerging Newcastle Disease Virus Isolates from the People’s Republic of China and Taiwan. *J Clin Microbiol* 2001, 39(10), 3512-3519.
- [17] Qin ZM, Tan LT, Xu HY, Ma BC, Wang YL, Yuan XY, Liu WJ. Pathotypical characterization and molecular epidemiology of newcastle disease virus isolates from different hosts in China from 1996 to 2005. *J Clin Microbiol* 2008, 46, 601–611.
- [18] Ewies SS, Ali A, Tamam SM, Madbouly HM. Molecular characterization of Newcastle disease virus (genotype VII) from broiler chickens in Egypt. *Beni-Suef Univ J Basic Appl Sci* 2017, 6(3), 232–237.
- [19] Roohani K, Tan SW, Yeap SK, Ideris A, Bejo MH, Omar AR. Characterisation of genotype VII Newcastle disease virus (NDV) isolated from NDV vaccinated chickens, and the efficacy of LaSota and recombinant genotype VII vaccines against challenge with velogenic NDV. *J Vet Sci* 2015, 16, 447–457.
- [20] Shahid Mahmood M, Siddique F, Hussain I, Ahmad SI, Rafique A. Thermostable vaccines for Newcastle disease: a review. *World’s Poultry Science Journal* 2014, 70(4), 829-838.
- [21] Dimitrov KM, Afonso CL, Yu Q, Miller PJ. Newcastle disease vaccines—A solved problem or a continuous challenge? *Vet Microbiol* 2017, 206, 126–136.
- [22] Andrews S, Makundi A, Mwanadota J, Shivakoti S, Shah BK, Poole EJ, Salt J, Stuke K. The co-administration of live fowlpox and Newcastle disease vaccines by non-invasive routes to chickens reared by smallholders in Tanzania and Nepal. *Research Square* 2022.
- [23] Acharya MA, Adhikari SK, Awasthi H, Jha A, Singh UM. Field Verification Trial of ND I-2 Vaccine in Nepal. *Nepalese Vet. J.* 2019, 36, 15-22.
- [24] Nyaupane S, Pokharel BB, Acharya MP. A Study to Estimate Longevity of Thermostable Newcastle Disease Vaccine (strain I-2) in Village Chickens of Nepal. *Adv Life Sci* 2016, 6, 45–48.
- [25] Animal Quarantine Office, Annual Quarantine Report 2020/21. Available online: [http://www.ktm.aqo.gov.np/downloadfile/Final%20Approval%20For%20Printing%20\(2\)_1659424832.pdf](http://www.ktm.aqo.gov.np/downloadfile/Final%20Approval%20For%20Printing%20(2)_1659424832.pdf) (accessed on 28 August 2022).

Prevalence of Gastro-intestinal Helminthiasis in Cattle of Madi Valley, Chitwan

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ABSTRACT

Cattle is one of the leading species of domestic livestock in Nepal. The helminthic diseases are common in livestock with varied occurrence. This study was carried out to find the prevalence and risk factor associated with gastro-intestinal helminth parasites in 304 fecal samples of cattle of Madi Valley, Chitwan, Nepal. Rectal sample were collected in zip lock plastic bag containing in 10% formalin and preserved in refrigerator before examination. Sedimentation and floatation techniques were used for the detection of helminth parasites. The overall prevalence of gastro-intestinal helminth was found to be 51.32%. The parasitic infection of trematode was 39%, cestode 13% and nematode 48%. In the trematode group, Fasciola sp. 4.61%, Paramphistomum sp. 15.13%, Dicrocoelium sp. 1.64%, Schistosoma sp. 0.66% and Bilharzia sp. 0.99% were prevalent. Among cestode, only Moniezia sp. was observed with infection rate of 7.89%. Nematode included Strongyloides sp. 3.95%, Trichostrongylus sp. 7.24%, Toxocara sp. 3.95%, Oesophagostomum sp. 1.97%, Haemonchus sp. 4.28%, Cooperia sp. 1.97%, Bunostomum sp 0.66%, Nematodirus sp 0.99% and others 3.29%. Mixed infections were observed in 17% of positive samples. Sex and age group showed statistically significant difference. Male have higher prevalence than that of female ($P < 0.05$). Potential risk factor like farmer's group, water source, body condition score, grazing and rearing system, purpose of animal rearing and last date of drenching showed significant association on prevalence of parasite. Cattle of Madi valley, Chitwan had shown wide range of helminthiasis in cattle.

Keywords: Cattle, Helminthiasis, Prevalence, Madi

INTRODUCTION

Livestock farming is an important agricultural sub-sector in Nepal (Khanna et al., 2022). Nepal has a population of 7.38 million cattle and 5.30 million buffalo (CBS, 2019) distributed over the country. Livestock farming act as the back bone of the Nepalese rural agriculture which maintain the livelihood of the 65% of the population (Chaudhary, 2018).

Parasitism is one of the major problems which reduce livestock productivity round the globe (Vercruysse & Claerebout, 2001). Gastro-intestinal helminth, has been the major cause for chronic illness and reduction of productive potential in cattle (Gunathilaka et al., 2018). Helminthes commonly infecting cattle includes trematode, cestode and nematode parasites (Bisset, 1994). These parasites infect the gastrointestinal tract and associated organs of ruminants with possibility to cause severe anemia (Holmes, 1987). The significance of helminths infection is increasing manifolds in developing countries (Rondelaud et al., 2001). The epidemiology of the disease is closely associated with environmental factor, temperature, humidity, rainfall and with the ecology and the infection status of the snail intermediate host, *Lymnaea* spp.

The reduction in feed intake and decrement of feed utilization due to associated parasitism cause the huge economic loss because of stunted weight gain, reduced milk production, low fertility, higher morbidity and associated effects (Jittapalapong et al., 2011). The control of helminthiasis in cattle revealed the positive milk yield response and lesser risk in zoonosis (Charlier et al., 2009).

The gastro-intestinal helminths, commonly found in cattle were identified as *Paramphistomum* sp., *Trichostrongyloid* sp., *Fasciola* sp., *Moniezia* sp., *Trichuris* sp., *Strongyloides* sp., *Taenia* sp., *Nimatodirus* sp., *Oesophagostomum* sp., *Toxocara* sp., *Bunostomum* sp., etc. (Ozidal et al., 2010; Jittapalapong et al., 2011; Farooq et al., 2012; Mahmuda et al., 2012). Within the Nepalese territory, wide varieties of helminth infections of fluke, tapeworms and roundworms are responsible for marked deleterious effects that tend to lower overall production due to morbidity and mortality (Mukhiya et al., 2007). The major endo-parasite in domestic animal causing severe economic problem in tropical climate of Chitwan, Nepal was found to be fluke infestation (Bista et al., 2018). Faciolosis, emerging zoonotic disease, in Nepal, has caused an overall economic loss of about 14.2 crore due to productivity loss in animals (Lohani & Rasaili, 1995). Jaiswal (2006) considered the *Fasciola* sp. as most important parasitic disease while Adhikari et al., (2003) reported *Paramphistomum* sp. caused serious illness.

Studies to determine the prevalence of intestinal parasites in cattle was not done in Madi valley yet. This study will be fruitful as it will give information regarding different

helminth parasites of cattle, a risk factor associated with gastro-intestinal helminth as well as update the previous findings on the prevalence of helminth parasites of cattle. This study will also form a base for the future investigators, those investigating the helminth related diseases of cattle.

MATERIALS AND METHODS

Study area

The study area, Madi is a municipality in Chitwan district of Nepal that lies in geographical coordinates of 27°27'5"N to 84°21'32"E. The study was based on laboratory testings after collection of samples on field level.

Sample Size

A total of 304 fecal samples of cattle of all age and sex group were collected from different area of Madi valley. During sample collection the precautions were taken to keep samples fresh, only rectal samples were collected in air tight container and fixed in 10% formalin. The questionnaire was used to collect information regarding various potential risk factors. Sample size was calculated with formulae given by Godden (2004) for infinite population (> 50,000).

$$SS = \frac{Z^2 (p)(1 - p)}{C^2}$$

Where, $z=1.96$ at the level of 95 % confidence level

P = expected prevalence previous prevalence is 26% (Sapkota et al., 2006)

C = desired prescription (0.05) at 95% confidence level

Fecal Examination

The fecal samples were collected, kept in preservatives and brought to laboratory and refrigerated. The fecal samples were examined by differential floatation technique (Juyal, 2003) and sedimentation technique (Soulsby, 1982).

Data analysis

The prevalence was calculated by dividing the number of samples showing positive for fecal examination by the total number of sample examined. Percentage (%) was used to measure prevalence. The data were entered in MS- Excel and analysis was done by SPSS Version 16. The chi – square test at 5% level of significance was used to evaluate statistically significance of the association of risk factors.

RESULTS

Out of 304 samples 156 (51.32%) were positive and the rest were negative for presence parasite's eggs on the fecal sample. Mixed infections were observed in 27 samples. The

parasitic infection of trematode was 86 (39%), cestode 28 (13%) and of nematode 86 (48%). The trematode identified with their prevalence percentage are as follows: *Fasciola* sp. 4.61%, *Paramphistomum* sp. 15.13%, *Dicrocoelium* sp. 1.64%, *Schistosoma* sp. 0.66% and *Bilharzia* sp. 0.99%. Among Cestode, only *Moniezia* species was observed with infection rate of 7.89%. Nematode included *Strongyloides* sp. 3.95%, *Trichostrongylus* sp. 7.24%, *Toxocara* sp. 3.95%, *Oesophagostomum* sp. 1.97%, *Haemonchus* sp. 4.28%, *Cooperia* sp. 1.97%, *Bunostomum* sp. 0.66%, *Nematodirus* sp. 0.99% and others 3.29% (Figure 1).

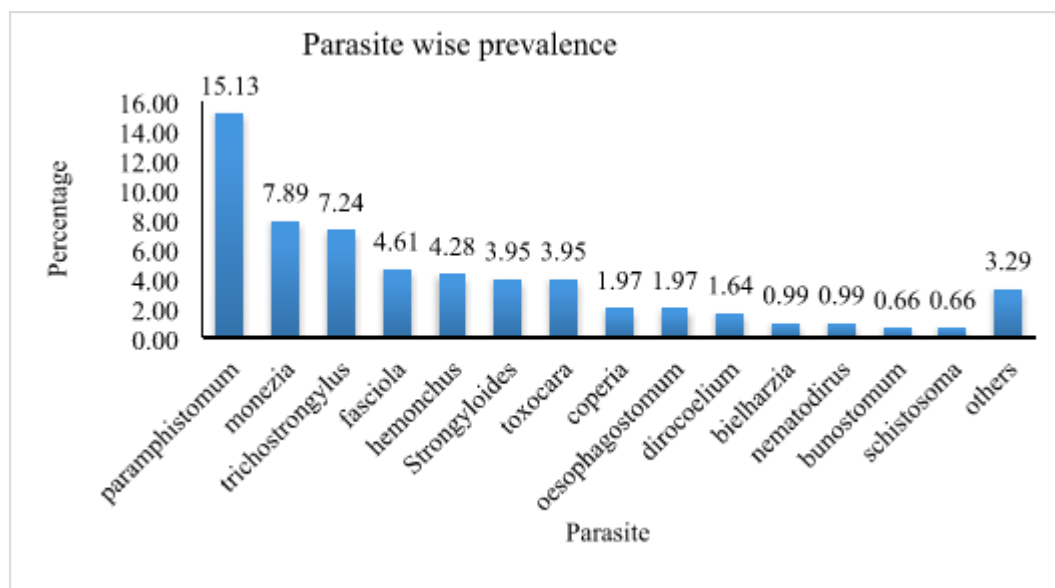


Figure 1. Bar diagram showing Parasite wise prevalence of parasites

All age group were affected as expressed in **Table 1**. Higher proportion in age group between 2 year to 4 year (58.12%), followed by below 2 year (57.5%) and age group above 4 year (38.46%) . Among the positive sample, (77/105) were male and (79/199) were females. Male have higher chance of getting infection then female (odd ratio=4.177). Prevalence in male (73.33%) then female (39.70%) is statically significant (**Table 2**).

Table 1: Age wise prevalence of parasite

Age group	Absent n (%)	Present n (%)	C h i - s q u a r e value	p- value	Remarks
Below 2 yrs	17 (42.5)	23 (57.5)	10.460	0.000	significant
2-4 yrs	67 (41.88)	93 (58.12)			
Above 4 yrs	64 (61.54)	40 (38.46)			

Table 2: Sex wise prevalence of parasite

	Result		Total	Chi-square value	Odd ratio	p value
	Positive N (%)	Negative N (%)				
Female	79 (39.7)	120 (60.3)	199	31.125	4.177	0.000** Significant
Male	77 (73.33)	28 (26.67)	105			

Table 3. Univariable analysis associated with detection of parasitic eggs

	Risk Factors		Positive N (%)	Negative N (%)	Total	Chi-square Test	Odd ratio	P-value	Remarks
1	Farmer's group	Upper caste	69 (40.12)	103 (59.88)	172	19.888	0.347	<0.001	Significant
		Lower caste	87 (65.91)	45 (34.09)	132				
2	Water source	Underground	76 (39.38)	117 (60.12)	193	30.152	0.252	<0.001	Significant
		Both (surface + underground)	80 (72.07)	31 (27.93)	111				
3	Body condition score	Good	26 (19.12)	110 (80.88)	136	1.021	14.47	<0.001	Significant
		Poor	130 (77.38)	38 (22.62)	168				
4	Feeding and grazing system	Stall only	70 (37.84)	115 (62.16)	185	34.364	4.281	<0.001	Significant
		Mix (stall + grazing)	86 (72.27)	33 (27.73)	119				
5	Purpose of animal use	Draft	53 (71.62)	21 (28.38)	74	25.186		<0.001	Significant
		Milk	41 (35.04)	76 (64.96)	117				
		Others	62 (54.87)	51 (45.13)	113				
6	Last date of drenching	Below 6 month	30 (27.25)	79 (72.75)	109	48.265	-	<0.001	significant
		6 -12 month	90 (73.17)	33 (26.83)	123				
		Above 12 month	36 (50)	36 (50)	72				

Figures in Parenthesis represents the percentage.

The risk factors (age, sex, rearing group, body condition score, water source, grazing and rearing system, last date of drenching, purpose of animal rearing) associated with parasitic infection were analyzed for the determine association of risks factor to the parasitic infection (**Table 3**). Lower cast group of ownres, Surface water as water sources of feeding, bad BCS, Draft animals, history of more than 6 mont of no drenching, are positively associated with presence of eggs of parasites on feces. All risk factors mentioned were seen to have significant effect on the parasitic infection ($p < 0.05$) at the 95 % level of significance.

DISCUSSION

The prevalence of helminth of this study i.e. 51% is similar to Pinilla Leon et al. (2019) that showed 56.3% prevalence in cattle and sheep in Columbia. Higher prevalence was seen in the cattle of the Surkhet district done by Parajuli (1993) and that of Shrestha (1996) where the prevalence rate was 75.91% in cattle of Kathmandu valley. Lower prevalence in lactating cattle and buffaloes of Kavrepalanchowk district i.e. 22% was found by Shrestha et al. (2020) and 41.6% in cattle of Ibadan, Nigeria by Adedipe et al. (2013). Prevalence of class wise parasite is similar to the Mukhia (2007) i.e. the prevalence of nematode, trematode and of cestode was 49.09%, 36.37% and 14.54% resp. in buffaloes brought to Kathmandu for slaughtering. Prevalence of nematodes is found to be lower as compared to the result demonstrated by Adedipe (2013) while overall prevalence was higher. This may be due to the low pathogenicity of nematodes in terms of Eggs Per Gram (EPG) than that of the trematode and cestode.

Higher prevalence of rumen fluke (28.41%) and lower prevalence of liver fluke (3.69%) than this study was obtained by Jittapalapong et al. (2011) in Thailand while contrasting result was found by Parajuli (1993) that prevalence of Fasciolosis and Paramphistomiasis were 42.2 % and 25.4 % respectively.

This study shows that the higher prevalence in age group between 2 to 4 year (58.12%), followed by below 2 years (57.5%) and age group above 4 years (38.46%) which is similar to findings of Gunathilaka et al. (2018) in Srilanka. Male have higher chance of getting infection then female (odd ratio=4.177). Prevalence in male (73.33%) then female (39.70%) is statically significant which is supported by Ameen et al. (2015) with higher prevalence in male then that of female in Nigeria whereas significant result with higher prevalence on female was obtain by pfukeniya et al. (2007) in Zimbabwe.

This result is supported by Muhammad et al. (2007) that higher prevalence in grazing animal then that of stall animals and using ponds and rivers/canals as drinking water were found to have significant influence ($P < 0.05$) on the prevalence in Toba Tek Singh district of Panjab, Pakistan. Female animals and animals with moderate body condition are more likely to be positive to helminth infection was found by Adedipe et.al, (2014) in Ibadan, Nigeria. Risk Factors like body condition and sex didn't show any effect on the prevalence of infections found by Aregay et.al, (2013) cattle of Ethiopia.

CONCLUSION

The study revealed gastrointestinal parasitism hazardous for dairy industry. This study identified *Paramphistomum* sp. *Moniezia* sp, *Trichostrongylus* sp. *Fasciola* sp were major species. Risk factor likes sex, age, farmer's group, water source, body condition score,

feeding system, use of animal and date of drenching had significant effect on prevalence of helminth parasite in cattle.

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REFERENCES

1. Adedipe, O. D., Uwalaka, E. C., Akinseye, V. O., Adediran, O. A., & Cadmus, S. I. B. (2014). Gastrointestinal helminths in slaughtered cattle in Ibadan, South-Western Nigeria. *Journal of veterinary medicine*, 2014.
2. Adhikari, B., Shrestha, B. & Shrestha, R. M. (2003). A study on the prevalence and diversity of *Fasciola* sp. in cattle and buffaloes in areas of Kathmandu valley. Central Veterinary Laboratory Annual Technical Report 1: 59-66
3. Ameen, S. A., Adedokun, R. a. M., & Akinola, S. O. (2015). Prevalence of Gastro-intestinal Parasites of Cattle in Ogbomoso, Oyo State. *International Journal of Applied Agriculture and Apiculture Research*, 11(1–2), 22–26.
4. Aregay, F., Bekele, J., Ferede, Y., & Hailemeleket, M. (2013). Study on the prevalence of bovine fasciolosis in and around Bahir Dar, Ethiopia. *Ethiopian Veterinary Journal*, 17(1), 1-11.
5. Bisset, S. A. (1994). Helminth parasites of economic importance in cattle in New Zealand. *New Zealand Journal of Zoology*, 21(1), 9-22.
6. Bista, S., Lamichhane, U., Singh, D. K., & Regmi, S. (2018). Overview of seasonal prevalence of liver fluke & rumen fluke infestation in cattle and buffalo of western Chitwan, Nepal. *J. Inst. Agric. Anim. Sci.* 35: 235-241
7. Charlier, J., Höglund, J., von Samson-Himmelstjerna, G., Dorny, P., & Vercruysse, J. (2009). Gastrointestinal nematode infections in adult dairy cattle: Impact on production, diagnosis and control. *Veterinary Parasitology*, 164(1), 70–79. <https://doi.org/10.1016/j.vetpar.2009.04.012>
8. Chaudhary, D. (2018). Agricultural Policies and Rural Development in Nepal: An Overview. *Research Nepal Journal of Development Studies*, 1(2), 34–46. <https://doi.org/10.3126/rnjds.v1i2.22425>
9. Diary, K. (2079). *Krishi Diary 2079*. Government of Nepal. Ministry of Agriculture and Livestock Development, Kathmandu, Nepal.
10. Farooq, Z., Mustaq, S., Iqbal, Z. & Akther, S. (2012). Parasitic helminths of domesticated and wild ruminants in Cholistan Desert of Pakistan. *International Journal of Agriculture and Biology* 14(1): 63-68.
11. Godden, B. (2004) Sample Size Formulas. <http://williamgodden.com/samplesizeformula.pdf>
12. Gunathilaka, N., Niroshana, D., Amarasinghe, D., & Udayanga, L. (2018). Prevalence of Gastrointestinal Parasitic Infections and Assessment of Deworming Program among Cattle and Buffaloes in Gampaha District, Sri Lanka. *BioMed Research International*, 2018, e3048373. <https://doi.org/10.1155/2018/3048373>
13. Holmes, P. H. (1987). Pathophysiology of parasitic infections. *Parasitology*, 94(S1), S29-S51.
14. Jaiswal, L.K. (2006). Study on fascioliasis in ruminants at Dhanusa district. *The blue cross* 8: 122.
15. Jittapalapong, S., Sangwaranond, A., Nimsuphan, B., Inpankaew, T., Phasuk, C., Pinyopanuwat,

- N., Chimnoi, W., Kengradomkij, C., Arunvipas, P., & Anakewith, T. (2011). Prevalence of Gastro-Intestinal Parasites of Dairy Cows in Thailand. *Kasetsart Journal - Natural Science*, 45, 40–45.
16. Juyal, P. (2003). *Diagnostic veterinary parasitology: An introduction*. New Delhi: New India publishing agency.
17. Khanal, P., Dhakal, R., Khanal, T., Pandey, D., Devkota, N. R., & Nielsen, M. O. (2022). Sustainable Livestock Production in Nepal: A Focus on Animal Nutrition Strategies. *Agriculture*, 12(5), 679.
18. Lohani, M. N., & Rasaili, D. P. (1995). Economic analysis of animal diseases in Nepal. *Proceedings of the 4th National Veterinary Conference*, 2123:8-21
19. Mahmuda, A., Mohammed, A.A., Alayande, M.O., Magaji, A.A., Salihu, M.D., Fabiyi, J.P. et al. (2012). Seasonal prevalence of gastrointestinal nematodes of calves in Sokoto northwestern Nigeria. *Scientific Journal of Veterinary Advanced* 1(3): 82-89
20. Muhammad, A. R., Zafar, I., Abdul, J., & Muhammad, Y. (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *Journal of Helminthology*. 81. 323.
21. Mukhia, G. (2007). *A Study On Intestinal Helminth Parasites of Buffaloes Brought ToSatungal (Kathmandu) For Slaughter Purpose* (Doctoral dissertation, Central Department of Zoology Institute Of Science and Technology Tribhuvan University Kirtipur, Kathmandu Nepal).
22. Ozdal, N., Gul, A., Ilhan, F. & Deger, S. (2010). Prevalence of Paramphistomum infection in cattle and sheep in Van Province, Deger. *Helminthologia* 47(1): 20-24
23. Parajuli, B. (1993). Prevalence of flukes in cattle and buffaloes in Surkhet district. *Proceedings of the 4th National Veterinary Conference of Nepal Veterinary Association*, 17-19 Nov, 1992, 2123:72-75
24. Pfukenyi, D. M., Mukaratirwa, S., Willingham, A. L., & Monrad, J. (2007). Epidemiological studies of parasitic gastrointestinal nematodes, cestodes and coccidia infections in cattle in the highveld and lowveld communal grazing areas of Zimbabwe.
25. Pinilla Leon, J. C., Delgado, N. U., & Florez, A. A. (2019). Prevalence of gastrointestinal parasites in cattle and sheep in three municipalities in the Colombian Northeastern Mountain. *Veterinary World*, 12(1), 48–54. <https://doi.org/10.14202/vetworld.2019.48-54>
26. Rondelaud, D., Vignoles, P., Abrous, M., & Dreyfuss, G. (2001). The definitive and intermediate hosts of *Fasciola hepatica* in the natural watercress beds in central France. *Parasitology Research*, 87(6), 475-478.
27. Sapkota, R. C., Shrestha, S., Bhusal, S., Adhikary, S., & Paudel, S. (2006). Prevalence of Helminth parasites in lactating cattle in Madhyapur Thimi Municipality of Bhaktapur. *Green Field Journal of Himalayan College of Agricultural Sciences and Technology (HICAST)*, Gathaghar, Bhaktapur, Nepal, 4(2):58-64
28. Shrestha, E. K., Thakur, R. P., Dhakal, I. P., & Mahato, S. N. (1992). Prevalence and treatment of Fasciolosis in cattle and buffaloes in Dhankuta district. *Veterinary Review, Pakhribas Agricultural Centre (PAC), Dhankuta, Nepal*, 7(2):47-49
29. Shrestha, S. P. (1996). Prevalence of Fasciolosis in farm animals in Far Western Development Region. *Proceedings of the 1st National Workshop on Livestock and Fisheries Research in Nepal, Khumaltar, Lalitpur*, 7-9 May, 1996, pp 223-226
30. Shrestha, U. T., Adhikari, N., Kafle, S., Shrestha, N., Banjara, M. R., Steneroden, K., Bowen, R., Rijal, K. R., Adhikari, B., & Ghimire, P. (2020). Effect of deworming on milk production in dairy cattle and buffaloes infected with gastrointestinal parasites in the Kavrepalanchowk district of central Nepal. *Veterinary Record Open*, 7(1), e000380. <https://doi.org/10.1136/vetreco-2019-000380>
31. Soulsby E.J.L (1982). *Helminths, Arthropods and Protozoa of Domestic Animals*. 7th Edition. The English Language Book Society and Bailliere Tindall.
32. Vercruysse, J., & Claerebout, E. (2001). Treatment vs non-treatment of helminth infections in cattle: defining the threshold. *Veterinary parasitology*, 98 (1), 195-214.

Study on Productive and Morphological traits of Goat Kids in different Ecozones of Rolpa district

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ABSTRACT

Goat is a domesticated small ruminant, reared by small farmers across the country primarily for meat production. Productivity of goat is often low, which could be related to several genetic and non-genetic factors. A field study was carried out to characterize, evaluate and estimate the effect of non-genetic factors on the hill goat kids during December, 2015 to August 2016 in different eco-zones of Rolpa district, mid-west Nepal. Altogether 150 goat kids were selected for this study. Morphological attributes productive performances based on field monitoring and measurements within different altitudes of Rolpa were collected. Least square analysis was performed using Harvey (1990) computer software package, and means were compared using DMRT. The mean body weight of goat kids at birth, pre-weaning, weaning at 5 months' age were 2.62, 6.34, 10.15 and 13.87 kg respectively. Birth weight of kids significantly differed with respect to altitude ($P < 0.01$). Coat colour ($P < 0.05$) of does and parity ($P < 0.001$) of dams. Similarly, kidding rates of does significantly differed ($P < 0.05$) with respect to colour and altitude. Body weight of the kid at weaning were significantly ($P < 0.05$) correlated to body length, heart girth and wither height. Thus, the results of this study suggest that the performance of low altitude goat flocks was better than mid and high altitude goat flocks in Rolpa district in terms of production and reproduction traits. The selection of the best performing kids for higher weight gain could be done on the basis of both birth and weaning weights. This result could be attributed to superior genotype along with better management practices adopted by the farmers in Rolpa district.

Keywords: Kids, wither, girth weight, genetic

INTRODUCTION

Rolpa covers an area of 1,879 km² with population (2016) of 221,177. Rolpa is drained southward by the Madi River from a complex of 3,000 to 4,000 meter ridges about 50 kilometers south of the Dhaulagiri Himalaya (Statoids, 2014). The Rolpa district lies at

the height of 701m to 3639 meters above the sea level. The total area is 189385 hectares out of which 59854.5 hectare land is used for crop farming, forest consists 84474 hectare, pasture consists 32698.8 hectare, wild plants and forages 9620.8 hectare and rivers and rocks 1251.9 hectares. The average temperature in Rolpa district is maximum (31.2 Celsius), minimum (3.6 Celsius) and annual rainfall is 441mm (CBS, 2016). The elevations of high, mid and low altitude from the sea level in Rolpa district are at the range of 3639, 1375 and 701 m respectively. Nepal is an agricultural country where about 66 percent of the population is involved in agricultural occupation. Agriculture contributes to around 27.1 percent of the gross domestic product (GDP) of Nepal. The livestock sector contributes about 11.5 percent of the total GDP and 25.7 percent of the agricultural GDP (AGDP) as reported by MOAD (2018). Goat farming is the most popular means of self-employment among the youths in the country. Among the agricultural commodities, livestock plays an important role in agricultural development and economic upliftment of the country. Goat farming has been practiced by a large section of population in rural areas of Nepal. The recent population of goat is about 13.4 million and total meat contribution was 7.1 thousand metric tonnes (contributing to 20.1% of total meat production in country) per year (MOAD, 2022). The rate of increment in goat population during last 15 years (2008 to 2018) was reported 3.74 percent per year (MOAD, 2018). Various breeds of goat breeds are reared in different ecological regions of Nepal. There are gradients of topography, environment and climatic conditions and each breed evolved is acclimatized to each corresponding topographical zone (Pradhan and Gurung1985). Four indigenous breeds of goat namely Chyangra, Sinhal, Khari and Terai are documented in Nepal goats. Chyangra (1%) are found at an altitude above 2400 masl in high Himalayans, Sinhal (16%)found in high hill ranging 1500-3000 masl, Khari (56%) are available across the hills 500-1500masl and Terai goats (27%) in Terai region below 500 masl (Pokharel and Neopane, 2008).

MATERIALS AND METHODS

Time and Location of the study

The study was carried out from September, 2015 to August 2016 in Rolpa district of mid-western Nepal.

Sampling procedure and sample size

The data were collected on the basis of Pocket areas of goats distributed within different altitude at Rolpa district. The selected high altitude (1500-1800 masl) gaupalikas were (Jaimakshala and Pakhapani) followed by mid altitude (1200-1400 masl) gaupalika (Gairigaun and Libang) and low altitude (800-1000 masl) gaupalika (Masina and Jhenam). Two wards from each VDCs were selected. Within the population, in each selected site, sampling goats' kids were identified randomly. A data recording format was developed to collect data and information related to growth performance, reproductive performance,

litter traits, morphological traits and production system of hill goats reared in the study area.

Statistical analysis

Collected data were entered in the computer using MS- Excel and converted into text documents i.e. Text (MS-DOS). To study the main causes of variation and effects of non-genetic factors on productive and reproductive traits, as well as to overcome the difficulty of disproportionate subclass numbers, data were analyzed by least squares procedure using Harvey, (1990) which is based on least squares technique of variance analysis. The pair wise comparison of the least square mean comparison was made using DMRT (Duncan's Multiple Range Test) (Duncan, 1955) as modified by Kramer (1957).

Models used to analyze the collected data

A fixed effect model given by Handerson (1953) was used to analyze the body weight of kids at different stages of growth.

Morphological traits

The morphological traits were measured by using the measuring tape.

Body length (BL) - Distance from shoulder joint to the pin bone.

Wither height (WH) - Distance from ground to the point of wither.

Heart girth (HG) - Body circumference at the chest immediately behind the fore Legs.

Models used to analyze the collected data

A fixed effect model given by Handerson (1953) was used to analyze the body weights of kids at different stages of growth.

Fixed effect model for weight and body measurement traits of kids

Following statistical model was used to analyze the litter traits, body weight and morphological traits of goat kids-

$$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + e_{ijklmn}$$

Where, μ is the overall mean

a_i is the effect of i^{th} altitude ($i=1,2$ and 3)

b_j is the effect of j^{th} type of colour ($j = 1, 2, 3$ and 4)

c_k is the effect of k^{th} type of age in month ($l= 1, 2, 3, 4$ and 5)

d_l is the effect of parity ($k= 1, 2, 3$ and 4)

f_m is the effect of m^{th} season of birth ($n=1$ and 2)

e_{ijklmn} is the random element (error mean) assumed to be normally and independently distributed among the sampled population.

RESULTS AND DISCUSSION

Birth weight of kids

The overall mean birth weight of hill goat kids in this study was 2.44 ± 0.91 kg (Table 1). Parajuli *et al.* (2014) reported the average birth weight of born kids were in the range of 2.83 kg to 2.62 kg at Nawalparasi. Kolachhapati (2006) also reported the birth weight of kids in hill goats of Surkhet, Udaypur and Kavre was in the range of 1.87-2.47 kg. The birth weight of kids in this study were similar to that of Indian Ganjam goats (2.25 kg) reported by Rao *et al.* (2007). The effects of various factors on the birth weight of kids are presented in (Table 1). Pandey *et al.* (2009) reported that the mean litter weight of 2.88 kg for 50% Kharai and Jamunapari.

Table 1. Least squares means for Birth weight (kg) of hill goat at Rolpa district, Nepal, 2015/16

Factors	LS $\pm$ SE	NO	Significant Level
Overall	2.44 $\pm$ 0.91	150	
Altitude			**
Low Altitude	2.62 $\pm$ 0.20 ^a	46	
Mid Altitude	2.18 $\pm$ 0.93 ^b	44	
High Altitude	2.53 $\pm$ 0.10 ^a	60	
Breed			***
Khari	2.14 $\pm$ 0.20 ^b	87	
Khapari	2.52 $\pm$ 0.17 ^a	47	
Khabari	2.66 $\pm$ 0.23 ^a	16	
Colour			*
Black	2.24 $\pm$ 0.20 ^b	69	
Brown	2.25 $\pm$ 0.18 ^b	57	
White	2.73 $\pm$ 0.26 ^a	8	
Mixed	2.55 $\pm$ 0.21 ^a	16	
Parity			**
1-2	2.17 $\pm$ 0.17 ^b	56	
3-6	2.41 $\pm$ 0.17 ^a	88	
Season			NS
Dry(Dec-May)	2.63 $\pm$ 0.14	143	
Wet(Jan-Nov)	2.26 $\pm$ 0.26	7	
Sex			NS
Male	2.47 $\pm$ 0.20	41	
Female	2.41 $\pm$ 0.17	109	

Note: * significant at 5% ($P < 0.05$), **significant at 1% ($P < 0.01$) ***significant at 0.1% ($P < 0.001$), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO are the numbers of observations.

Body weight of kids

The overall body weight of kids from (5 month) age was 13.33 ± 0.91 kg. The numbers of kids above five months age were found in less number during data collection. So, they were not analyzed in this research. The effects of various factors on the overall weight of kids (1-5month) age are presented in Table (2). Regarding the birth season, the market requires ranges on the age of the kids between 4 to 8 months, which usually coincides with the summer period. Consequently, the improvement of the production of meat of kids during this early age has an important economic impact on the income of the ambulant herds (Gaddouret *et al.*, 2007). Under pastoral harsh conditions with restricted and irregular resources, goat productivity is highly influenced by non-genetic factors because of the extensive grazing management (Mahjoub *et al.*, 2005).

Pre-weaning (2 month) weight

The average pre-weaning weight of hill kids at 2 months was 6.34 ± 0.90 kg (Table 2). Rao *et al.* (2007) also reported similar value of pre-weaning weight (6.8 kg) of Ganjam goats in India. Parajuli *et al.* (2014) reported that the pre-weaning weight of kids was 6.84 kg in Nawalparasi.

Weaning (4 month) weight

The overall mean weaning weight of kids was about 10.15 ± 0.89 kg (Table 2). The weaning weight determines the economic value of the goats too. So, it is necessary to identify the weaning weights in the goat kids. Nearly similar value of average weaning weight of native Hill-goats (10.06 kg) was reported by Kolachhapati (2006).

Post-weaning (5 month) weight

The overall Post weaning weight of kids was about 13.67 ± 0.74 kg (Table 2). Bhattarai *et al.* (2011) also reported similar results of mean body weight of kids at post-weaning 13.87 ± 0.94 . However, lower values of weaning weight of Khari was reported by Uprety and Khanal (1997), Neopane (1997) but Rao *et al.* (2007), Rai and Singh (2005) reported higher values.

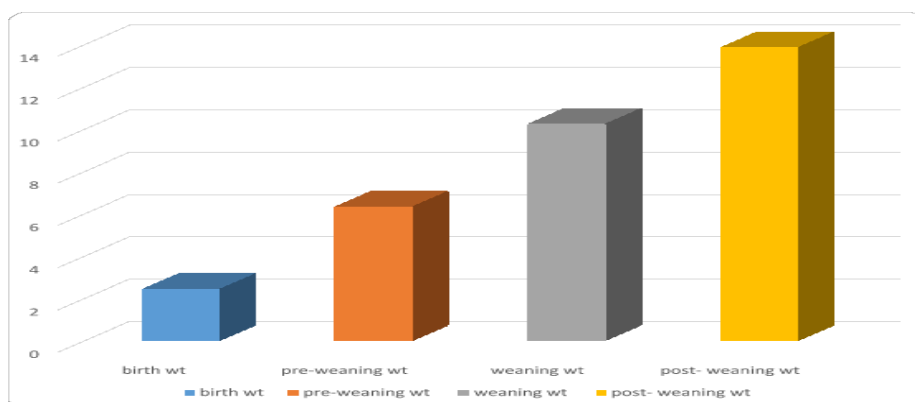


Figure 2: Weight of kid from birth to Post-weaning at Rolpa district, Nepal, 2015/16
 Table 2. Least Square means overall body weight(kg) of kids in Rolpa district, Nepal, 2015/16

Factors	LS±SE	N0	Significant-Level
Overall	13.33±0.91	150	
Altitude			***
Low Altitude	13.02±0.10 ^b	46	
Mid Altitude	15.02 ±0.93 ^a	44	
High Altitude	11.96 ±0.13 ^b	60	
Breed			*
Khari	12.74±0.14 ^b	87	
Khapari	14.51±0.89 ^a	47	
Khabari	12.94±0.11 ^{ab}	16	
Colour			**
Black	10.88±0.12 ^b	69	
Brown	12.66 ±0.91 ^{ab}	57	
White	15.87 ±0.13 ^a	8	
Mixed	11.92 ±0.12 ^{ab}	16	
Age			***
1 Month	5.43±0.87 ^c	50	
2 Month	6.34±0.91 ^c	28	
3 Month	7.15±0.11 ^c	12	
4 Month	10.15±0.89 ^b	37	
5 Month	13.67±0.74 ^a	17	
Parity			NS
1-2	13.00±0.86	56	
3-6	13.81±0.83	88	
More than 6 Year	13.18±0.14	6	
Season			NS
Dry(Dec-May)	13.16±0.71	143	
Wet(Jan-Nov)	13.50±0.13	7	
Sex			NS
Male	13.46±0.10	41	
Female	13.20±0.89	109	

Note: * significant at 5% (P<0.05), **significant at 1% (P<0.01), ***significant at 0.1% (P<0.001), NS-non significant, LS Mean- Least Square Mean, SE- Standard Error, NO are the number of observations.

Body Length of kid

The mean body length of kids at one, two (pre-weaning), three, four (weaning) and five months (post weaning) kids were 32.86±2.18, 36.66±2.26, 42.90±0.25, 46.49±2.23 and

51.05±2.24 cm respectively. The numbers of kids above five months of age were found less in numbers during sample collection. So, they were not analyzed in this research. The detail of various factor effecting body lengths are presented in Table (3). Mabrouket *al.* (2011) reported similar values of goat kids body length at one, two, three, four and five months (32, 36, 42, 46 and 47 cm) respectively in Tunisia. Okafar *et al.* (2016) reported the body length (BL) of West African Dwarf kids ranged from 22.00 to 28.00 cm (mean, 24.52 ± 0.29 cm) at birth, 52.00 to 60.00cm (mean, 56.18 ± 0.33 cm) at 4month, and 60.00 to 67.00 cm (mean, 63.55 ± 0.26 cm) at 6 months in Nigeria.

Table 3. Least squares means for body length (cm) of the goat kids in Rolpa district, Nepal, 2015/16

Factors	LS±SE	NO	Significant Level
Overall	58.87 ±2.26	150	
Altitude			NS
Low Altitude	55.19±2.26	46	
Mid Altitude	51.86±0.40	44	
High Altitude	54.61±2.48	60	
Breed			*
Khari	49.87 ±2.23 ^b	87	
Khapari	58.29 ±2.51 ^a	47	
Khabari	50.47 ±0.27 ^{ab}	16	
Colour			*
Black	53.08 ±2.48 ^b	69	
Brown	50.87 ±2.26 ^a	57	
White	57.88 ±0.33 ^{ab}	8	
Mixed	53.69 ±0.25 ^{ab}	16	
Age			***
1Month	32.86 ±2.18 ^c	50	
2 Month	36.66 ±2.26 ^{bc}	34	
3 Month	42.90 ±0.25 ^{ab}	12	
4 Month	46.49 ±2.26 ^a	37	
5 Month	51.05 ±2.24 ^a	17	
Parity			***
1-2	54.35 ±2.13 ^b	56	
3-6	59.61 ±0.34 ^a	88	
More Than 6 Year	59.02 ±1.77 ^{ab}	6	
Season			NS
Dry(Dec-May)	54.60±1.77	143	
Wet(Jan-Nov)	53.18±0.32	7	
Sex			**
Male	55.52 ±2.48 ^a	41	
Female	52.24 ±2.20 ^b	109	

Note: * significant at 5% (P<0.05), **significant at 1% (P<0.01) ***significant at 0.1% (P<0.001), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Heart girth of kid

Average heart girth of kid at pre-weaning was observed as 46.07 ± 1.39 cm that reached up to the range of 51.75 ± 1.37 cm until weaning. The numbers of kids above five months age were found in less number during data collection so, they were not analyzed in this research. The heart girths of kid are presented in Table (4).

Table 4. Least square means for heart girth (cm) of goat kid in Rolpa district, Nepal, 2015/16

Factors	LS $\pm$ SE	NO	Significant Level
Overall	50.62 $\pm$ 1.37	150	
Altitude			*
Low Altitude	51.28 $\pm$ 1.54 ^a	46	
Mid Altitude	51.35 $\pm$ 1.39 ^a	44	
High Altitude	49.25 $\pm$ 1.49 ^b	60	
Breed			NS
Khari	49.78 $\pm$ 2.18	87	
Khapari	50.54 $\pm$ 1.52	47	
Khabari	51.53 $\pm$ 1.75	16	
Colour			NS
Black	49.63 $\pm$ 1.49	69	
Brown	50.57 $\pm$ 1.37	57	
White	51.30 $\pm$ 2.03	8	
Mixed	50.97 $\pm$ 1.65	16	
Age			***
1Month	42.18 $\pm$ 1.3 ^c	50	
2 Month	46.07 $\pm$ 1.39 ^b	34	
3 Month	49.53 $\pm$ 1.65 ^b	12	
4 Month	51.75 $\pm$ 1.37 ^a	37	
5 Month	54.15 $\pm$ 1.34 ^a	17	
Parity			NS
1-2	50.59 $\pm$ 1.29	56	
3-6	50.24 $\pm$ 1.27	88	
More than 6 Year	51.02 $\pm$ 2.18	6	
Season			NS
Dry(Dec-May)	50.11 $\pm$ 1.04	143	
Wet(Jan-Nov)	51.13 $\pm$ 2.03	7	
Sex			NS
Male	51.02 $\pm$ 1.29	41	
Female	50.21 $\pm$ 1.34	109	

Note: * significant at 5% ($P < 0.05$), ***significant at 0.1% ($P < 0.001$), NS-non significant, LSD-Least significant difference, LS mean- Least square mean, SE- Standard error of

mean. NO is the number of observations.

Wither height of kids

The average wither height of kids from one to five month ages was 43.28 ± 2.13 cm. The various factors affecting average wither height from one to five months' age of kids are presented in the Table (5). Animals have a balanced relationship between body weights and body measurements. From this standpoint, live weight is determined to the nearest kilogram (kg) and thereafter it could be evaluated for selling. On the other hand, productivity in livestock industry can be determined by using some phenotypic measurements. The present investigation was designed to obtain some basic morphometric information and to relate body weight with different body measurements of goats.

Table 5. Least squares means for kid wither height (cm) in Rolpa district, Nepal, 2015/16

Factors	LS $\pm$ SE	NO	Significant Level
Overall	43.78 $\pm$ 2.13	150	
Altitude			***
Low Altitude	42.79 $\pm$ 2.43 ^a	46	
Mid Altitude	48.36 $\pm$ 2.18 ^a	44	
High Altitude	40.23 $\pm$ 2.33 ^b	60	
Breed			NS
Khari	43.86 $\pm$ 2.1	87	
Khapari	42.74 $\pm$ 2.36	47	
Khabari	44.75 $\pm$ 0.25	16	
Colour			NS
Black	43.81 $\pm$ 2.33	69	
Brown	42.08 $\pm$ 2.13	57	
White	47.62 $\pm$ 1.27	8	
Mixed	41.70 $\pm$ 0.25	16	
Age			***
1Month	37.13 $\pm$ 2.13 ^c	50	
2 Month	40.03 $\pm$ 0.25 ^b	34	
3 Month	47.26 $\pm$ 2.10 ^b	12	
4 Month	49.15 $\pm$ 0.68 ^a	37	
5 Month	52.20 $\pm$ 2.10 ^a	17	
Parity			NS
1-2	42.01 $\pm$ 2.03	56	
3-6	44.52 $\pm$ 2.0	88	
More than 6 Year	44.83 $\pm$ 0.33	6	
Season			NS
Dry(Dec-May)	42.90 $\pm$ 1.67	143	
Wet(Jun-Nov)	44.67 $\pm$ 0.30	7	

Sex			*
Male	45.11 $\pm$ 2.03 ^a	41	
Female	42.46 $\pm$ 0.05 ^b	109	

Note: * significant at 5% ($P < 0.05$), ***significant at 0.1% ($P < 0.001$), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO are the numbers of observations.

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Phenotypic Correlation

Phenotypic correlation between body weight and its measurements at pre-weaning

The phenotypic correlation coefficients between body weight of kids at weaning and their respective body measurements required in breeding. The coefficient of correlation of body length ($r = 0.63$), heart girth ($r = 0.65$) and wither height with ($r = 0.59$) with body weight at weaning was found to be significantly ($P < 0.05$) and positively correlated.

Phenotypic correlation among body weight and its measurements at post weaning age of 5 months

Body length and Heart girth were significantly ($P < 0.05$) and positively correlated with the post-weaning weight of hill goat, with the coefficient of 0.66 and 0.68, respectively. The coefficient of correlation of wither height ($r = 0.64$) with body weight at Post-weaning was observed to be significantly ($P < 0.01$) and positively correlated with post-weaning weight of the kids with the higher value of 'r' compared to body length and wither height (Table 46).

Accordingly, the correlation coefficient of heart girth was greater compared to that of body length and wither height in weaning and post weaning weight of kids. Heart girth was more strongly correlated to the body weight of kids with the greater coefficient of correlation than that of body length and that of wither height.

Weaker degrees of association wither height with body weight at different age might be due to less contribution of legs for muscle mass deposition on the body as compared to the contribution made by increased heart girth and body length as reported by Kuwar (2000) and Popesco (1984).

High correlation existed between heart girth and wither height with respect to the live body weight of kids indicated the functional phenotypic relationship between these physical characteristics of body measurements as indicated by Badiet *al.* (2002) and Hassen and Ciroma (1992).

CONCLUSION

This study suggest that the performance of low altitude goat flocks was better than mid and high altitude goat flocks in Rolpa district in terms of production and reproduction traits. The selection of the best performance of kids could be done on the basis of both birth and weaning weights to obtain higher weight at later stages. This result could be attributed to superior genotype along with better management practices followed by the farmers in Rolpa district.

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REFERENCES

1. Bhattarai, N and S. Sapkota. 2011. Effect of Non genetic Factors on Weight Traits of Local Tarai Goats under Farmers' Managed Condition. *Nepal Journal of Science and Technology* (12): 51-54.
2. Central Bureau of Statistics (CBS). 2016. National Population and Housing Census 2011 Volume 03. Kathmandu: Government of Nepal, National Planning Secretariat. 28pp.
3. Gaddour, A.; Najari S.; Ouni, M. 2007. Kid's growth of pure breeds and crossed caprine genotypes in the coastal oases of southern Tunisia. *Research Journal of Agronomy* (2): 51-58pp.
4. Harvey W.R. 1990. Least-square analysis of data with unequal subclass numbers. United States Department of Agriculture, Agricultural Research Service, ARS 20, USA.
5. Kolachhapati, M.R. 2006. Phenological study of hill goats under different management systems and nutritional regimes. Thesis Phd. Institute of Agriculture and Animal Sciences, Rampur, Chitwan. 148p.
6. Mahjoub, O. and Hameed, M.S. 2005. Performance of Omani goats fed diets containing various metabolisable energy densities. *Small Ruminant Research* (58): 175-180pp
7. MOAD. 2018. Selected indicators of Nepalese agriculture and population. Government of Nepal. Ministry of Agriculture and Cooperatives. Agribusiness promotion and statistical division. SinghaDarbar, Kathmandu, Nepal
8. MOAD. 2022. Selected indicators of Nepalese agriculture and population. Government of Nepal. Ministry of Agriculture and Cooperatives. Agribusiness promotion and statistical division. SinghaDarbar, Kathmandu, Nepal

9. Moubarak O.N., N.J. Sghaier, R.G. Amor, B.A. E. Amel and J.V. Delgado. 2011. The effect of non-genetic factors on the early body weights of Tunisian local goats. *Brazilian Journal of Animal Science* (39): 5p
10. Neopane, S. P. 1997. The Genetics of Productivity Traits in a Nepalese Hill-Goat Herd. PhD Thesis, University of London, UK. pp. 278.
11. Okafor, P. C., C.C. Ogbu and H. N dofor.2016. Reproductive and Early Growth traits of Intensively Reared West African Dwarf (WAD) Kids in a Humid Tropical Environment. *International Journal of Livestock Research* 6 (2) 53-68pp.
12. Pandey SR, M.R. Kolacchapati, N.R. Devkota and S.P. Neopane. 2009. Effect of genetic groups on litter and dam traits of goat under the western hill condition of Nepal. *Journal of institute of Agriculture and Animal Sciences(IAAS), Rampur, Chitwan* (30): 87-96pp.
13. Parajuli, A. K.,M.R. Kolachhapati, N.R. Bhattarai, N.R. Devkota.2014. Effect of non-genetic factors on productive performance of hill goat in Nawalparasi, Nepal. *Nepalese Journal of Agricultural Sciences*. 198-203pp.
14. Pokharel PK and SP Neopane.2008. Indigenous Goats of Nepal. National Animal Science Research Institute.Nepal Agricultural Research Council. Khumaltar, Lalitpur. 3-8pp.
15. Rai B. and M.K. Singh. 2005. Production performance of Jakhrana goats in its home tract. *Indian J. of Anim. Sci.* 75(10): 1176-78.
16. Rao P.K., M.K. Singh, B. Rai, and R. Roy.2007. Performance of Ganjam Goat under field conditions. *Indian Journal of Animal science* 77(2): 184-186pp.
17. Statoids. 2014. Districts of Nepal.Gwillim Law. Land adminstration and management mapping, surveying and aerial photopgraphy, Major refrences projects. Finn map.
18. Upreti, C.R.1998/99. Comparative performance of seven goat breeds (Khari, Sinhal. K*J, K*B, K*Ki, Jamunapari, Barbari) at ARS, Bandipur. Annual Technical Report. Agriculture Research station (goat) Bandipur. pp. 14-24.
19. Neopane, S. P. 1997. The Genetics of Productivity Traits in a Nepalese Hill-Goat Herd. PhD Thesis, University of London, UK. pp. 278.

Antibiotic Residue in Raw Milk Collected from Dairy Farms and Markets in Nepal

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ABSTRACT

Antibiotic residue in food has been a growing public health problem due to the increasing global threats of antimicrobial resistance. Animal products including milk and meats might also contain residues of antibiotics as administering antibiotics in animals to treat infection is a common practice. However, the level of the presence of residues are not frequently quantified in Nepal. The objective of this study is to estimate the level of residues in milk collected from dairy farms and markets. A total of 935 raw milk samples were collected from 14 districts of 5 provinces namely Koshi, Madhesh, Bagmati, Gandaki and Lumbini province. Samples were tested at the Veterinary Standards and Drug Regulatory Laboratory (VSDRL) from June 2021 to Dec 2022 first using Rapid Test Kit followed by Gentamicin ELISA Kit for quantification. The result showed antimicrobial residue was present in 1.6% of the milk samples (15/935). On further quantification with ELISA, only 4 samples (0.42%) were positive for Gentamicin antibiotic which were below the MRL level. Extensive research with larger sample size and national representative samples are needed to understand the exact scenario of antibiotic residue in milk.

Keywords: Antibiotics, Gentamicin, ELISA, MRL

INTRODUCTION

Milk is a source of high quality protein and bioavailable amino acids, several vitamins, and minerals (Smith et.al., 2021). In low-middle-income countries, milk is an important food source for nurturing the human population with high risks of nutritional deficiencies (Dror & Allen, 2011). Intake of animal food sources including milk can stimulate growth and weight gain in growing children by providing energy, protein, and micronutrients

(Hoppe et al., 2006). In developing countries, milk is the most consumed dairy product and its demand is further increasing with the increased population, urbanization, and awareness among the people (FAO, 2022).

Globally, the use of antibiotics is increasing rapidly for therapeutic and prophylactic purposes along with the growing dairy industry (Kümmerer, 2008). The introduction of antibiotics in 1940s revolutionized medicine by saving millions of lives of peoples from infectious disease. However, soon after the introduction of each new type of antibiotic, bacteria also evolved, gaining the potency to withstand the effect of the antibiotic that previously killed them, and to multiply in the presence of those antibiotics, thus becoming resistant (Pogurschi et al., 2015). Antibiotics are used widely in food-producing animals to improve productivity by lowering the morbidity and mortality (Oliver et al., 2011). The use of antimicrobials in livestock globally is double as compared to humans (Aarestrup, 2012) with approximately 80% of all the food-producing animals receiving medication for part or most of their lives (Lee et al., 2001). Antibiotics are used in the dairy sector not only to control, treat and prevent infection in animals but also for enhancing animal growth and increase feed efficiency (Tollefson & Miller, 2000). Though the use of antibiotics in livestock animals has numerous benefits, its injudicious use in animals leads to the excretion of antibiotics in animal products such as milk, meat, and eggs for a certain period of time as residues (Zhang et al., 2009). These residues have a serious impact on the dairy industry and human health when present above the maximum residue limit (Priyanka et al., 2017). The maximum residue limit is the maximum permissible level of drug or chemical concentration in the food or feeds intended to be used for consumption (Sachi et al., 2019). Various long-term and short-term effects on human health such as allergy, ototoxicity, hepatotoxicity, reproductive disorders, bone marrow toxicity, etc can be seen as a result of the consumption of antibiotic residues (Padol et al., 2015).

The major concern associated with the presence of antibiotic residues above MRL in food products such as milk is the emergence of foodborne AMR. The continuous exposure of bacteria to antibiotic residues can generate antibiotic-resistant bacteria which can complicate the treatment of both human and animal diseases. (Bengtsson-Palme et al., 2018). Antibiotic resistance is a major concern to global health and food security as it is estimated to cause 10 million deaths each year by 2050 (O'Neill, 2014).

In Nepal, the dairy industry contributes to 9% of the total GDP (two third of Livestock GDP). Dairy industry in Nepal has also grown rapidly in the past years with a total production of 2.47 million metric tons of milk in 2020/21 which shows an increment of 7.77% compared to the last fiscal year (DLS, 2020). Several studies on the antibiotic residue in meat and milk of different districts has been done before by Thapaliya et al., 2013, Khanal et al., 2018, Gompo et al., 2020. There is a government regulatory laboratory; Veterinary Standards and Drug Regulatory Laboratory (VSDRL), with a mandate of

testing drug residue in meat, milk and egg. Also, Nepal has signed the signatory of Jaipur Declaration on AMR (2011), is a member of stakeholders of tripartite association (WHO, WOA and FAO) for of Global Action Plan for control of AMR and has defined focal point for antimicrobial use, it is important for the assessment of antibiotic residues in food and the environment to know about the status of antimicrobial use and to control the emerging risk of antimicrobial resistance. Thus, this study aims to provide a recent data on the antibiotic residue in milk of major dairy hub of Nepal in order to have a picture of antibiotic residue scenario in animal products in the country.

MATERIALS AND METHODS

The present study was conducted at the Veterinary Standards and Drug Regulatory Laboratory (VSDRL) from June 2021 to Dec 2022 as a part of routine testing of the laboratory.

Sample collection

A total of 935 raw milk samples were collected from 5 provinces of Nepal using random sampling method. Out of the total samples, 36 samples were collected from Koshi province, 99 samples were collected from Madhesh province, 460 samples were collected from Bagmati province, 247 samples were collected from Gandaki province and 93 samples were collected from Lumbini province. Samples were collected in sterile collection tubes and transported to the laboratory in icebox where they were further stored at -20°C before testing.

Sample processing

Samples were processed within seven days of sample collection. The raw milk samples were heated in a water bath at 82°C for 2 minutes to destroy heat labile natural inhibitors and microorganisms contaminated in raw milk as per the guidelines provided in test kit.

Test for presence and absence of drug residue

The milk samples were tested using Rapid Test Kit for determination of drug residues in milk and milk products by Asian Medic Co. Ltd., Thailand. The test kit has 91.7% accuracy, 100% sensitivity and 90.5% specificity in detecting the presence of 12 kinds of antibiotic drug residues in milk.

Procedure: 0.1 ml of milk sample was added to the prepared tubes and 0.1 ml of UHT fresh milk was added into another prepared tube for negative control. All the tubes were incubated for 2 hours 30 minutes in water bath at 64°C, keeping medium in the tube under water level. Then, the colour change of medium in sample tubes were observed. A clear colour change from purple to yellow indicated the absence of antimicrobial residues. A purple colour indicated the presence of antibiotic residues and the level of purple colour

indicated the quantity of drug residues.

ELISA for the quantification of gentamycin in positive samples

The positive samples from rapid test were further tested with Gentamicin ELISA kit (Elabscience Biotechnology Inc., United States) for quantification of Gentamycin antibiotics in the milk samples.

Preparation of sample for ELISA: 1 gm of the sample was combined with 1 ml of 1% trichloroacetic acid solution and mixed by vortex for one minute. Then the sample was centrifuged at 4000 rpm for 5 minutes at room temperature. 950µl of sample solution was added to 50 µl of the obtained intermediate liquid and was mixed properly. The final mixture was then used for analysis.

Assay procedure: Before the assay, all the reagents and samples were brought to room temperature and a record of sample wells and standard wells was prepared. 50 µl of standard solution and sample solution were placed in respective wells. 50 µl of HRP conjugate and 50 µl of antibody working solution were added to each well and were mixed thoroughly. The plate was then covered and incubated for 30 minutes at 25°C. After 30 minutes, the cover was removed and the wells were washed with 260 µl of wash buffer for 4 times. After this, the plate was dried by inverting and patting it against thick clean absorbent paper until the wells were dried and no bubbles were seen. Then 50 µl of substrate reagent was added to each well and the plate was oscillated for 15 seconds to ensure proper mixing. The plate was then incubated for 15 minutes at 25°C in the dark. Finally, 50 µl of stop solution was added to each well and the plate was gently oscillated for 10-15 seconds to ensure the complete mixing of the reagents.

The optical density was measured at 450nm using a microplate reader (Multiscan ex, Thermo, USA). The final results were calculated by obtaining the optical density values and calculating the absorbance percentage.

$$\text{Absorbance \%} = \frac{\text{average absorbance of standard or sample}}{\text{average absorbance of 0 ppb standard solution}} \times 100\%$$

A calibration curve was plotted between the standard concentration and optical density. The obtained values were cross verified using a software (Ridasoft Win.NET)

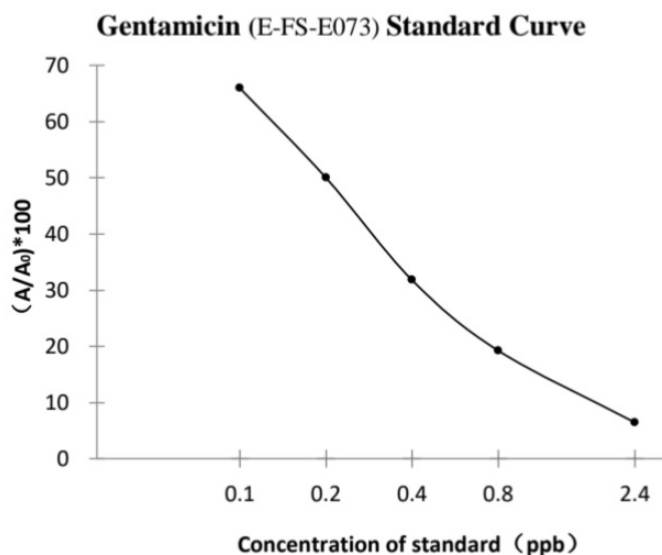


Figure 1: Gentamycin standard curve

Data analysis

The data were entered in Microsoft Excel version 2303 and data were visualized in bar graphs and tables using MS Excel and the maps were created using QGIS version 3.22.5.

RESULTS AND DISCUSSION

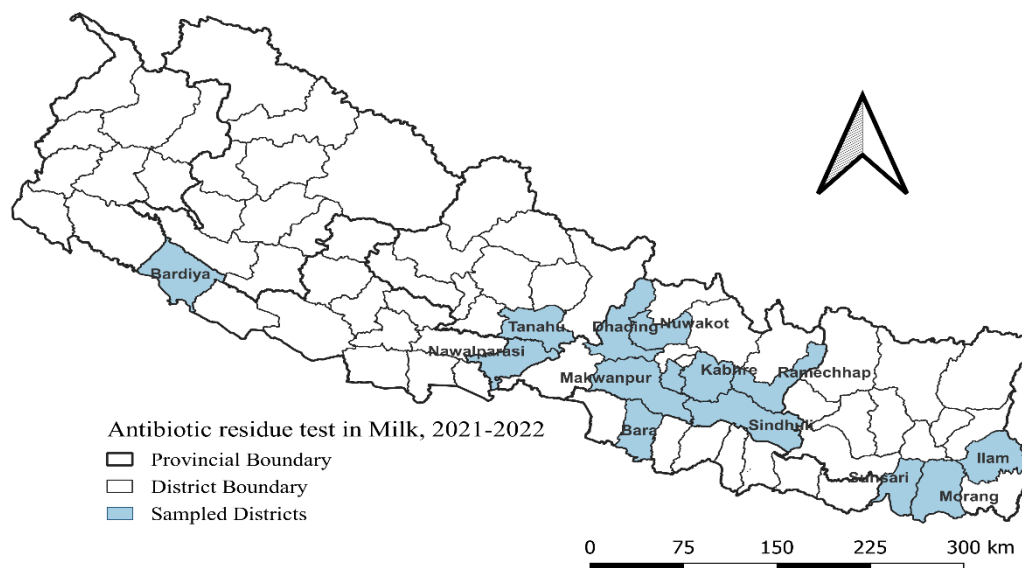
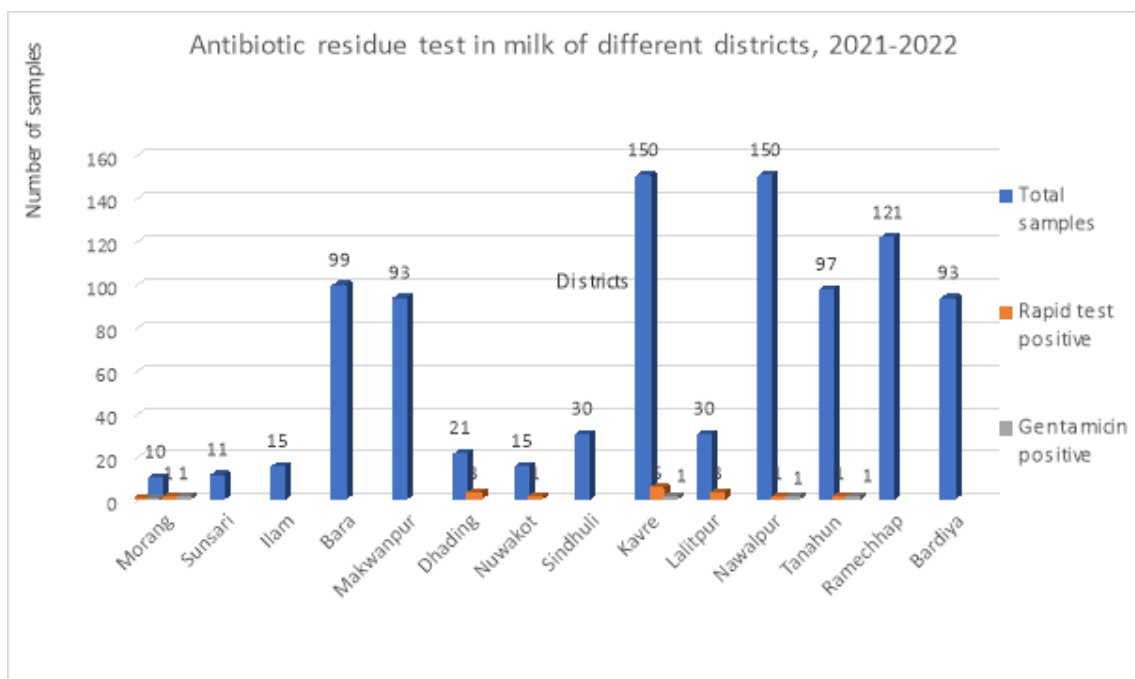


Table 1: Milk samples collected from different provinces

Province	Number of samples collected	Number of samples positive (Rapid test)	Percentage of sample positive
Bagmati	460	12	2.61
Gandaki	247	2	0.81
Koshi	36	1	2.78
Lumbini	93	0	0.00
Madhesh	99	0	0.00
Total	935	15	1.60

A total of 935 milk samples were tested for the presence of antibiotic residue. Overall, 1.6 % (n=15) of samples were positive for the presence of antibiotic residue. Milk samples from Bagmati province were seen highly positive.



Out of 14 districts sampled, Kavre district had highest number of samples (n=5) positive for antibiotics in milk, followed by Dhading and Lalitpur (n=3 each).

The positive samples were further tested with Gentamicin ELISA test kit. Out of 15 positive samples, four samples were positive for Gentamicin antibiotic. The Gentamicin positive samples were from Kavre, Morang, Nawalpur and Tanahu. The antibiotic level ranged from 2.25 to 3 ppb which is less than recommended Maximum Residue Limit (MRL) of 100 ppb for Gentamicin in milk as per the European Union and Codex Alimentarius Commission.

This study was done on laboratory data of recent two years of VSDRL. The rapid test result shows an overall of 1.6% sample positive for presence of antibiotics in milk. The result is less than other similar studies. Thapaliya et al., 2013 found 17.3% of market milk positive for antibiotic residues that were collected from 4 districts of Nepal. Similarly, Khanal et al., 2018 found 23% milk samples, positive for the presence of antibiotic residues while Bhusal et al., 2020 found a 6% prevalence of antibiotic residues in milk samples from Kathmandu valley. Gompo et al., 2020 detected antibiotic residues in 55% of the milk samples collected from the Kaski district. The higher percentage positive samples in milk of Kaski and Kathmandu districts may be because of intensive farming practices in such urban areas where the use of antibiotics might be relatively higher. Likewise, our study also shows a higher percentage of sample positive in Dhading (14%), Lalitpur (10%) and Morang (10%) districts. The decrease in overall percentage of antibiotic positive milk samples also may be the result of awareness among the farmers regarding the withdrawal period of drugs, prudent use of antimicrobials by veterinarians and number of samples. However, the data is likely to be underestimated as the major milk producing districts are not included in the study. Also, two provinces are not sampled at all and only one district representing the Lumbini province is included in the study. This demands the need for an extensive study covering all regions of the country to have data and to know about the actual scenario of antibiotic residue in milk.

CONCLUSION

Antimicrobial residue in milk is one of the growing issue for the human health. This study provides a recent data to know the current situation of antimicrobial residue, and provide a baseline for the further study on antimicrobial use in animals. The result shows the presence of antimicrobial residue in milk in 1.6% of the 935 samples collected from different 14 districts of Nepal. On further quantification with ELISA, only 4 samples were positive for Gentamicin antibiotic which were below the MRL level. This comparatively less percent positive milk for antibiotic residue than previous studies may be the result of the proper use of antimicrobials by veterinarians, increased awareness among the farmers regarding the withdrawal period of drugs, and increased testing of drug residues in milk. However, extensive research with larger sample size including highly productive districts and representing all the provinces is needed to know about the exact scenario of antibiotic residue in milk.

REFERENCES

- Aarestrup, F. (2012). Sustainable farming: Get pigs off antibiotics. *Nature*, 486(7404), 465–466. <https://doi.org/10.1038/486465A>
- Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews*, 42(1). <https://academic.oup.com/femsre/article-abstract/42/1/fux053/4563583>

- Bhusal, D. R., Chhetri, B., & Subedi, J. R. (2020). Determination of Antibiotics Residues in Milk Samples Collected in the Different Sites of Kathmandu, Nepal. *Asian Journal of Dairy and Food Research*, OF. <https://doi.org/10.18805/AJDFR.DR-186>
- Britannica. (2022). Milk. *Encyclopedia Britannica*. <https://www.britannica.com/topic/milk>
- Dror, D. K., & Allen, L. H. (2011). The Importance of Milk and other Animal-Source Foods for Children in Low-Income Countries. *Http://Dx.Doi.Org/10.1177/156482651103200307*, 32(3), 227–243. <https://doi.org/10.1177/156482651103200307>
- FAO. (2022). Gateway to dairy production and products. <https://www.fao.org/dairy-production-products/products/en/>
- Gombo, T. R., Sapkota, R., Subedi, M., Koirala, P., & Bhatta, D. D. (2020). Monitoring of Antibiotic Residues in Chicken Meat, Cow and Buffalo Milk Samples in Nepal. *International Journal of Applied Sciences and Biotechnology*, 8(3), 355–362. <https://doi.org/10.3126/IJASBT.V8I3.31314>
- Hoppe, C., Mølgaard, C., & Michaelsen, K. F. (2006). Cow's Milk and Linear Growth in Industrialized and Developing Countries. *Annual Review of Nutrition*, 26, 131–173. <https://doi.org/10.1146/ANNUREV.NUTR.26.010506.103757>
- Khanal, B. K. S., Sadiq, M. B., Singh, M., & Anal, A. K. (2018). Screening of antibiotic residues in fresh milk of kathmandu valley, nepal. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, 53(1), 57–86. <https://doi.org/10.1080/03601234.2017.1375832>
- Kümmerer, K. (2008). *Pharmaceuticals in the environment: sources, fate, effects and risks*. Springer Science and Business Media. <https://books.google.com/ks?hl=en&lr=&id=hS1EAAAAQBAJ&oi=fnd&pg=PR28&dq=+K%C3%BCmmerer+HTK.+Pharmaceuticals+in+the+environment+%E2%80%93+sources,+fate,+effects+and+risks.+Aquat+Toxicol.+2001%3B71:391%E2%80%9332.+%5B+Google+Scholar%5D&ots=wNEHwHLCgz&sig=GNJXMKUeDEDncctmyKc7R3aq2c4>
- Lee, M. H., Lee, H. J., & Ryu, P. D. (2001). Public Health Risks: Chemical and Antibiotic Residues. *Asian-Australasian Journal of Animal Sciences*, 14(3), 402–413. <https://doi.org/10.5713/AJAS.2001.402>
- MoALD. (2022). Statistical Information on Nepalese Agriculture. <https://moald.gov.np/wp-content/uploads/2022/07/STATISTICAL-INFORMATION-ON-NEPALESE-AGRICULTURE-2077-78.pdf>
- Oliver, S. P., Murinda, S. E., & Jayarao, B. M. (2011). Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. *Foodborne Pathogens and Disease*, 8(3), 337–355. <https://doi.org/10.1089/FPD.2010.0730>
- O'Neill, J. (2014). Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. *The Review on Antimicrobial Resistance*.
- Padol, A. R., Malapure, C., Dimple, V. D., & Kamdi, B. (2015). Occurrence , Public Health Implications and Detection of Antibacterial Drug Residues in Cow Milk. *Environ. We Int. J. Sci. Tech*, 10, 7–28.
- Priyanka, P., Sheoran, M., & Ganguly, S. (2017). Antibiotic residues in milk-a serious public health hazard. *J Environ Life Sci*, 2(4), 99–102. https://www.academia.edu/download/55174563/Priyanka_et_al.2017_JELS_Online.pdf
- Sachi, S., Ferdous, J., Sikder, M. H., & Azizul Karim Hussani, S. M. (2019). Antibiotic residues in milk: Past, present, and future. *Journal of Advanced Veterinary and Animal Research*, 6(3), 315. <https://doi.org/10.5455/JAVAR.2019.F350>
- Smith, N. W., Fletcher, A. J., Hill, J. P., & McNabb, W. C. (2021, December 20). Modeling the Contribution of Milk to Global Nutrition. *Frontiers*. <https://doi.org/10.3389/fnut.2021.716100>
- Thapaliya, M., Karki, T. B., & Sedai, D. (2013). Sulfonamides and Penicillin Residue in Market Milk. *Journal of Food Science and Technology Nepal*, 8, 60–64. <https://doi.org/10.3126/JFSTN.V8I0.11751>
- Tollefson, L., & Miller, M. A. (2000). Antibiotic use in food animals: controlling the human health

impact. *Journal of AOAC International*, 83(2), 245–254. <https://academic.oup.com/jaoac/article-abstract/83/2/245/5656315>

- Virto, M., Santamarina-García, G., Amores, G., & Hernández, I. (2022). Antibiotics in Dairy Production: Where Is the Problem? <https://doi.org/10.3390/dairy3030039>
- Zhang, H., Ren, Y., & Bao, X. (2009). Simultaneous determination of (fluoro) quinolones antibacterials residues in bovine milk using ultra performance liquid chromatography–tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 49(2), 367–374. <https://www.sciencedirect.com/science/article/pii/S0731708508005803>

Observation of Rearing System of Small Ruminants in Jumla District

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ABSTRACT

Small ruminant production is one of the main sources of meat in Jumla district and contributes in the food security of the nation. A study was carried out at Guthichaur and surrounding areas of Jumla district to observe the rearing system and management practices of small ruminant. Twenty farmers from Guthichaur, Jumla with a flock size of 100 to 350 animals were interviewed about the rearing system, management practices, and health problems. Sheep was a major livestock commodity and most of the farmers raised sheep and goat together in transhumance system (migratory sheep flock). Baruwāl, Bhyanglung and their crosses in case of sheep while Sinhal in case of goat were found to be common in the study areas. From the beginning of summer season (Falgun-Jestha), the animals were grazed in the nearby field during the day and kept in shed at night. In winter, where it snows, they were kept in shed and fed hay and stored corn due to limited grazing area. At night, some farmers kept their sheep, goats in shed while some farmers kept their sheep outside in field. From third week of Jestha to fourth week of Bhadra, farmers moved their flock to high hills called Patan to graze on locally available herbs. Farmers performed open castration on their animals by themselves and besides deworming the debilitated animals, they generally didn't deworm or vaccinate their animals. Some of the major health problems observed in the animals were plant poisoning, parasitic infestations, scabies and lice infestation, pneumonia, abortion, uterine prolapse, vaginal prolapsed, and inbreeding. Farmers kept the same male for seed material for many years and were found to be unfazed by the consequence of inbreeding.

Keywords: Rearing system, small ruminant (sheep and goat), transhumance system, Jumla

INTRODUCTION

Small ruminant production is one of the main sources of meat in Jumla district and contributes in the country's food security. Sheep is the major livestock commodity in Jumla district compared to goat. Farmers raise sheep in transhumance system (Tiwari

et. al., 2020), also called as migratory sheep flock, popular in Himalayan regions, where farmers practice seasonal migration of their animal for pasture. Farmers adopting this rearing system generally don't practice regular vaccination, deworming, and health management practices that lead to various health burden in the animals. Previous study has reported ovine fascioliasis in fecal test (34%), slaughtered sheep (6.7%) and post mortem examinations (23.5%) with mature flukes apparent in liver and gall bladder of inspected sheep (Sah et. al., 2020). Observation of the transhumance rearing system adopted by the indigenous people in the remote area like Jumla is essential to understand their husbandry practices, identify the challenges they face for the health management of their flock, and the constraint they face to improve the nutritional need for their animals. Understanding of the annual cycle of transhumance rearing system in Jumla can help the government to formulate essential policies and converge the focus that could uplift the livelihood of the farmers and support the food security of the nation. This study highlights the transhumance rearing system and management practices including health status of small ruminant in the surrounding areas of Jumla.-

METHODOLOGY

Observational study of the rearing system of small ruminant was carried out in Guthichaur and surrounding areas of Jumla. Twenty farmers' group from Guthichaur, Jumla with a flock size of 100 to 350 animals were interviewed. Among the groups of farmers, eight had combined their smaller flock to a larger one. Farmers were interviewed in field and at the river basins. They were asked about the rearing system/pattern, feeding, vaccination and deworming schedule, general health problems etc.

Brief description about study area:

- Sheep and Goat Research Program (SGRP), Guthichaur Rural Municipality ward no- 2, Jumla and surrounding areas
- 19 Km from District head quarter (Khalanga) Jumla.
- Rainfall: 755 mm (June-Sep: 85%)
- Temperature: Max.27.3°C to Min. -5.7°C
- Snowfall: December to March
- Relative humidity: 60-84%
- Sunshine hour: 9.3 hrs in winter
- Latitude: 29°17'72.5" N
- Longitude: 82°40'83.4" E
- Elevation: 2700 MASL (Meters Above Sea Level)

Data analysis

Data were collected, compiled and analyzed in descriptive way by using MS Excel and SPSS.

RESULTS AND DISCUSSION

Farmers raised sheep and goat in transhumance system (migratory sheep flock). Sheep was the major livestock commodity followed by goat. Baruwai, Bhyanglung and their crosses in case of sheep while Sinhal in case of goat were found to be common in the study areas. Limited improved breeds like Romney marsh and Coopworth cross were also found among some farmers as Sheep and Goat Research Program introduced the breeds a few years ago. Flock sized ranged from 100-350 to 15-50 animals).

Transhumance rearing system of ruminants (migratory sheep flock)

From the beginning of summer season (Falgun-Jestha), sheep were grazed all day in the nearby field. At night, some farmers kept their sheep and goats in shed while some kept their sheep in the field outside the shed. In winter, when it snows, they were kept in shed and fed hay and stored corn due to limited grazing area

During the rainy season (from 3rd week of Jestha to 4th week of Bhadra) most of the farmers moved their sheep to upper hills or mountain area (Patan) where the animals were grazed mostly on herbs and small shrubs available in the area. Buki (*Anaphalis contorta*), a highly nutritious grass, was found abundant in those areas. Observation from this study was found similar to what reported by Sapkota et. al. (2020). Generally, they take their sheep to upper hill in Jestha/Ashar and bring their flock to river basins in the first to second week of Bhadra (Sapkota et. al., 2020). Migratory sheep movement observed in this study ranged from elevation of 1250m to 4500 m during various seasons similar to reported elsewhere (Ghimire and Chapagain, 2020). Farmers with larger flock took care of their flock by themselves, whereas, those with smaller flock combined 4-5 flock into a larger flock. Farmers combining their smaller flock into a larger one did grazing duty on rotation with 2-3 farmers staying in the shed for a week or 10 days.

By the middle to 4th week of Bhadra, animals were brought down to the river basin and kept in the Jungle or in the growing field under the open sky until Ashoj as reported elsewhere (Sapkota et. al., 2020). From Ashoj, when vegetation starts becoming scarce, they made the flock size smaller and took to their houses. They reared the animals in small flocks until beginning of the next warm season completing the annual grazing cycle.

Practice of feeding salt

Since winter was very dry in those areas and animals did not get green grasses throughout the year, farmers offered salt to their sheep and goats once or twice every month to overcome the mineral deficiency. They poured some salt on grass or on a rectangular stone to let the sheep get their essential minerals.

Practice of wool shearing

Wool Shearing was done twice a year: once in Falgun-Chaitra and next in Bhadra-Ashoj. Wool shearing was done with shearing scissors in SGRP, Guthichaur farm, and in the villages. Some farmers sheared wool even with sickle. With shearing scissors, one person could shear 6-7 sheep in a day.

General health practices

Deworming and Vaccination

Farmers generally didn't deworm or vaccinate their sheep except for the weak and debilitated ones. They didn't routinely vaccinate their sheep against PPR and FMD although some were aware of the benefit of vaccination, however, they were found cooperative when some NGOs or District veterinary hospital offered the vaccine.

Castration

Majority of farmers performed open castration sheep by themselves at the age of 4/5 months. Only some farmers castrated their sheep through closed castration method using Burdizzo castrator. In open surgical castration, they did not ligate the blood vessels. They burnt the feces of sheep to ash and applied in to the scrotum after castration, whereas, some farmers used turmeric powder, antibiotic powder, povidine iodine e.t.c as antiseptic. Consequently, they have lost their castrated sheep due to heavy bleeding and infection. Farmers near Sheep and Goat Research Program castrated their sheep with Burdizzo castrator by help of animal health technician.

Major health problems

Major health problems faced by farmers were plant poisoning, parasitic infestations, scabies and lice infestation, pneumonia, abortion, uterine prolapse, vaginal prolapsed and inbreeding etc. A few published reports showed that Pneumonia, Fascioliasis, other parasites, abortion, prolapse, plant poisoning etc were the major health problems in sheep in Jumla (Sah et. al., 2020; 2021; 2022).

Parasitic infestations

To overcome the problem of parasitic infestation, they applied cypermethrin (Tick out) solution soaked cloth on to the animals during Chaitra to Baishak (small scale dipping). Some farmers dewormed their animals once or twice in 2-3 years period. Generally, they used Albendazole, Oxytetracycline, Piperazine etc. for deworming. They dewormed their sheep after lambing season during Magh to Falgun. In SGRP premises, Fasciola is endemic, and tapeworms were also found in abundance (Sah et. al., 2020).

Plant Poisoning

A specific type of herbal plant (locally called bikh- *Aconitum* species) is found in the bank of rivers/streams. After consumption of this plant, animal becomes lethargic, and foaming

from mouth, recumbency and bloating were apparent, eventually leading to death, if untreated immediately. This plant grows in the beginning of warm season where green fodder is scanty increasing the risk of sheep consuming the poisonous plant. Every year, almost 4-5 sheep die in each flock because of this plant poisoning. Pandey and Gyawali (2012) also reported that poisonous plants often account for the loss up to 10-15% of lamb per year in the moving flock, especially during the spring season near the summer pasture. Atropine sulphate injection @ 0.4 mg/kg b.wt. and sodium thiosulphate @ 5-10 gm dissolved in 100-200 ml water were used to counteract the poisoning. Oral drenching of sodium thiosulphate immediately after poisoning reduced the mortality to 90-95 %.

Uterine and vaginal Prolapse

Sporadic cases of uterine and vaginal prolapsed cases were common in those areas. One sheep in nearby farm at Kolte had uterine prolapse during the study period.

Pneumonia

Because of the cold weather, onset of pneumonia gradually leading to death was observed in the sheep. Additionally, since the animals were kept in the shed during winter, they also suffered from suffocation due to poor ventilation. Even during the rainy season when the sheep were moved to Patan, they were kept under open sky in cold environment putting the animal in risk of pneumonia. The shepherds had to carry some general medicines for first aid in Patan as there were no veterinary services available. A post mortem examination of a dead sheep showed yellowish fibrin like materials surrounding the lungs and multiple necrotic foci throughout the lungs.

Inbreeding problems

Farmers kept the same male for seed material in the flock for many years. It was difficult to convince them that inbreeding occurs when the same male is kept in herd for many years. Farmers perceived that large sized rams were better than smaller ones and they kept same large ram in their flock for many years. Adhikari et al., 2103 mentioned in a study that most of the flocks in migratory system used the same breeding buck for 4-5 years without considering inbreeding affects. The average body weight of one-year-old male sheep in farmers' possession weighing 12-13 kg compared to 20-22 kg at Sheep and Goat Research Program (SGRP) further questions the breeding quality of sheep among the farmers.

CONCLUSION

Transhumance rearing system of small ruminant is in practice in the hilly regions of Nepal including Jumla. A seasonal movement of livestock between agro-ecological zones for pastures have been adopted by the farmers while still facing numerous challenges and constraints. Indigenous husbandry practices performed by the farmers in Jumla to

move the flock to the high hills of Patan to access the nutritious grasses, ad lib grazing, while dodging the hot climate in the lower altitude let them take the benefit of unused natural green fodder. However, their potential contribution in the country's food security along with their grappled thriving situation need to be highlighted more to improve the livelihood of the farmers, herd-health management and economy. Awareness on disease management and good husbandry practices, timely technical support and guidance, accessible and affordable veterinary services, linkage to livestock/meat market, and other promotional business ideas from the animal products and by-products could be some of the challenges that need to be addressed from the central government and local sector as well.

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REFERENCES

1. Adhikari D., Adhikari, D.P., Sharma, M.P., Nepali, D.B., Kolachhapati, M.R., Tiwari, M.R. and Ghimire P. and Chapagain, P.S. (2020). Livestock Movement in Gaurisankhar Valley, Dolakha, Nepal. *The Geographic Base*, **31**;7:15-23.
2. Pandey L.N. and Gyawali, R. (2012). Constraints and potential of goat and sheep production under transhumance management system in the high mountainous regions of Nepal. *Research and Development Strategies for Goat Enterprises in Nepal*. pp. 92-101.
3. Sapkota, S. (2013). In *Migratory System of Barawal Sheep Production in Nepal: Problem and Prospect*. Proceedings of the 9th National Workshop on Livestock and Fisheries Research in Nepal, pp.191-196.
4. Sapkota A. Sapkota, B. Khatiwada, S. and Neupane, G. (2020). Transhumant Goat and Sheep Husbandry Practices in High Hills of Annapurna Conservation Area. *International Journal of Environment, Agriculture and Biotechnology*, **5**(5).
5. Sah R.P., Yadav, M.P., Kanu, S.P. and Rijal, T.R. (2020). Study on ovine fascioliasis: Case study, associated risk factors and economic significance at Sheep and Goat Research Program, Guthichaur, Jumla, Nepal. *International Journal of Veterinary Sciences and Animal Husbandry*, **5**(4):164-168.
6. Sah R.P., Yadav, M.P. and Kanu, S.P. (2021). Study on association of different animal and management factors on occurrence of Pneumonia in sheep in Jumla. *Nepalese Journal of Agricultural Sciences*, **21**:110-118.
7. Sah R.P., Yadav, M.P., Kanu, S.P., Ali, Md S.N. and Talukder, Md H. (2022). Cross sectional study of diseases and disorders in sheep at Sheep and Goat Research Program, Guthichaur, Jumla, Nepal. *Nepalese Journal of Agricultural Sciences*, **22**:65-71.
8. Tiwari K.R., Sitaula, B.K., Bajracharya, R.M., Raut, N., Bhusal, P. and Sengel, M. (2020). Vulnerability of Pastoralism: A Case Study from the High Mountains of Nepal. *Sustainability*, **12**(7):27-37.

Molecular Detection of *Anaplasma* in Cattle of Morang, Rupandehi and Surkhet districts of Nepal

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ABSTRACT

Anaplasma, a vector transmitted rickettial parasite, infects blood cells of bovine causing asymptomatic to clinical bovine anaplasmosis. This study was conducted to estimate the prevalence of *Anaplasma* sp. in blood samples of dairy cattle in Morang, Rupandehi, and Surkhet districts of Nepal and to determine the risk factors and biochemical alterations of the respective animal. In total, 120 blood samples (40 from each district) were randomly collected from dairy cattle. The blood smears were prepared and fixed with 10% methanol on the field, and fresh blood was collected in EDTA tubes for molecular analysis and in clot activating tubes to yield serum for biochemical tests. 9 (7.5%) blood samples had produced the 577 base pairs of DNA fragments specific for the 16S rRNA gene of *Anaplasma* sp. by PCR amplification. 5 (4.16%) blood samples in the smear were found positive by microscopic examination. Serum biochemical profile was not different between *Anaplasma* sp. positive cattle and negative cattle ($p > 0.05$). The prevalence rate between Jersey and Holstein Friesian crossbred was also not different ($p > 0.05$). There was a higher prevalence among cattle aged more than 2 years compared to cattle less than 2 years ($p < 0.05$) indicating older cattle were more susceptible to the *Anaplasma* infection. In conclusion, serum biochemical alterations and risk factors should be considered in order to achieve prognosis and initiation of an appropriate therapeutic regimen for a favorable outcome of the tick-borne haemoparasitic diseases along with molecular detection for improved detection.

Keywords: Haemoparasites, Polymerase Chain Reaction, Tick Borne Disease

INTRODUCTION

Nepal has 6 indigenous breeds of cattle belonging to *Bos indicus* except Lulu (*Bos taurus*). Dairy cattle comprise 14.60% of the total cattle population in Nepal (AITC 2020). Tick borne diseases (TBDs) are among the major constraints to the livestock industry in developing countries that causes paralysis or toxicosis and physical damage to livestock

with significant economic losses (Rajput et al., 2005; Durrani et al., 2008, Zulfiqar et al., 2012). The impact is felt by large-scale production systems but is potentially even more devastating for smallholding rural farmers and communities. In addition to clinical episodes of tick borne diseases, productivity loss will also occur in animals that appear healthy but are infected, and play a critical role in the epidemiology. The local environment is also a critical epidemiological factor, because various tick species have been distributed to transmit pathogens to susceptible animals in Nepal (Shrestha et al., 2005; Thakuri et al., 1990).

Bovine anaplasmosis is also known as gall sickness and the disease causing agents are *Anaplasma marginale*, *A. centrale*, *A. phagocytophilum*, *A. bovis* and *A. platy*, among which *A. marginale* is responsible for clinical bovine anaplasmosis (Aubry & Geale, 2011; Theiler, 1910). More than seventeen tick species acts as vectors including *Hyalomma*, *Dermacentor*, *Ixodes*, *Argas*, *Rhipicephalus* (Ashraf et al., 2013; Fyumagwa et al., 2009). In tick, the infection may occur intra-stadial, trans-stadial or rarely via trans-ovarian. Sometime the bites of dipterous flies' act as mechanical transmitters. Trans-placental infection occurs during the acute phase of infection in dam about second and third trimester (Kocan et al., 2010). During diagnosis of *Anaplasma* sp., they appear as dense, homogeneously staining blue purple inclusions (0.3-1) μm in diameter. Inclusion bodies contain 1-8 initial bodies (0.3-0.4) μm known as individual Rickettiale (Merck, 2010).

Most of the research works are based on the sero-prevalence and other microscopic diagnostic techniques for screening of vector borne parasitic diseases (Yadav, 2015; Gautam et al. 2019). However, pathogen species can be misidentified and due to this, and a lack of sensitivity, the level of carrier animals can be underestimated by microscopy. The introduction of PCR based molecular techniques, the sensitivity, specificity and reproducibility for detection of *Anaplasma* sp has been improved. The analytical sensitivity of PCR based method has been estimated at 0.0001% infected erythrocytes. A nested PCR has been used to identify *A. marginale* carrier cattle with capability of identifying as few as 30 infected erythrocytes per ml of blood, well below the lowest levels in carrier. Real-time PCR assays may target one of the several genes (Carrelli et al., 2007; Decaro et al., 2008) or 16S rRNA (Reinbold et al. 2010) and are reported to achieve a level of analytical sensitivity equivalent to nested conventional PCR (Carrelli et al., 2007; Decaro et al., 2008; Reinbold et al., 2010).

As limited information is available regarding the prevalence of *Anaplasma* sp infection in ruminants from Nepal, the present study was designed to report the prevalence of *Anaplasma* sp in blood samples of dairy cattle from Morang, Rupandehi, Surkhet districts. Furthermore, the present study will provide a baseline data regarding risk factors associated with *Anaplasma* sp infection and the potential effect of the parasite on the

selected parameters of serum biochemical profile of dairy cattle in Nepal.

MATERIALS AND METHODS

Sample and data collection

Blood samples were collected from 120 cattle of different dairy farms of Morang (40), Rupandehi (40), Surkhet (40) districts and collected in Serum and EDTA vials maintaining cold chain in cool box. Blood smears were prepared at field and fixed with 10% methanol. After staining with Giemsa-Stain the slides were observed under oil immersion (100X) at NCRP laboratory. A questionnaire was filled at the spot in order to gather data of risk factors associated with bovine anaplasmosis.

DNA Extraction

DNA extraction was carried out by using protocol provided in QIAGEN tissue and blood test kits, in central biotechnology laboratory of Agriculture and Forestry University (AFU), Rampur. The quality and quantity of DNA was detected in Nano-drop-spectrophotometer. About 1 µl of extracted sample was allowed to Nano-drop-spectrophotometer.

The extracted DNA sample having the value 1.8 (260nm /280nm) were subjected to Polymerase Chain Reaction (PCR) in BIORAD T100 Thermal cycler. Mixture for PCR composed of Nuclease Free Water (NFW) = 7 µl, Master Mix (MM) = 10 µl, Forward primer (F) = 1 µl, Reverse primer (R) = 1 µl and Extracted DNA (60ng/ µl) = 1 µl. Fwd: 5'AGAGTTTGATCCTGGCTCAG 3' Rev: 5'GTTAAGCCCTGGTATTTCAC 3' primer is used for *Anaplasma* sp. Initial denaturation at 95°C for 5 min Denaturation (40cycles) at 94°C for 30 sec Annealing at 55°C for 30sec, Elongation at 72°C for 90sec Final extension at 72°C for 5 min.

Gel-electrophoresis was performed using 1.5% agarose 1X TBE in gel electrophoresis tank (CLEAVER Scientific Ltd.). Ingredients for Gel-E-Run, for each well, the well mixed solution of Loading Dye = 1 µl (6X) and PCR sample = 5 µl was dropped gently in the well of gel whereas ladder of (100bp) = 6 µl was dropped into single well (generally into first and last well). The setting of current in gel-electrophoresis was 400 Amp, 80Volt for 1.5 hr. And then subjected to L.E.D. ORBIT SHAKER in the solution of EtBr2 (15min) and distilled water (5min) and the gel was observed under the clear view UV-Transilluminator.

Serological analysis for serum bio-chemistry parameters in blood samples from dairy cattle i.e. Urea Nitrogen and Serum Glutamic Pyruvic Transaminase (SGPT) were determined in *Anaplasma* sp positive and negative blood samples of cattle by using BIOBASE EL-

10A Elisa microplate reader and diagnostic kits manufactured by Tulip Diagnostics (P) Ltd. (Spain).

Statistical Analysis

SPSS version-19 was used for statistical analysis. Cattle were categorized into two age groups, less than 2 years and more than 2 years old. Association between the presence of *Anaplasma* sp and two epidemiological parameters, viz. breed and age were analyzed using the Fisher's exact test (for 2 x 2 tables). Independent T-test was applied to compare the biochemical profile between parasite positive and negative animals.

RESULTS

Prevalence of *Anaplasma* spp

Anaplasma sp was detected in 9 out of 120 collected blood samples (7.5%) from dairy cattle during present study as they amplified a 577 base pair product of 16S rRNA gene specific for *Anaplasma* sp (Figure 1). Whereas only 5 blood samples (4.17% of total) in smear were found positive under 100X oil immersion.

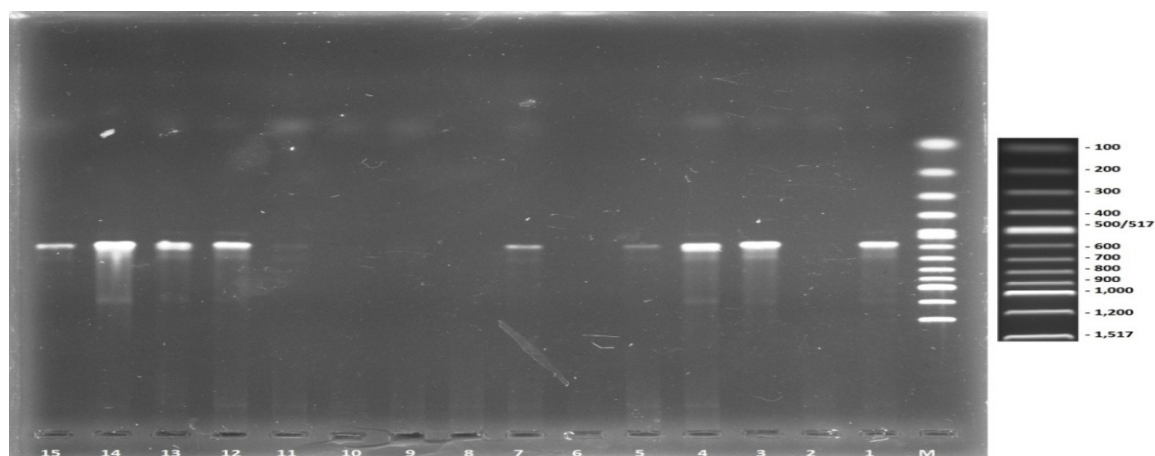


Figure. 1 Identification of *Anaplasma* sp. from blood sample of cattle by Polymerase Chain Reaction (PCR) Lane, M: DNA Marker 100bp. Lane, 1,3,4,5,7,12,13,14,15 Positive samples with base pair 577.

District wise Prevalence of *Anaplasma* spp.

Table 1. Results of *Anaplasma* sp. infection in dairy cattle of Morang, Rupandehi and Surkhet districts of Nepal by PCR.

Districts	Sample (N)	<i>Anaplasma</i> sp +ve	<i>Anaplasma</i> sp -ve
Morang	40	5	35
Rupandehi	40	3	37

Surkhet	40	1	39
Total	120	9	111

Risk factors associated with *Anaplasma spp.*

Prevalence of *Anaplasma sp* was not different by breed (Jersey Cross or Holstein Friesian Cross) ($P > 0.05$). While, cattle aged more than 2 years old were more susceptible compared to cattle less than 2 years of age ($P < 0.05$) (Table 2).

Table 2. Association between prevalence of *Anaplasma spp.*, in blood samples of dairy cattle collected from Morang, Rupandehi and Surkhet districts and animal characteristics studied during present study. P values represent the output of Fisher's exact test.

Subject		Parameters	Sample (N)	<i>Anaplasma sp</i> +ve	<i>Anaplasma sp</i> -ve	P-value
Dairy cattle of Nepal	Breed	Jersey crossed	42	5	37	$P > 0.05$
		Holstein Friesian crossed	29	4	25	
		Total	71	9	62	
	Age	>2 year	49	9	40	$P < 0.05^*$
		<2 year	22	0	22	
		Total	71	9	62	

Analysis of biochemical profile

The comparison of serum biochemical profiles found to be non-significant for all studied parameters between *Anaplasma spp.* positive and negative animals SGPT and Nitrogen Urea level remain unchanged i.e. $P > 0.05$ (Table 3).

Table 3. Comparison of various studied parameters biochemical profile between *Anaplasma sp* positive and negative cattle samples from of dairy cattle collected from Morang, Rupandehi and Surkhet districts. P-value represents the result of independent t-test calculated for each parameter

Dairy cattle of Nepal	Parameter	<i>Anaplasma sp</i> +ve	<i>Anaplasma sp</i> -ve	P-value
	SGPT (U/L)	33.30±10.47	31.48±10.00	$P > 0.05$
	Nitrogen Urea(mg/dl)	17.93±1.60	17.27±0.68	$P > 0.05$

DISCUSSION

Ticks and tick-borne diseases affect the productivity of bovines in tropical and subtropical regions of the world, leading to adverse impact on the cattle farming industry. Report of Food and Agriculture Organization (FAO) estimates, there are 50% production losses

because of their fatality and adverse effects on animal productivity in the developing countries due to different tick borne diseases including anaplasmosis (Nasir 2000; Akhter et al. 2010).

As limited information's are available in literature regarding *Anaplasma* sp incidence from Nepal, present research was designed to report the prevalence of genera *Anaplasma* in blood samples of dairy cattle from Morang, Rupandehi and Surkhet districts. The primers used in the present study can amplify the highly conserved sequence of 16S rRNA gene used to perform PCR for amplification. In the present study, the prevalence of *Anaplasma* sp. was found to be 7.5% and 4.17% by PCR and microscopic techniques respectively. This is the first research report regarding anaplasmosis adopting the molecular technique in cattle of Nepal. Pradeep et al (2019) performed a study on bovine anaplasmosis in which 7% (14/199) were positive for the presence of the *Anaplasma* sp. by successful amplification of the 16S rRNA genus-specific primers, inclusions of *A. marginale* were observed in 3% (6/199) blood smears in south India. These results are similar to our findings. The first report from central Punjab of Pakistan using molecular technique by Iqbal et al. (2019) showed the presence of two *Anaplasma* sp. with an overall prevalence rate of 10.44% which is slightly higher than our finding.

Prevalence rate of the *A. marginale* in total 211 bovines was 18.48% and 6.64% ($P < 0.05$) as per the PCR and Giemsa stained blood smear, respectively by Kumar et al. (2019) in Gujrat India. The results are higher than our findings. It may be due variation in geographical location and sample size. Shrestha SP and UM Singh (1999) conducted a research study to find the prevalence of protozoan diseases in the eastern terai by microscopic examination of blood smears of 83 crossbred dairy cattle in which 5 (6.02%) found positive for *Anaplasma* sp. which is slightly greater; this might be due to blood samples they collected were from febrile animals. Debbarma et al (2020) studied a total of 310 cattle blood smears samples and altogether 108 samples were found to harbor different blood parasites, out of which 5.8% *Anaplasma* sp. were present.

According to Anbu et al. (2020) PCR assay was significantly higher sensitivity in detection of tick borne diseases than microscopic examination in clinically suspected cattle and reported 20.78% infected cattle with *Anaplasma* sp. The infection of *Anaplasma* sp. (27.77%) was higher in less than 2 year of age group. Kaur et al (2020) reported the 23.74% (66/278) infected cattle with *A. marginale*. The highest prevalence of parasitic infection was found in animals having greater than 3 years of age (37.50%) followed by 1 to 3 years (28.57%) and less than 1 year of age (16.98%) in north India.

Debbarma et al (2020) observed that a significant increase in the activity of SGPT (32.30 ± 3.697 and BUN (23.05 ± 1.07) in infected cattle in comparison to healthy cattle. Whereas, the concentration of serum glucose level (57.94 ± 2.111) was significantly decreased

in infected group comparison to healthy group. A study conducted by Nasreldin et al (2020) found the significantly elevated serum alanine amino transferase, aspartate amino transferase and urea in blood from parasite-infected cattle compared with controls. The blood glucose level was significantly decreased in infected cattle compared with controls. In our research and the research report of Hussain et al (2015) it was found that serum biochemical profile unaffected when compared between the *Anaplasma* sp. positive and negative animals. It is because the sample collection was performed from most of the apparently healthy cattle.

Velusamy et al. (2014) found that there was a significantly ($p < 0.05$) high prevalence of haemoprotozoan diseases in Holstein Friesian (HF) and Jersey cross breeds than indigenous breed. We have included only HF and Jersey cross breed and the result was non-significant among two breeds. The occurrence of these haemoprotozoan diseases was found to be high among the age groups of 2 to 7 years in cross-bred animals and below 2 years in indigenous animals which is similar concurrent with our findings.

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DECLARATIONS

Funding

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Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical Approval

The publish data and results in the research were approved by NARC. The NARC Research Ethics Committee of Nepal has confirmed that no ethical approval is required.

Consent to participate

For this research all the involving animal subjects were given freely and respective animal

owners were informed prior to sampling.

REFERENCES

1. Anbu, R. K. R., Mangalanathan, V. B., Ganapathy, S., & Alagesan, S. K. (2020). Epidemiological Studies on Bovine Tick-Borne Haemoparasitic Diseases in Chennai.
2. Aubry, P., & Geale, D. W. (2011). A review of bovine anaplasmosis. *Transboundary and emerging diseases*, 58(1), 1-30.
3. Carelli, G., Decaro, N., Lorusso, A., Elia, G., Lorusso, E., Mari, V., & Buonavoglia, C. (2007). Detection and quantification of *Anaplasma marginale* DNA in blood samples of cattle by real-time PCR. *Veterinary microbiology*, 124(1-2), 107-114.
4. Debbarma, A., Pandit, S., Jas, R., Baidya, S., Batabyal, S., & Bachan, M. (2020). Alterations of serum biochemical parameter in cattle naturally infected with tick-borne haemoparasitic diseases in West Bengal, India. *Int J Livest Res*, 1.
5. Decaro, N., Carelli, G., Lorusso, E., Lucente, M. S., Greco, G., Lorusso, A., & Buonavoglia, C. (2008). Duplex real-time polymerase chain reaction for simultaneous detection and quantification of *Anaplasma marginale* and *Anaplasma centrale*. *Journal of Veterinary Diagnostic Investigation*, 20(5), 606-611.
6. Fyumagwa, R. D., Simmler, P., Meli, M. L., Hoare, R., Hofmann-Lehmann, R., & Lutz, H. (2009). Prevalence of *Anaplasma marginale* in different tick species from Ngorongoro Crater, Tanzania. *Veterinary parasitology*, 161(1-2), 154-157.
7. Gautam, R., Parajuli, K., & Sherchand, J. B. (2019). Epidemiology, risk factors and seasonal variation of scrub typhus fever in Central Nepal. *Tropical medicine and infectious disease*, 4(1), 27.
8. Hussain, M. F., Qamar, M., Malik, M. I., Hussain, M., Saeed, Z., Shaikh, R. S., & Iqbal, F. (2017). Molecular detection of *Anaplasma* in apparently healthy Cholistan breed of cattle from the Bahawalpur district, Pakistan. *Tropical Biomedicine*, 34(1), 37-44.
9. Iqbal, N., Mukhtar, M. U., Yang, J., Sajid, M. S., Niu, Q., Guan, G., & Yin, H. (2019). First molecular evidence of *Anaplasma bovis* and *Anaplasma phagocytophilum* in bovine from central Punjab, Pakistan. *Pathogens*, 8(3), 155.
10. Kaur, R., Yadav, A., Rafiqi, S. I., Godara, R., Sudan, V., Chakraborty, D., & Katoch, R. (2021). Epidemiology, haematology and molecular characterization of haemoprotozoan and rickettsial organisms causing infections in cattle of Jammu region, North India. *BMC veterinary research*, 17(1), 1-12.
11. Kumar, N., Solanki, J. B., Varghese, A., Jadav, M. M., Das, B., Patel, M. D., & Patel, D. C. (2019). Molecular assessment of *Anaplasma marginale* in bovine and *Rhipicephalus (Boophilus) microplus* tick of endemic tribal belt of coastal South Gujarat, India. *Acta parasitologica*, 64(4), 700-709.
12. Nasreldin, N., Ewida, R. M., Hamdon, H., & Elnaker, Y. F. (2020). Molecular diagnosis and biochemical studies of tick-borne diseases (anaplasmosis and babesiosis) in Aberdeen Angus Cattle in New Valley, Egypt. *Veterinary World*, 13(9), 1884.
13. Rentería-Solís, Z., Birka, S., Schmäsche, R., Król, N., & Obiegala, A. (2018). First detection of *Baylisascaris procyonis* in wild raccoons (*Procyon lotor*) from Leipzig, Saxony, Eastern Germany. *Parasitology research*, 117(10), 3289-3292.
14. Reinbold, J. B., Coetzee, J. F., Sirigireddy, K. R., & Ganta, R. R. (2010). Detection of *Anaplasma marginale* and *A. phagocytophilum* in bovine peripheral blood samples by duplex real-time reverse transcriptase PCR assay. *Journal of clinical microbiology*, 48(7), 2424-2432.
15. Shrestha, S. P., & Singh, U. M. (1999, June). Study on blood protozoan disease in cattle of eastern Terai region. In *The 3rd National Workshop on Livestock and Fisheries Research in Nepal*, Agricultural Research Station, Lumle, Kaski, Nepal (pp. 26-28).
16. Shrestha, S. P., Deo, S. N., Acharya, M. P., & Kunwar, B. L. (2005). Mapping of livestock tick fauna

- of different agro-climatic zones of Nepal. Nepalese Veterinary Journal, 28, 73-77.
17. Velusamy, R., Rani, N., Ponnudurai, G., Harikrishnan, T. J., Anna, T., Arunachalam, K., ... & Anbarasi, P. (2014). Influence of season, age and breed on prevalence of haemoprotozoan diseases in cattle of Tamil Nadu, India. Veterinary World, 7(8), 574-578.
 18. Yadav, S. K. (2015). Prevalence of Blood Protozoan Parasites of Cattle, Buffaloes and Goats of three Vdcs of Siraha District, Nepal (Doctoral dissertation, Central Department of Zoology Institute of Science and Technology Tribhuvan University Kirtipur, Kathmandu).

Study of Canine Babesiosis in Pet and Stray Dogs of Kathmandu Valley

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ABSTRACT

Canine babesiosis is caused by protozoan parasites which is usually transmitted via various species of ticks. The parasites ultimately parasitize the erythrocytes of host body, resulting in fever, anemia, and hemoglobinuria. From June 2021 to September 2021, a cross-sectional study using the purposive sampling method was conducted. A total of 140 samples were collected from dogs and were examined for the presence of Babesia spp. using thin blood smear test stained in Giemsa. Regression analysis was done to compare categorical and outcome variables. The overall prevalence of babesiosis was found to be 14.3%. The result showed significantly higher prevalence of babesiosis in tick infected dogs ($p < 0.05$). Sex, age, type and breed wise prevalence were found to be statistically insignificant ($p > 0.05$). In hematological parameters, RBC, Hb, PCV, and platelets declined significantly in positive cases ($p < 0.05$). In TLC, the changes in the Babesia positive cases were statistically insignificant ($p > 0.05$), whereas in DLC of the infected cases, changes only in neutrophils and lymphocytes were statistically significant ($p < 0.05$). The mean temperature in positive cases was found to be 103.44 ± 0.69 . This research intends to add some information on the status of babesiosis inside Kathmandu valley with majorly concluding ticks as major cause of diseases. Tick control is therefore critical in order to reduce the prevalence.

Keywords: Prevalence, Disease, Ticks, Hematology

INTRODUCTION

Canine Babesiosis is one of the major tick-borne illnesses in dogs caused by protozoan parasite. (Irwin, 2010). This disease represents a considerable diagnostic challenge for veterinarians, as the clinical signs are often diffused and overlapped (Andersson et al., 2017). Due to the possible transmission of protozoa, human babesiosis has also been lately recognized as an emerging zoonosis with considerable attention (Schnittger et al., 2012). Ticks play an important role in transmitting parasites to the host body during

their blood meal (Shaw et al., 2001). Members of the genus *Babesia*, which are typically transmitted by vector ticks of the *Rhipicephalus* species, *Haemaphysalis* species, and *Dermacentor* species, are one of the major protozoan disease causing agents in canine species (Schnittger et al., 2012). *Babesia* multiplies in erythrocytes by asynchronous binary fission, resulting in considerable pleomorphism (Schetters et al., 2009). The three large species of *Babesia*, namely *B. canis*, *B. vogeli*, and *B. rossi* and the three small species, namely *B. gibsoni*, *B. microti*, and *B. annae* are documented (Irwin, 2010). However, the most frequently found species infecting dogs are *B. canis* and *B. gibsoni* in Europe and Asian countries, respectively (Bhaskaran Ravi et al., 2016). The common clinical features that are observed during infection are fever, lethargy, lymphadenopathy, muscle pain, and hemoglobinuria (Welc-Faleciak et al., 2009). The hematological parameters in disease cases generally show marked thrombocytopenia and a decrease in packed cell volume (PCV) (Schetters et al., 2009). In total leukocytic count (TLC) there is an insignificant change, whereas the differential leukocytic count (DLC) shows marked neutropenia, eosinophilia, and lymphocytosis with an insignificant change in monocytes and basophils (Hornabrook & Field, 1969). Since, the diagnosis of such diseases by blood smear examination is not a common procedure in the Nepalese context, the disease cases are mostly identified symptomatically and get supportive treatment (Phyual et al., 2006).

Table 1: List of drugs used in treatment of canine babesiosis (Schoeman, 2009)

Drug	Dose	Route of administriion	Duration of therapy
Diminazene aceturate (Berenil®)	3.5 mg/kg	IM/SC	One dose
Trypan blue (Trypan blue®)	10 mg/kg	Strictly IV	One dose followed by imidocarb or diminazene 1 week later
Imidocarb dipropionate (Imizol®)	7 mg/kg	IM	Two doses, 14 days apart

Hence, Giemsa-stained blood-smear method was used to study the prevalence of canine babesiosis among the pet and stray dogs in Kathmandu valley. Additionally, hematological parameters between the *Babesia* positive and negative individuals compared in the study.

MATERIALS AND METHODS

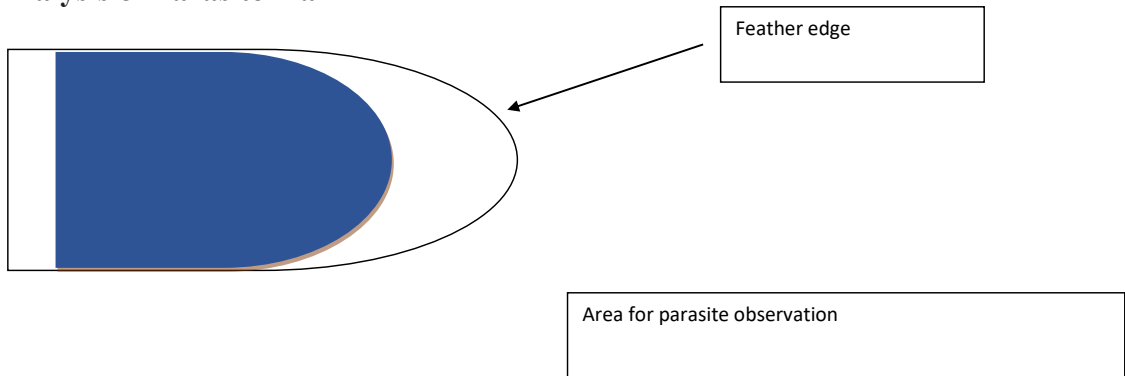
Sampling technique

Random sampling was done to take sample from dogs and the basic parameters such as age, sex and tick infestation status were considered during sampling. Altogether 140 blood samples were collected visiting different places of Kathmandu valley from 70 domesticated and 70 stray dogs.

Giemsa Technique

Giemsa stained blood films examination under light microscopy is considered to be the gold standard for the diagnosis of *Canine Babesiosis* (Njunda *et al.*, 2013). Around 3 ml of blood was collected in a clean ethylenediamine tetraacetic acid (EDTA) tube either from the saphenous, cephalic, or juglar veins with a 22G * 1” needle. Whenever the lab work had to be postponed, it was kept at -4C. The blood was taken to the lab for a complete blood count (CBC) with the help of a hematology analyzer followed by the preparation of blood smear. A drop of blood was taken on a clean glass slide, and by using another slide tilted at an angle of 45 degrees, a thin smear was prepared and air dried immediately. The smear was fixed in methyl alcohol for around 5 minutes. The fixed slide was stained in working solution of Giemsa stain for 35-40 minutes. The slide was washed and dried for examination using a compound microscope under oil immersion.

Analysis of Parasitemia



Positive cases

Babesia spp. are pear-shaped species, which are seemed to be blue due to Giemsa-staining pointed by arrow inside the RBC.

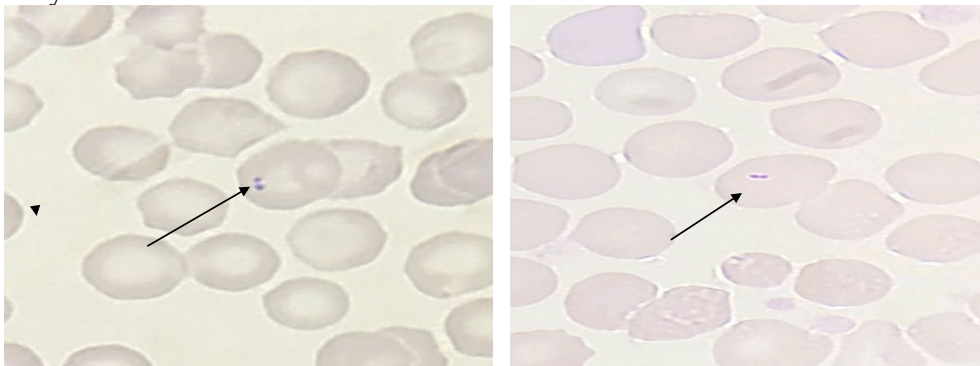


Figure 1: Presence of *Babesia spp* inside RBC

RESULTS

A total of 140 blood samples were collected from pet and stray dogs of CRVH and different animal welfare centers respectively in three districts of Kathmandu valley. Out of 140 blood samples examined, 20 (14.3%) were found to be positive and 120 (85.7%) were negative. In the case of in 60 tick infected sample, 15(33.3%)were found to be positive and 80 samples from the dogs having no ticks present at their body during sampling, 5(6.7%) were found to be positive.

Age-wise prevalence

Out of 140 blood samples, 12 were from dogs of younger than 1 year, 101 were from dogs aged between 1 and 5 years, 23 were from dogs aged between 6 and 10 years, and 4 were from dogs of older than 10 years. Among the 12 samples of dogs younger than 1 year, 1(8.33%) was found positive, and among 101 samples of dogs aged between 1 and 5 years, 13 (14.77%) were found positive. Similarly, among the 23 samples of dogs having an age between 6 and 10 years, 6 (26.08%) were found positive, and among the 4 samples from the dogs with age above 10 years, 0 were found positive. The present study indicated a higher prevalence percentage in the age groups of 6-10 years and 1-5 years, respectively. However, the result found was statistically insignificant ($p=0.351$) meaning that the differences in age group do not play an important role in the occurrence of babesiosis. The effect of the age was only 0.6% on disease occurrence.

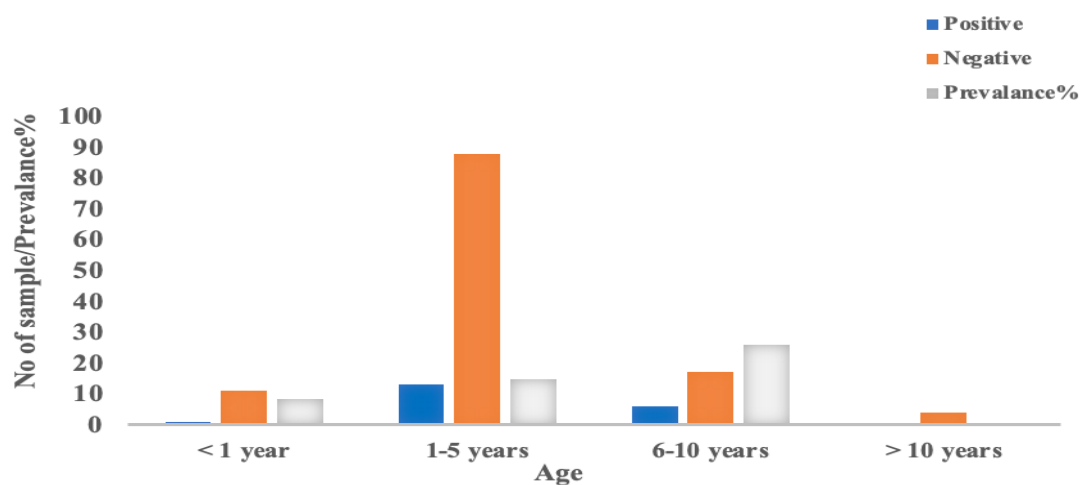


Figure 2: Prevalence of babesiosis on the basis of age

Sex-wise prevalence

Out of 140 blood samples, 81 samples were from male dogs and 59 samples were from female dogs. Among 81 samples from male dogs, 10 (14.08%) were found positive, and

among 59 samples from female dogs, 10 (20.41%) were found positive. The present study showed a higher prevalence percentage in females than in males. However, the result was found to be statistically insignificant ($p=0.446$) meaning that the sexes do not play an important role in the occurrence of babesiosis. The effect of sex was only 0.4% on disease occurrence, given by R square.

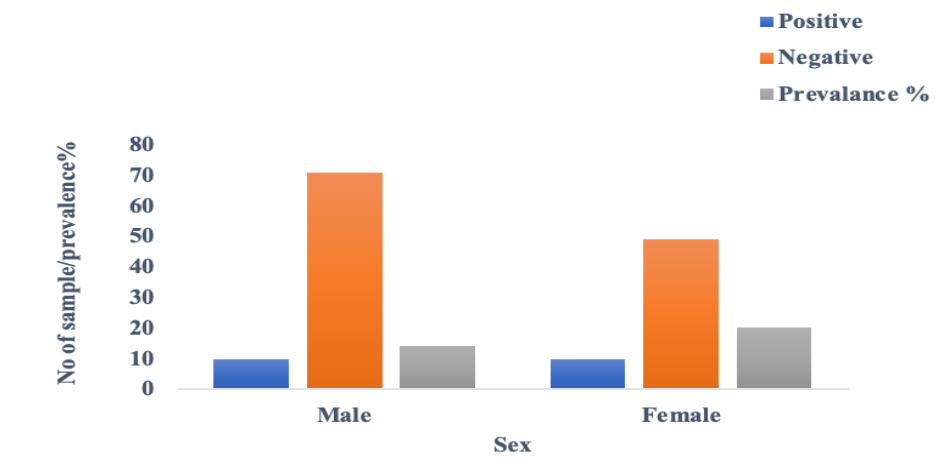


Figure 3: Prevalence of babesiosis on the basis of sex

Prevalence on the basis of presence of ticks

Out of 140 blood samples, 60 were from tick infected dogs, and 80 didn't have tick infestations. Among 60 tick-infected dogs, 15 (33.3%) were found positive, and among 80, 5 (6.7%) were found positive. Out of 5 dogs, that were babesia positive having no ticks present at the time of sample collection, 3 were from street dogs and 2 were from pet dogs. The present study indicated a higher prevalence percentage of *babesiosis* in tick-infected dogs, and the result was found to be statistically significant ($p= 0.002$) meaning that the presence of ticks in the body plays an important role in the occurrence of babesiosis.

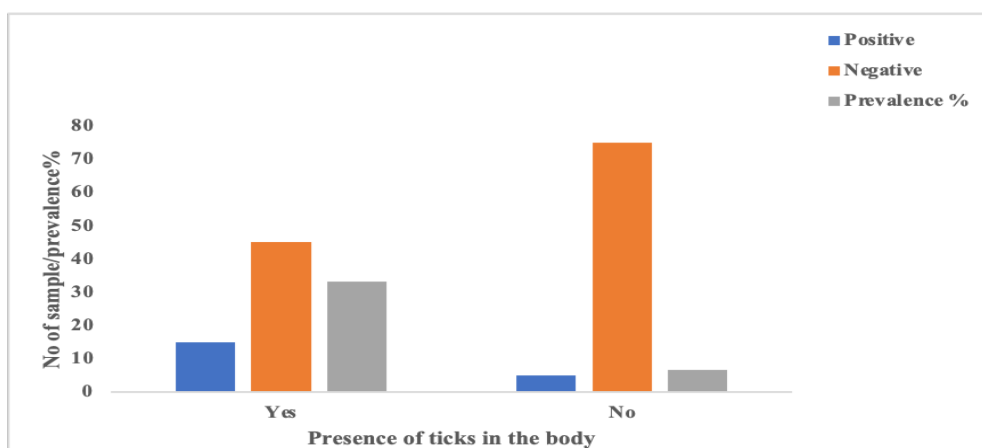


Figure 4: Prevalence of Babesiosis on the basis of presence of ticks in the body

Prevalence on the basis of types of dogs

Out of 140 blood samples, 70 were from pets and 70 were from stray dogs. Among 70 samples of pet dogs, 9 (14.75%) were found positive, and among 70 samples of stray dogs, 11 (18.6%) were found positive. The present study showed that stray dogs had a higher prevalence percentage of *Babesiosis* than pet dogs. However, the result was found to be statistically insignificant ($p=0.632$) meaning that the types of dogs do not play an important role in the occurrence of babesiosis.

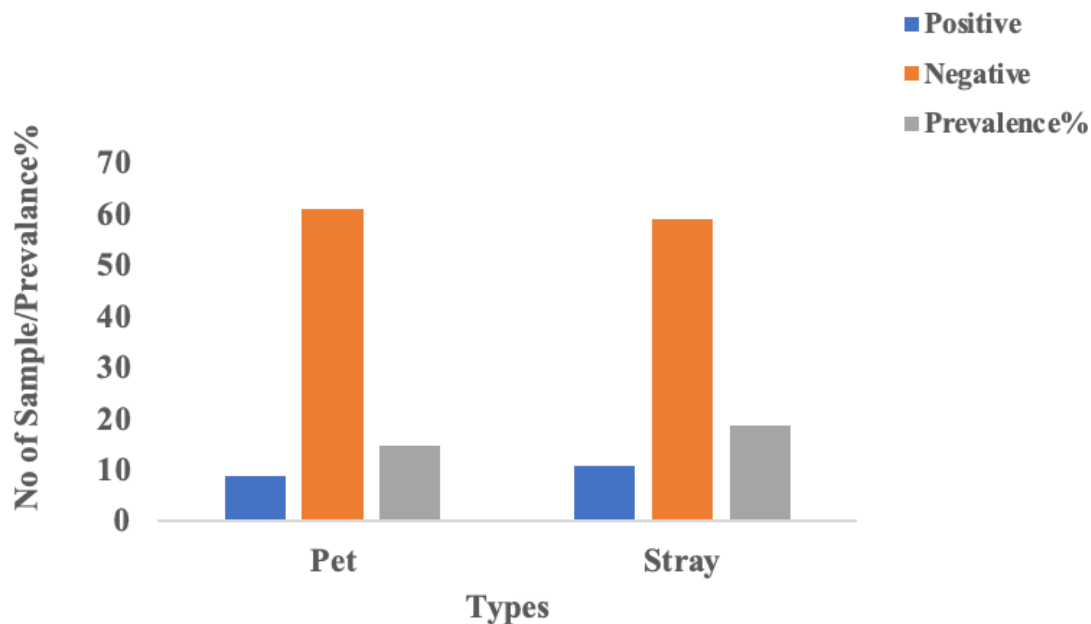


Figure 5: Prevalence of babesiosis on the basis of types of dog

Prevalence on the basis of breed

Out of 140 blood samples, 60 samples were of pure breed, 83 were mongrel and 16 were of cross breed. The breed that showed true characters of certain breed were classified as pure breed, the breed that showed mixed character of two pure breeds were classified as mongrel and the breed which were not characterized as specific breed or mixed one were classified as local breed. Among 60 samples from pure breed, 9(17.65%) were found positive, among 83 samples from mongrel, 10(13.7%) were found positive and among 16 samples from cross breed 1(6.67%) were found positive. The present study indicated the higher prevalence percentage in pure breed followed by mongrel and cross breed dogs. However, the result was found to be statistically insignificant ($p=0.631$). meaning that the nature of breed of dog do not have important role in the occurrence of babesiosis.

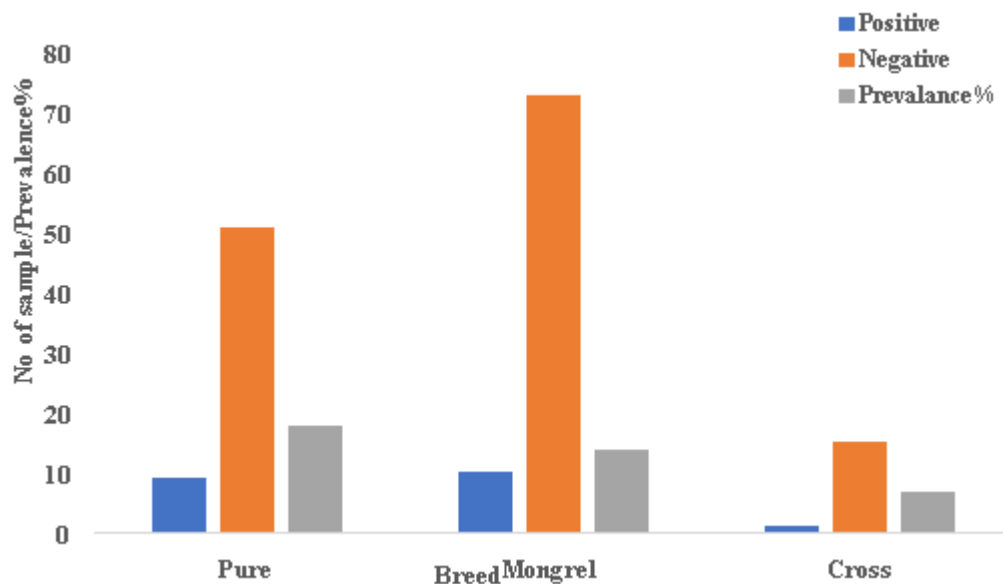


Figure 6: Prevalence of babesiosis on the basis of breed of dog

Comparison of hematological parameters

The hematological parameters of *Babesia* infected and non-infected dogs were analyzed. The result found that the mean value for RBC was $5.06 \pm 1.10 \times 10^{12}/l$, Hb was 11.29 ± 2.21 gm/dl, PCV was $32.29 \pm 2.61\%$, TLC was $13.75 \pm 1.64 \times 10^{12}/l$, neutrophil was $59.52 \pm 9.08\%$, lymphocyte was $31.97 \pm 8.73\%$, monocyte was $4.34 \pm 1.10\%$, eosinophil was $3.66 \pm 0.38\%$, basophil was $0.55 \pm 0.05\%$ and platelet was 235.35 ± 42.23 in non-infected dogs. Similarly, the result found that the mean value for RBC was $5.50 \pm 1.14 \times 10^{12}/l$, Hb was 9.57 ± 2.31 gm/dl, PCV was $27.63 \pm 1.72\%$, TLC was $11.99 \pm 1.38 \times 10^9/l$, neutrophil was $44.7 \pm 7.17\%$, lymphocyte was $45.55 \pm 7.65\%$, monocyte was $5.1 \pm 1.55\%$, eosinophil was $4.15 \pm 0.13\%$, basophil was $0.5 \pm 0.06\%$ and platelet was 150.4 ± 18.68 in infected dogs. The result showed that mean value of RBC, PCV, Hb, and platelet decrease significantly ($p < 0.05$) with the presence of disease in the body. Similarly, R square value of 5.8%, 6.9%, 6.0%, and 20.2% specified the percentage responsible for decrease in RBC, PCV, Hb, and platelet, respectively. In the case of TLC, the mean value decreased insignificantly ($p > 0.05$), whereas in DLC, the mean value of neutrophil decreased significantly ($p < 0.05$) with R square 7.1% and lymphocyte increased significantly ($p < 0.05$) with R square 6.2%. The fluctuation in monocytes, basophils, and eosinophils was insignificant ($p > 0.05$).

Table 2: Comparison of complete blood count in *Babesia spp.* infected cases and in non-infected cases (Reference Source: MSD Veterinary Manual, 11th edition)

Parameters and reference values	Non-infected cases	Infected cases	P-value	R square
RBC (5.6-8.7*10 ¹² /l)	5.06±1.10	5.50±1.14	0.04	5.8
Hb (11.9-18.9gm/dl)	11.29±2.21	9.57±2.31	0.02	6.9
PCV (35-57%)	32.29±2.61	27.63±1.72	0.04	6.0
TLC (5-14.1*10 ⁹ /l)	13.75±1.64	11.99±1.38	0.198	1.2
Neutrophil % (58-85%)	59.52±9.08	44.7±7.17	0.01	7.1
Lymphocytes% (8-24%)	31.97±8.73	45.55±7.65	0.03	6.2
Monocytes% (2-10%)	4.34±1.10	5.1±1.55	0.126	1.7
Eosinophil% (0-9%)	3.66±0.38	4.15±0.13	0.393	0.5
Basophil% (0-1%)	0.55±0.05	0.5±0.06	0.696	0.5
Platelets (211-621*10 ⁹ /l)	235.35±42.23	150.4±18.68	0.000	20.2

The mean temperature was 101.33±1.28 °F in non-infected dogs and 103.44±0.69°F in infected dogs. The present study indicated a greater increase in temperature in infected dogs than in non-infected ones, and the result was found to be statistically significant ($p=0.01$) which clearly explains that there is a rise in the body temperature with the presence of the disease. The effect of babesiosis in the increment of body temperature of body temperature was 4.7% given by R square.

DISCUSSION

Out of 140 samples, the overall prevalence of babesiosis was found to be 14.3%. This prevalence is higher as compared with the findings of Maharjan *et al.* (2014) who found a prevalence of babesiosis of 9.09% in feral dogs in Lalitpur district. This may be due to seasonal variation differences in the selection of dogs. But the result resembles the findings of Díaz-Regañón *et al.* (2020) who found the prevalence of babesiosis at 15.71% in stray dogs in Nepal.

Although statistically insignificant, the prevalence of disease was found higher in the age group of 6-10 years, followed by 1-5 years. The findings are similar to those of the study reported by Mahalingaiah *et al.* (2017), who found higher prevalence in the age group of 1-8 years and less in dogs of age groups less than 1 year and more than 8 years. According to the study done by Schoeman (2005), babesiosis occurs mostly in young dogs, although dogs of all ages can be affected. The differences in the result might be due to the unequal selection of dogs of various age groups.

This study revealed that there is no significant difference in the sex-wise prevalence of babesiosis, but female dogs have a higher prevalence than male ones. The findings resemble those of studies conducted by Shitta *et al.* (2012) and Phuyal *et al.* (2017), who found higher prevalence of *Babesia spp.* in females than in males. Likewise, prevalence of babesiosis in tick infected dogs was higher than in non-infected dogs and the result was also found to be statistically significant which is slightly higher than the findings of (Obeta *et al.*, 2020) who found 17.3% prevalence in tick-infested dogs and 3.5% prevalence in non-tick infested dogs. The higher prevalence could be attributed to the location differences in which the study was carried out.

There was no significant relationship between disease prevalence and dog type; however, street dogs had a higher risk of infection than pet dogs due to exposure to an unsanitary environment. The findings agree with the study conducted by Díaz-Regañón *et al.* (2020) and Bhattacharjee & Sarmah (2013), who states that stray animals are prone to vector borne diseases as they lack basic animal health care.

In case of breed, prevalence was higher in pure breed followed by mongrel and cross breed, however the result was statistically insignificant and the findings resembles with the results obtained by Okubango *et al.* (2013). But the study contradicted to the findings made by Opara *et al.* (2017) who found higher prevalence on mongrel dogs. This discrepancy could be attributed to the fact that the study was focused specifically in local dogs unlike this study which focused on all types of dogs.

The above results showed that the mean temperature in positive cases was $103.44 \pm 0.69^{\circ}\text{F}$ which was higher than in normal cases and statistically significant. The result agreed with the study conducted by Richardson *et al.* (1964), who state that the rise in body temperature is due to a biological response to the blood parasitic diseases. The hematological parameters of the study revealed statistically significant lower RBC, Hb, and PCV in infected cases than in non-infected cases. This finding agrees with the findings of Zamokas *et al.* (2014), who also observed a lower mean RBC, Hb, and PCV in the infected dogs than in non-infected dogs. The result could be attributed to the higher production of superoxide in the infected cells and, thus clarifying the RBC damage due to oxidative damage (Otsuka *et al.*, 2001).

It has been reported statistically no significant difference in the TLC between infected and non-infected dogs. This study resembled the findings of Phuyal et al. (2017), which also observed minor differences in TLC. In differential leucocyte count (DLC), there was a statistically significant difference in neutrophil counts and lymphocyte counts between infected and non-infected cases, whereas monocyte, eosinophil, and basophil counts did not vary significantly. Neutropenia was observed in infected cases, which satisfies the findings of Weiser et al. (1991), which stated neutropenia is usually present in a hemoprotozoan infection. Similarly, lymphocytosis was observed in positive cases which resembled with the findings of Hornabrook & Field (1969) which observed lymphocyte increase in atypical shape.

The hematological parameter of the study revealed statistically significant lower platelets in infected cases than in non-infected ones. This finding resembles studies conducted by Phuyal et al. (2017), Schetters et al. (2009), Kumar & Kumar (2020), and Irwin (2010), which describe thrombocytopenia as a hallmark of disease prevalence.

CONCLUSION

Overall prevalence of canine babesiosis was 14.3% examined by blood smear examination. Although rapid test kits are developed for the diagnosis, they are expensive to carry out in case of research based study. So, Geimsa stained blood smear analysis by direct microscopy is one of the useful tool, that is relatively cheap and effective for recognizing infectious diseases like *babesiosis* (Nayal et.al., 2012). A higher prevalence of the disease could be attributed to the increased exposure to vector ticks. Hence, proper control measures for the control of vectors should be followed in dogs to decrease the prevalence of hemoparasites in Nepal. For this, the active involvement and cooperation of concerned authorities from the veterinary and ailing professions is required.

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REFERENCES

1. Adaszek L., Obara-Galek J., Piech T., Winiarczyk M., Kalinowski M., Winiarczyk S. (2016): Possible vertical transmission of *Babesia canis canis* from a bitch to her puppies: a case report. *Veterinarni Medicina*, 61: 263-266. <https://doi.org/10.17221/8881-VETMED>
2. Andersson, M.O., Tolf, C., Tamba, P. et al. Canine tick-borne diseases in pet dogs from

- Romania. *Parasites Vectors* **10**, 155 (2017). <https://doi.org/10.1186/s13071-017-2092-x>
3. Bhaskaran Ravi, L., Bhoopathy, D., & Subbiah, V. (2016). Escalations in the incidence of canine babesiosis over a period of 8 years (2006-2013) in Chennai, Tamil Nadu, India. *Journal of parasitic diseases: official organ of the Indian Society for Parasitology*, *40*(4), 1239–1242. <https://doi.org/10.1007/s12639-015-0659-z>
4. Bhattacharjee K, Sarmah PC, (2013). Prevalence of haemoparasites in pet, working and stray dogs of Assam and North-East India: A hospital based study. *Vet. World*. *6*(11): 874-878. <https://doi.org/10.14202/vetworld.2013.874-878>
5. Petra, B., Josipa, K., Renata, B. & Vladimir, M. (2018). Canine Babesiosis: Where Do We Stand?. *Acta Veterinaria*, *68*(2) 127-160. <https://doi.org/10.2478/acve-2018-0011>
6. Cruthers, L. R. (2019). *Protozoa, Sarcostomastigophora, Hemoflagellates* (Vol. 1). <https://doi.org/10.1016/B978-0-12-813890-8.00005-5>
7. Díaz-Regañón, D., Agulla, B., Piya, B., Fernández-Ruiz, N., Villaescusa, A., García-Sancho, M., Rodríguez-Franco, F., & Sainz, Á. (2020). Stray dogs in Nepal have high prevalence of vector-borne pathogens: a molecular survey. *Parasites & vectors*, *13*(1), 174. <https://doi.org/10.1186/s13071-020-04057-7>
8. Hornabrook, R. W., & Field, E. J. (1969). Clinical and pathological findings in kuru. *Lancet (London, England)*, *1*(7594), 576. [https://doi.org/10.1016/s0140-6736\(69\)91992-8](https://doi.org/10.1016/s0140-6736(69)91992-8)
9. Irwin P. J. (2010). Canine babesiosis. *The Veterinary clinics of North America. Small animal practice*, *40*(6), 1141–1156. <https://doi.org/10.1016/j.cvsm.2010.08.001>
10. Kumar, P., & Kumar, A. (2020). Therapeutic management and Haemato-biochemical changes of Babesiosis in dogs.
11. Mahalingaiah, M. K. C., Asoor, M., Thimmaiah, R. P., Narayanaswamy, H. D., Mukartal, S. Y., Elattuvalappil, A. M., ... & Singh, S. (2017). Prevalence of canine babesiosis in different breeds of dogs in and around Bengaluru. *Adv. Anim. Vet. Sci*, *5*(3), 140-144.
12. Njunda, A. L., Assob, N. J. C., Nsagha, S. D., Kamga, F. H. L., Mokenyu, M. D., & Kwenti, E. T. (2013). Comparison of capillary and venous blood using blood film microscopy in the detection of malaria parasites: a hospital based study. *Sci J Microbiol*, *2*(5), 89-94.
13. Okubanjo, O., Adeshina, O., Jatau, I., & Natala, A. (2013). Prevalence of *Babesia canis* and *Hepatozoon canis* in Zaria, Nigeria. *Sokoto Journal of Veterinary Sciences*, *11*(2), 15–20. <https://doi.org/10.4314/sokjvs.v11i2.3>
14. Rihardson, U.F. and Kendall, S.B. (1964). Veterinary protozoology. 3rd edn. The English Language Book Society & Oliver & Boyd Ltd, Edinburgh (pp.45 & 151).
15. Sanchez, E., Vannier, E., Wormser, G. P., & Hu, L. T. (2016). Diagnosis, treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: A review. *JAMA - Journal of the American Medical Association*, *315*(16), 1767–1777. <https://doi.org/10.1001/jama.2016.2884>
16. Schetters, T. P., Kleuskens, J. A. G. M., Van De Crommert, J., De Leeuw, P. W. J., Finizio, A. L., & Gorenflot, A. (2009). Systemic inflammatory responses in dogs experimentally infected with *Babesia canis*; a haematological study. *Veterinary parasitology*, *162*(1-2), 7-15. <https://doi.org/10.1016/j.vetpar.2009.02.012>
17. Shitta, K. B., James-Rugu, N. N., & Azonci, A. H. (2012). *Babesia canis* infection in dogs in Jos North and Jos South LGAs of Plateau State, Nigeria. *International Journal Science Technology Development Study*, *7*, 42-47.
18. Leonhard, S., Rodriguez Anabel, E., Monica, F. C., & Morrison David, A. (2012). Babesia: A world emerging. *Infection, Genetics and Evolution*, *12*(8), 1788-1809. <https://doi.org/10.1016/j.meegid.2012.07.004>
19. Shaw, S. E., Day, M. J., Birtles, R. J., & Breitschwerdt, E. B. (2001). Tick-borne infectious diseases of dogs. *Trends in parasitology*, *17*(2), 74-80. [https://doi.org/10.1016/S1471-4922\(00\)01856-0](https://doi.org/10.1016/S1471-4922(00)01856-0)
20. Phuyal, S., Jha, V. C., & Subedi, M. (2017). Prevalence of blood parasites in dogs of Kathmandu Valley. *Nepalese Veterinary Journal*, *34*, 107-112.

21. Welc-Falęciak, R., Rodo, A., Siński, E., & Bajer, A. (2009). Babesia canis and other tick-borne infections in dogs in Central Poland. *Veterinary parasitology*, 166(3-4), 191-198. <https://doi.org/10.1016/j.vetpar.2009.09.038>
22. Zamokas, G., Grigonis, A., Karvelienė, B., Daunoras, G., Babickaitė, L., & Šapalienė, I. (2014). Importance of haematological changes in diagnosing canine babesiosis. *Veterinarija ir zootechnika*, 67(89).
23. Mehlhorn, H., & Shein, E. (1984). The piroplasms: life cycle and sexual stages. *Advances in parasitology*, 23, 37–103. [https://doi.org/10.1016/s0065-308x\(08\)60285-7](https://doi.org/10.1016/s0065-308x(08)60285-7)
24. Obeta, S. S., Ibrahim, B., Lawal, I. A., Natala, J. A., Ogo, N. I., & Balogun, E. O. (2020). Prevalence of canine babesiosis and their risk factors among asymptomatic dogs in the federal capital territory, Abuja, Nigeria. *Parasite Epidemiology and Control*, 11, e00186.
25. Nayel, M., El-Dakhly, K. M., Aboulaila, M., Elsify, A., Hassan, H., Ibrahim, E., ... & Yanai, T. (2012). The use of different diagnostic tools for Babesia and Theileria parasites in cattle in Menofia, Egypt. *Parasitology research*, 111, 1019-1024. <https://doi.org/10.1007/s00436-012-2926-6>

Prevalence of Fish Tapeworm – *Diphyllbothrium latum* in Commercial Fish Farms of Chitwan District, Nepal

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ABSTRACT

Diphyllbothrium latum, commonly known as “fish- tapeworm”, has a zoonotic importance as it causes Diphyllbothriasis in humans. Fish, one of the highly consumed meat sources by human, also acts as second intermediate host for *D. latum*. There are several reports indicating consumption of *D. latum* infested poorly cooked fish causing Diphyllbothriasis in human. Thus, *D. latum* in fish can be considered as a major risk factor for Diphyllbothriasis in humans. Despite being a public health concern, no scientific data regarding the prevalence of *D. latum* in fish population of commercial fish farms of Nepal exists. The objective of this study was to bridge this knowledge gap by estimating the prevalence of *D. latum* in commercial fish farms of Chitwan district of Nepal. The study was conducted in six municipalities of Chitwan, Nepal. Out of 46 registered fish farms of Chitwan district, 42 farms were sampled in this study. The fish sampled from each farm were brought to the Histology laboratory of Department of Aquatic Resource Management of Agriculture and Forestry University, Nepal maintaining cold chain system and were dissected for feces collection. For qualitative parasitological examination of feces, sedimentation and centrifugal flotation method were used. Out of 42 farms sampled, 16 (38.10%) fish farms were found positive for presence of any kind of gastrointestinal parasites. Out 42 commercial fish farms examined, 9 (21.4%) farms were found positive for *D. latum*. Besides, *D. latum* fish farms were also found positive for *Contracaecum* sp. (19%), *Coccidia* (11.9%) and *Eustrongyloides* (2.4%). This research indicated that the prevalence of *D. latum* was more than 20% in commercial fish farms of Chitwan district showing possibility of zoonotic transmission to human population. Management practices of fish farmers must include ways to prevent *D. latum* infestation in fish population. Moreover, general public should be made aware about prevalence of *D. latum* in fish and its possible risk to human health along with its preventive measures for safe fish consumption.

Keywords: *Diphyllbothrium latum*, public health, zoonotic

INTRODUCTION

Total pond fish production in Nepal in 2020/2021 AD was 73,693 metric ton of which 3,580 metric tons was produced alone in Bagmati province (MOALD, 2022). Among 13 districts of Bagmati province, Chitwan district holds the highest percentage of fish production of around 84.77% (MOALD, 2022). The demand for fish is increasing in developing countries like Nepal due to its rich nutritional value (Ljubojevic et al., 2015). *Diphyllbothrium latum* (Phylum: Cestode, Family: Platyhelminthes), a parasite of fish, are zoonotic in nature and is a public health concern (Sampaio et al., 2005). The disease caused by fish tape worm *Diphyllbothrium latum* is known as Diphyllbothriasis. Although *Diphyllbothrium latum* was previously considered as a non-tropical disease, in recent years, its cases are also reported in tropical regions (Ramana et al., 2009). Cases of Diphyllbothriasis has been reported in India and Bangladesh in both fish, animal and human population (Ramana et al., 2009; Samad 2013). Definitive first and second intermediary hosts of *Diphyllbothrium* spp. include humans, mammals and birds that eat fish. In Nepal, parasitic zoonosis like Diphyllbothriasis has been considered as probable endemic (Devleeschauwer et al., 2014). Prevalence of *Diphyllbothrium latum* in Nepal was reported in pet and stray dogs of Rupandehi district (Yadav and Shrestha, 2017). Similarly, fecal sample from people of Bote and Darai communities of Tanahun district of Nepal which is adjoining district of Chitwan was also found to be positive for *D. latum* eggs (Thapa, 2000). *D. latum* has also been found in intestines of Common carp fingerlings in Kathmandu valley of Nepal (Rai, 2002) indicating possible prevalence of *D. latum* in commercial fish population of Nepal. Although being important topic of public health concern, no data is available regarding prevalence of *Diphyllbothrium latum* in commercial fish farms of Nepal and its zoonotic risk and not much studied have been done about it so far. The objective of this study is to estimate the prevalence of fish tapeworm, *D. latum*, in commercial fish farms of Chitwan district, Nepal.

MATERIALS AND METHODS

Study location

All six municipalities of Chitwan district i.e., Bharatpur Metropolitan, Ratnanagar, Madi, Kalika, Khairahani and Rapti Municipality were included in this study (Figure 1). Chitwan lies in the central part of the Bagmati province of Nepal. It lies at latitude 27.5291° N and longitude 84.3542° E. All study locations were situated in plain lands of Chitwan.

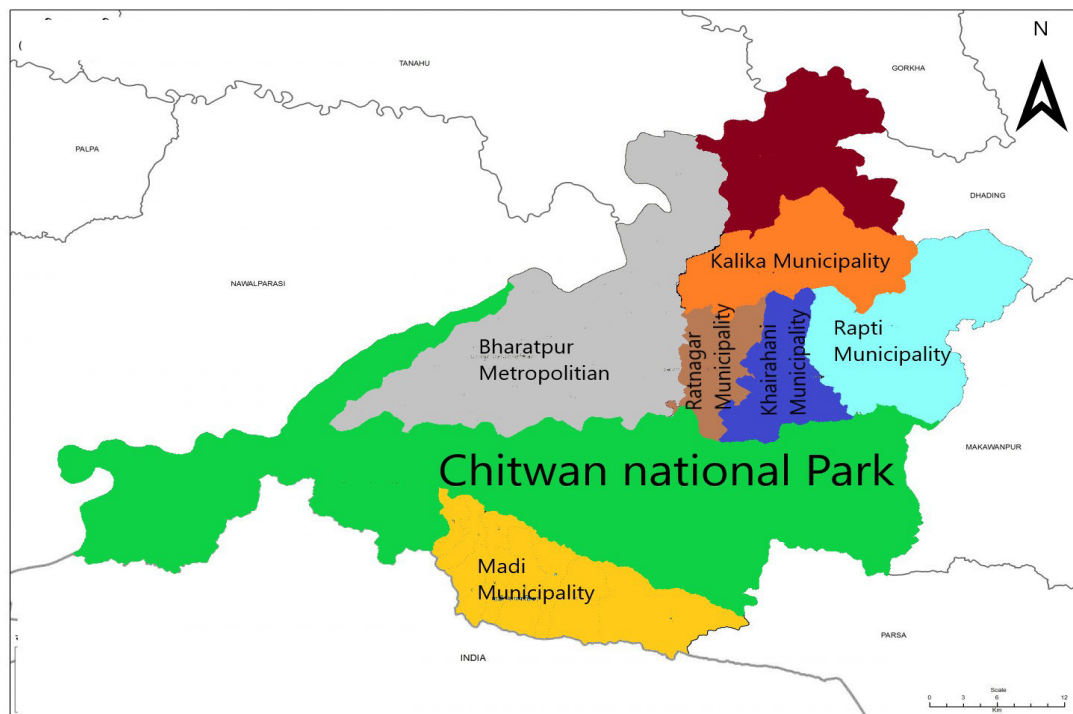


Figure.1. Map showing Chitwan district and its municipalities.

Sample

size

to the data at the Veterinary Hospital and Livestock Expert Center (VHLEC), Chitwan, a responsible governmental organization for keeping records of registered farms, showed that the total number of registered commercial fish farm tin Chitwan in 2021 A.D. was 46. Sample size was calculated based on formula: $n = N * X / (X + N - 1)$, $X = Z^2 P(1 - P) / d^2$ (Daniel, 1999)

Where,

Z=value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)

P= 50%, because no research of this kind has been done in Nepal.

d= 5% of precision

N=46, Total number of fish farms based on data provided by VHLEC, Chitwan.

Using above mentioned parameters, total number of fish farms to be sampled is 42 at 95% confidence level and 5% precision.

Sample collection

Sampling was done during mid-August to mid-September in 2021. Sample collection was done using simple random sampling method. Owners from selected commercial fish farms were shortly interviewed relating to fish disease and management before sample (fish) collection.

Out of total 42 commercial fish farm sampled, 13 was from Bharatpur Metropolitan city, 11 was from Madi Municipality, 10 from Ratnanagar Municipality, 1 from Kalika Municipality, 8 from Khairahani Municipality and 3 from Rapti Municipality as shown in Table1.

Table 1. Table showing location and number of commercial fish farm in the study

Location of fish farms	Number of fish farms
Bharatpur Metropolitan City	13
Madi Municipality	11
Ratnanagar Municipality	10
Kalika Municipality	1
Khairahani Municipality	8
Rapti Municipality	3
Total	42

Fish from each farm were selected according to that the number of ponds, one fish sample from 1-4 pond, 2 fish samples from 5-8 pond. Fish was brought to the Histology laboratory of Department of Aquatic Resource Management of Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Chitwan, Nepal. Fish was then dissected aseptically for collecting fecal samples and each sample was labelled with unique identification number. Fecal sample was subjected to parasitological examination in the same day.

Fecal examination

Prevalence of parasite will be confirmed through two parasitological methods i.e., sedimentation and flotation. Both methods will be used for each fecal sample. For sedimentation method 3 gram of fecal sample will be mixed with 42 ml of water, macerated and passed through a strainer and sedimented for 15minutes. The supernatant was discarded and 2 drop of sediment will be poured to a glass slide for microscopy. Similarly, for flotation technique, the sediment will be mixed with 10ml saturated salt solution and centrifuged. The uppermost 2 drop of supernatant will be taken for microscopy. Parasite would be identified based on its morphology (Soulsby, 1982)

Data analysis

The data collected from questionnaire discussion and microscopic examination was analyzed using IBM SPSS version 26.0 and MS- Excel 2016.

RESULTS

Eight different species of fish were sampled from 42 commercial fish farms. Common carp (31%) was highest sampled fish followed by Pangas (19%), Naini (16.7%), Grass carp (11.9%), Bighead carp (7.1%), Rohu (7.1%), Catfish (4.8%) and Silver carp (2.4%).

Table.2. Different fish species sampled for fecal examination and their frequency

Fish Species	Frequency	Percentage (%)
Bighead carp (<i>Hypophthalmichthys nobilis</i>)	3	7.1
Catfish (<i>Clarias batrachus</i>)	2	4.8
Common carp (<i>Cyprinus carpio</i>)	13	31
Grass carp (<i>Ctenopharyngodon idella</i>)	5	11.9
Naini (<i>Cirrhinus mrigala</i>)	7	16.7
Pangas (<i>Pangasius sp.</i>)	8	19
Rohu (<i>Labeo rohita</i>)	3	7.1
Silver carp (<i>Hypophthalmichthys molitrix</i>)	1	2.4
Total	42	100

Out of 42 farms visited, 16 (38.1%), fish farms were found positive for presence of any kind of gastrointestinal parasites whereas 26 (61.9%) sampled fish farms were free from any kind gastrointestinal parasite prevalence.

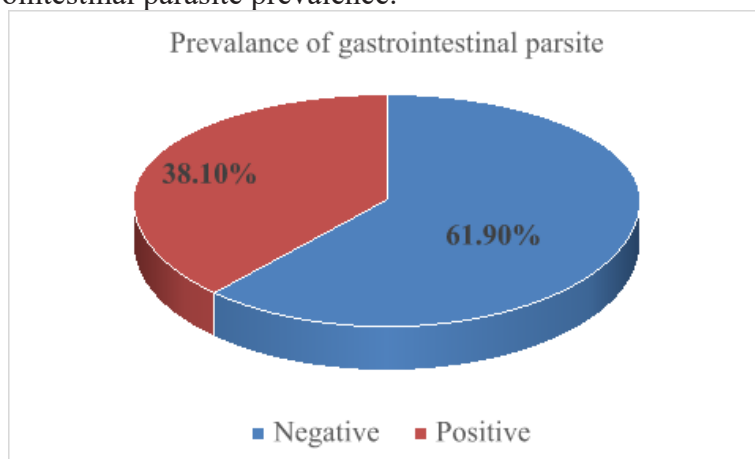


Figure 2. Pie chart showing prevalence of any kind of gastrointestinal parasite.

Through fecal examination of sampled fish, out of 42 fish farms, 9(21.4%) was found to be positive for *Diphyllbothrium latum*, 8 (19%) for *Contracaecum spp.*, 5 (11.9%) for *Coccidia sp.* and 1 (2.4%) was found to be positive for *Eustrongyloides sp.*

Table.3. Gastrointestinal parasite and their prevalence percentage

Parasite species	Frequency	Individual parasitic prevalence (%)
<i>Diphylobothrium latum</i>	9	21.4%
<i>Contracaecum</i> sp.	8	19%
<i>Coccidia</i> sp.	5	11.9%
<i>Eustrongyloides</i> sp.	1	2.4%

Table below shows fish species sampled, its sampling frequency and its parasite species wise frequency.

Table.4. Fish species with parasite species wise frequency

Fish Species	Sampling Frequency	<i>Diphylobothrium latum</i>	<i>Eustrongyloides</i> sp.	<i>Contracaecum</i> sp.	<i>Coccidia</i> sp.
Bighead carp (<i>Hypophthalmichthys nobilis</i>)	3	1	0	0	0
Catfish (<i>Clarias batrachus</i>)	2		0	0	0
Common carp (<i>Cyprinus carpio</i>)	13	5	1	4	2
Grass carp (<i>Ctenopharyngodon idella</i>)	5	0	0	1	0
Naini (<i>Cirrhinus mrigala</i>)	7	0	0	0	2
Pangas (<i>Pangasius</i> sp.)	8	0	0	2	1
Rohu (<i>Labeo rohita</i>)	3	3	0	1	0
Silver carp (<i>Hypophthalmichthys molitrix</i>)	1	0	0	0	0
Total	42	9	1	8	5

DISCUSSION

Overall gastrointestinal parasite in fish population prevalence was greater than that reported by Bibi et al. (2018) who reported 26.3% overall gastrointestinal parasite prevalence from Chenab River Pakistan. Similarly, prevalence of *D. latum* was higher than that reported by Bibi et al. (2018) who reported 8% *D. latum* prevalence and Udechukwu et al. (2017) who reported 16.7% *D. latum* prevalence in *Clarias gariepinus* (African catfish). *D. latum* prevalence was lower than that reported by Uddin et al. (1980) who reported 82% prevalence of *D. latum* from *Harpodonnetheus* sampled from the Feni and Muhuri rivers at the confluent of the Bay of Bengal. *Contracaecum* sp. prevalence was lower than that reported by Naha et al. (2019) in Jew-

Fish (*Otolithoides Pama*). Similarly, prevalence of contracaecum was similar i.e, 17.95% for *Contracaecum osculatum* in *Bliccabjoerkna* and lower i.e., 0.99% for *Contracaecum* sp. larva in *Iranocichlahormuzensis*. (Pajooki et al., 2011)

CONCLUSION

This research indicated that gastrointestinal parasite *Diphyllbothrium latum* is widely prevalent in commercial fish farms of Chitwan district. *D. latum*, being a zoonotic parasite, has a public health importance., Therefore, further studies need to be done with larger sample size to understand the true burden of *D. latum* in fish farms of Nepal. Public should be made aware about presence of *D. latum* in fish population and should also be made aware about safe fish cooking and consumption methods against *D. latum*.

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REFERENCES

1. Daniel, W.W., (Ed). (1999). Biostatistics: a foundation for analysis in the health sciences (7th ed.). New York: John Wiley & Sons.
2. Devleesschauwer, B., Ale, A., Torgerson, P., Praet, N., Maertens De Noordhout, C., Pandey, B. D., Pun, S. B., Lake, R., Vercruyse, J., Joshi, D. D., Havelaar, A. H., Duchateau, L., Dorny, P., & Speybroeck, N. (2014). The Burden of Parasitic Zoonoses in Nepal: A Systematic Review. *PLoS Neglected Tropical Diseases*, 8(1), e2634. <https://doi.org/10.1371/journal.pntd.0002634>
3. Yadav, K.K., and Shrestha, B. (2017) Prevalence of Zoonotic Gastrointestinal Helminth Parasites in Pet and Stray Dogs of Rupandehi District, Nepal. *Microbiol Infect Dis*. 1(2), 1-7
4. Rai, P. (2002) Study on the fish parasites of exotic fished (rainbow trout and carp) and their control measures in different culture systems [MSc dissertation]. Kathmandu: Tribhuvan University. 44 p.
5. Thapa, R.B. (2000) Prevalence of intestinal helminth parasites in general and Taenia spp in detail,

- particularly in Bote and Darai communities of Vyash Municipality-5, Kumaltari, Tanahun district of Nepal [MSc dissertation]. Kathmandu: Tribhuvan University. 91 p.
6. MOALD. (2022) *Statistical Information on Nepalese Agriculture 2020/2021*. Singha Durbar, Kathmandu: Ministry of Agriculture & Livestock Development.
 7. Bibi, F., Qaisrani, S. N., Ayaz, M., Nazir, M., Ahmad, A. N., Muhammad Awais, M., Khan, B. N., & Akhtar, M. (2018). Occurrence of endoparasites in some selected fishes of Chenab River, Pakistan. *Bangladesh Journal of Zoology*, 46(1), 53–61. <https://doi.org/10.3329/bjz.v46i1.37626>
 8. Ljubojevic, D., Novakov, N., Djordjevic, V., Radosavljevic, V., Pelic, M., & Cirkovic, M. (2015). Potential parasitic hazards for humans in fish meat. *Procedia Food Science*, 5, 172–175. <https://doi.org/10.1016/j.profoo.2015.09.049>
 9. Naha, P. R., Rahman, M. R., Sarker, J., and Islam, S. M. R. (2019). Prevalence and Intensity of *Contracaecum* sp. in Jew-Fish (*Otolithoides Pama*). *Scholars Journal of Agriculture and Veterinary Sciences*, 6(7), 176–182. 10.21276/sjavs.2019.6.7.3
 10. Pazooki, J., Mansouri-Habibabadi, Z., Masoumian, M. & Aghae-Moghadam, A. (2011). Survey on the metazoan parasites in *Neogobius* fishes from southeastern part of the Caspian Sea. *Iranian Journal of Fisheries Sciences* 10, 95– 108.
 11. Ramana, K., Rao, S., and Kumar, V. (2011) Diphyllbothriasis in a nine-year-old child in India: A case report. *Journal of Medical Case Reports*. DOI: 10.1186/1752-1947-5-332.
 12. Samad, M. A. (2013). Public health threat caused by Zoonotic Diseases in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 9(2), 95–120. <https://doi.org/10.3329/bjvm.v9i2.13451>
 13. Sampaio, J. L. M., Piana de Andrade, V., Lucas, M. C., Fung, L., Gagliardi, S. M. B., Santos, S. R. P., Mendes, C. M. F., Eduardo, M. B. P., & Dick, T. (2005). Diphyllbothriasis, Brazil. *Emerging Infectious Diseases*, 11(10), 1598–1600. <https://doi.org/10.3201/eid1110.050377>
 14. Soulsby, E. J. L., (Ed). (1982). *Helminths, arthropods and protozoa of domesticated animals* (7th ed.). London: Baillière Tindall
 15. Uddin, M., Dewan, M. L., Hossain, M. I., and Huq, M. M. 1980. Occurrence of *Diphyllbothrium latum* larvae (plerocercoid) in loitya (*Harpodonnehereus*) fish. *Bangladesh Vet J.* 14, 33-35
 16. Udechukwu, C. U., Panda, S. M., Sunday, I. D., & Bello, F. A. (2018). Parasites associated with *Clarias gariepinus* (African catfish) from dam, plastic and concrete ponds in Bauchi metropolis, Bauchi State, Nigeria. *GSC Biological and Pharmaceutical Sciences*, 2(2), 001–005. <https://doi.org/10.30574/gscbps.2018.2.2.0003>

Study of Productive, Reproductive and Morphological Traits of Bucks in Different Eco-zones of Lumbini Province, Nepal

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ABSTRACT

Goat is a domesticated small ruminant, primarily reared by small farmers for meat production, across the country. The productivity of goat is often low, which could be related to several genetic and non-genetic factors. A field study was carried out to characterize, evaluate and estimate the effect of non-genetic factors on the productive and reproductive performance of hill goats in different eco-zones at Rolpa district of Lumbini province. Altogether 43 bucks of different age groups were identified for this study. Morphological attributes, productive, and reproductive performance were collected based on field monitoring and measurements within different altitudes of Rolpa district. Least square analysis was performed using Harvey (1990) computer software package, and means were compared using DMRT. The measurements of morphological traits were higher in male as compared to females. Results revealed the age at first service of bucks (336 days), the average body weight of buck was (38.76 kg), the overall body length of buck from one to four year was 78.01 ± 0.35 cm, overall heart girth of one to four years' age buck was 77.57 ± 1.65 cm, average wither height from one to four-year-old buck was 71.88 ± 2.13 cm, overall average Scrotal circumference of bucks (1-4 years) were 24.38 ± 0.71 cm respectively. The results of this study suggest that the performance of low-altitude goat flocks was better than mid and high-altitude goat flocks in the Rolpa district in terms of production and reproduction traits. The selection of the best performance of buck could be done on the basis of their traits for breeding. This result could be attributed to superior genotype along with better management practices followed by the farmers in Rolpa district.

Keywords: Buck, Weight, breed, genetic

INTRODUCTION

Nepal is an agricultural country where about 66 percent of its population is involved in agricultural occupation. Agriculture contributes to around 27.1 percent of the gross domestic product (GDP) of Nepal, of which, the livestock sector contributes about 11.5

percent of the total GDP and 25.7 percent of the agricultural GDP (AGDP) as reported by MOAD (2018). Goat farming is being the most popular means of self-employment among the youths in the country. Current statistics regarding to goat population indicated that there are more than 11.64 million of goats in the country (MOAD, 2018). Among the agricultural commodities, livestock plays an important role in agricultural development and economic upliftment of the country. Goat farming has been practiced by a large section of population in rural areas of Nepal. The recent population of goat is about 11.64 million and total meat contribution was 6.9 thousand metric ton per year (MOALD, 2018). The rate of increment in goat population during last 15 years (2008 to 2018) was reported 3.74 percent per year contributing about 20.1% to the total meat production in the country (MOALD, 2018). Goats breed in Nepal are quite different with locational difference. There are gradients of topography, environment and climatic conditions vary from South to North, and each breed evolved is acclimatized corresponding to each topographical zone (Pradhan and Gurung1985). In Nepal, there are four commonly documented breed of goats as Chyangra, Sinhal, Khari and Terai goats. Chyangra goats are found in 2400 meters in high Himalayans, while Sinhal found in high hill ranging 1500-3000 meters from sea level. Khari are available across the hills of Nepal while Terai goats are available in Terai region of Nepal. Chyangra (1%), Sinhal (16%), Khari/hill goat (56%) and remaining 27% are of Terai and other breeds (Pokharel and Neopane, 2008).

MATERIALS AND METHODS

This section deals with the site of study, data collection and recording procedures, data analysis techniques, description of data sets and models used for analyzing the recorded traits.

Location of the study

This study was carried out in Rolpa district of Lumbini province. Rolpa covers an area of 1,879 km² with population (2016) of 221,177. Rolpa is drained southward by the Madi River from a complex of 3,000 to 4,000 meter ridges about 50 kilometers south of the Dhaulagiri Himalaya (Statoids, 2014). The Rolpa district lies at the height of 701m to 3639 meters above the sea level. The total area is 189385 hectares out of which 59854.5 hectares land is used for crop farming, forest consists 84474 hectares, pasture consists 32698.8 hectare, wild plants and forages 9620.8 hectare and rivers and rocks 1251.9 hectares. The average temperature in Rolpa district is maximum (31.2 Celsius), minimum (3.6 Celsius) and annual rainfall is 441mm (CBS, 2016)

Sampling procedure and sample size

The datas were collected on the basis of Pocket areas of goats distributed within different altitude at Rolpa district. The selected high altitude VDCS were (Jaimakshala and

Pakhapani) followed by mid altitude VDCS (Gairigaun and Libang) and low altitude VDCS (Masina and Jhenam). The elevations of high, mid and low altitude from the sea level in Rolpa district are at the range of 3639, 1375 and 701 m respectively. The elevation of selected areas for this research low, mid and high altitudes were 800-1000, 1200-1400 and 1500-1800 m respectively from the sea level. The two wards were selected from each VDCS. From each ward buck reproductive and productive parameters were recorded. The productive parameters include bucks were body weight, wither height, body length, heart girth, Scrotal circumference length etc. The reproductive parameters of buck age at first mating (puberty) etc. Within the population, in each selected site, sampling goats were identified randomly. A data recording format was developed to collect data and information related to growth performance, reproductive performance, litter traits, morphological traits and production system of hill goats reared in the study area.

Statistical analysis

Collected data were entered in the computer using MS- Excel and converted into text documents i.e. Text (MS-DOS). To study the main causes of variation and effects of non-genetic factors on productive and reproductive traits, as well as to overcome the difficulty of disproportionate subclass numbers, data were analyzed by least squares procedure using Harvey, (1990) which is based on least squares technique of variance analysis. The pair wise comparison of the least square mean comparison was made using DMRT (Duncan's Multiple Range Test) (Duncan, 1955) as modified by Kramer (1957).

Models used to analyze the collected data

A fixed effect model given by Handerson (1953) was used to analyze the body weights of male goat at different stages of growth.

Model I (fixed effect model) for buck weight, body measurements and reproductive traits

$$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + e_{ijklmn}$$

Where, μ is the overall mean

a_i is the effect of i^{th} altitude ($i=1,2$ and 3)

b_j is the effect of j^{th} type of breed ($j = 1, 2$ and 3)

c_k is the effect of k^{th} type of colour ($k= 1,2,3$ and 4)

d_l is the effect of age ($k= 1,2,3$ and 4)

e_{ijkl} is the random element assumed (error mean) to be normally and independently distributed among the sampled population.

RESULTS AND DISCUSSION

This chapter describes the study results focusing to the growth performance, morphological traits, reproductive traits as well as production systems of goats.

Body weight of bucks

The overall average body weight of buck was 38.76 ± 0.35 kg as mentioned in detail Table (1). The data were collected and analyzed from one to four-year-old bucks. The bucks were also in crossed form like as does. Joshi *et al.* (2003) also reported Khari, Khapari and Khabari breed bucks were $28-40$, 52 ± 4.80 , and 43.5 ± 4.9 kg respectively. Various factors such as altitude color and age were considered in this study for the body weight of the bucks.

Table 1. Least square means for body weight (kg) of buck

Factors	LS $\pm$ SE	NO	Significant Level
Overall	38.76 ± 0.35	43	
Altitude			NS
LA	39.74 ± 0.48	17	
MA	37.39 ± 0.39	14	
HA	39.15 ± 0.41	12	
Breed			**
Khari	35.68 ± 0.55^b	29	
Khari dominant	37.29 ± 1.04^b	8	
J a m u n a p a r i dominant	45.32 ± 0.63^a	6	
Colour			NS
Black	40.78 ± 0.66	27	
Brown	36.57 ± 0.73	8	
Mixed	38.94 ± 0.81	8	
Age			***
1 Year	34.86 ± 0.48^c	13	
2 Year	36.08 ± 0.30^c	12	
3 Year	41.64 ± 0.53^b	15	
4 Year	46.46 ± 0.96^a	3	

Note: **significant at 1% ($P < 0.01$) ***significant at 0.1% ($P < 0.001$), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Body Length of buck

The overall body length of buck from one to four year was 78.01 ± 0.35 cm. The various non- genetic factors affected the body length of buck in Rolpa district. The various factors affecting the body length of bucks are presented in the Table (2). The acute shortage of genetically superior buck throughout the country is one of the major constraints of goat production in Nepal. Existing haphazard breeding system may lead to the extinction of the genetic potential of goats which requires selection of superior buck. During selection

of buck attention should be given on the age, growth rate, body weight and soundness of the sexual organs and morphometric traits. In such case, body length and heart girth may be used as good reliable predictors to assess live weight (Islam *et al.*, 1991).

Table 2. Least squares means for body length (cm) measurements of the bucks

Factors	LS±SE	NO	Significant Level
Overall	78.01 ±0.35	43	
Altitude			NS
LA	78.77±0.35	17	
MA	73.46±0.33	14	
HA	74.58±0.35	12	
Breed			NS
Khari	70.28±0.40	29	
Khari dominant	79.43±0.38	8	
J a m u n a p a r i dominant	71.28±0.45	6	
Colour			NS
Black	74.8±0.48	27	
Brown	79.07±0.53	8	
Mixed	75.05±0.58	8	
Age			***
1 Year	63.60 ±0.33 ^c	13	
2 Year	70.89 ±0.38 ^b	12	
3 Year	79.54 ±0.32 ^a	15	
4 Year	81.39 ±1.80 ^a	3	

Note: ***significant at 0.1% ($P < 0.001$), NS-non significant, LSD-Least significant difference, LS mean- Least square mean, SE- Standard error of mean. NO are the numbers of observations.

Heart girth of buck

The overall heart girth of one to four years' age buck was 77.57±1.65 cm. The various factors affecting the heart girth of buck are presented in Table (3). The acute shortage of genetically superior buck throughout the country is one of the major constraint of goat production in Nepal. Existing haphazard breeding system may lead to the extinction of the genetic potential of goats which requires selection of superior buck. During selection of buck attention should be given on the age, growth rate, body weight and soundness of the sexual organs and morphometric traits. In such case, body length and heart girth may be used as good reliable predictors to assess live weight (Islam *et al.*, 1991). The present investigation was designed to obtain some basic morphometric information and to relate body weight with different body measurements of goats.

Table 3. Least square means for heart girth (cm) of buck

Factors	LS±SE	NO	Significant Level
Overall	77.57 ±1.65	43	
Altitude			NS
LA	78.30±2.28	17	
MA	77.19±2.20	14	
HA	77.24±2.29	12	
Breed			*
Khari	77.86 ±0.25 ^b	29	
Khari dominant	80.46 ±0.48 ^a	8	
J a m u n a p a r i dominant	81.35 ±0.27 ^a	6	
Colour			NS
Black	77.97±0.30	27	
Brown	76.07±0.33	8	
Mixed	78.66±0.35	8	
Age			**
1 Year	68.65 ±2.23 ^c	13	
2 Year	76.12 ±2.48 ^b	12	
3 Year	82.55 ±2.51 ^a	15	
4 Year	82.95 ±1.67 ^a	3	

Note: * significant at 5% ($P<0.05$), **significant at 1% ($P<0.01$), NS-non significant, LSD-Least significant difference, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Buck wither height

The average wither height from one to four-year-old buck was 71.88 ± 2.13 cm in this study. The various factors affecting the wither height from one to four-year-old bucks are presented in the Table (4). During selection of buck attention should be given on the age, growth rate, body weight and soundness of the sexual organs and morphometric traits. In such case, body length, wither height and heart girth may be used as good reliable predictors to assess live weight (Islam *et al.*, 1991). The present investigation was designed to obtain some basic morphometric information and to relate body weight with different body measurements of goats.

Table 4. Least squares means for wither height (cm) of bucks

Factors	LS±SE	NO	Significant Level
Overall	71.88 ±2.13	43	
Altitude			NS
LA	71.67±0.53	17	

MA	71.47±0.68	14	
HA	72.99±0.96	12	
Breed			**
Khari	71.51 ±0.83 ^b	29	
Khari dominant	78.44 ±1.52 ^a	8	
Jamunapari dominant	74.19 ±0.88 ^{ab}	6	
Colour			NS
Black	74.32±2.43	27	
Brown	72.72±1.09	8	
Mixed	69.11±1.21	8	
Age			NS
1 Year	63.65±0.68	13	
2 Year	72.79±0.76	12	
3 Year	73.20±0.73	15	
4 Year	78.58±1.37	3	

Note: **significant at 1% ($P < 0.01$), NS-non significant, LSD-Least significant difference, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Scrotal circumferences of buck

The overall average scrotal circumference of bucks (1-4 years) were 24.38±0.71 cm as mentioned in Table (5). Testicular sizes of animals are important for identification of those with adequate sperm production. Abba *et al.* (2015) observed scrotal circumference (17.00 –21.80) cm in Nigerian goats. Bezerra *et al.* (2009) also reported scrotal circumference of Boers goats on average 7.9 ± 0.8 to 25.7 ± 2 cm in Brazil. This is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum (Akpa *et al.*, 2010). Raji *et al.* (2008) reported 23.17cm for Red Sokoto bucks at 2 years of age. These are higher than that of the present study at similar age group. Higher results had also been reported by Keith *et al.* (2009) and Mekasha *et al.* (2008). However, similar to what is obtained in the present study was the results of Ugwu (2009) who reported 17.25 ± 0.76 cm in west African dwarf. The variability may be due to breed difference, contemporary group level, age, weight and height of bucks (Bourdon and Brinks, 1986). Kabiraj *et al.* (2011) also reported that Scrotal Circumference for 0.5 to 1.0, 1.5 to 2.0 and 2.5 to 3.0 years were 17.50±0.65, 21.38±0.43 and 22.88±0.66 respectively. These values were higher than those obtained in this current study. This is probably as a result of breed difference, differences in the age and number of bucks, dam's age at first breeding and rate of rebreeding as well as the general body size of the bucks. The SC was measured according to the method of Coulter and Foote (1979). The testes were first retracted into the lower part of the scrotum for measurement of the SC. In order to prevent the two testes from separation, the thumb and finger were placed on the sides rather than the front or

back of the scrotum. Then a flexible measuring tape was looped and placed around the greatest diameter of the scrotum and pulled so that the tape was firmly in contact with the entire circumference. The scrotal circumference is important traits considering in context of breeding of bucks.

Table 5. Least square means of Scrotal circumference (cm) of buck

Factors	LS±SE	NO	Significant Level
Overall	24.38 ±0.71	43	
Altitude			NS
LA	24.91±2.43	17	
MA	23.41±2.36	14	
HA	24.81±2.43	12	
Breed			NS
Khari	24.13±1.11	29	
Khari dominant	23.19±2.05	8	
Jamunapari dominant	25.73±1.24	6	
Colour			NS
Black	24.66±1.32	27	
Brown	25.09±1.44	8	
Mixed	23.39±1.60	8	
Age			NS
1 Year	22.63±0.93	13	
2 Year	24.33±1.90	12	
3 Year	25.14±1.04	15	
4 Year	25.45±1.06	3	

Note: NS-non significant, LS mean- Least square mean, LSD-Least significant difference, SE- Standard error of mean. NO are the number of observations.

Age of First service of buck

The overall age of buck during first service at Rolpa district is 336.93±1.04 days. Male goats up to 12 months of age are sometimes referred to as “buckling.” Adult male goats can weigh anywhere between 100 to 350 pounds, depending on their breed, health and nutritional status. Although they can come into puberty and breed does as early as 4 months of age, waiting until a buck is a year of age to start using him for breeding is best. The number of does a buck can breed during the breeding season is often referred to as “Buck Power” (Noble, 2004). The sexual behavior of goats is an important factor for flock breeding efficiency and productivity in goat farming. Male fertility is a vital issue because numerous does are generally mated to a single buck. On the other hand, if sexual behaviors are expressed and semen is collected at earlier ages, these could be used in breeding programs to shorten the generation interval. Therefore, evaluation of male fertility using a serving capacity test prior to mating is good practice to reach breeding success. In goat bucks, courting (latency and amount of courting anogenital

sniffing, vocalization, nudging, and flehmen) and copulatory (mounting and ejaculation) behaviors are evaluated using a sexual performance test that is part of a serving capacity test (Darwish and Mahboub 2011). The various factors affecting age of first service of buck are presented in Table(6).

Table 6. Least square means for Age (days) of first service of Buck

Factors	LS±SE	NO	Significant Level
Overall	336.93 ±1.04	43	
Altitude			NS
LA	321.90±1.44	17	
MA	334.80±1.39	14	
HA	344.54±1.49	12	
Breed			NS
Khari	306.49±1.67	29	
Khari dominant	361.89±0.30	8	
Jamunapari dominant	340.85±1.85	6	
Colour			NS
Black	346.98±1.98	27	
Brown	307.71±2.13	8	
Mixed	355.19±2.38	8	
Age			NS
1 Year	316.84±1.39	13	
2 Year	324.15±1.57	12	
3 Year	356.71±1.67	15	
4 Year	350.02±0.27	3	

Note: NS-non significant, LS mean- Least square mean, LSD-Least significant difference, SE- Standard error of mean. NO are the numbers of observations.

DISCUSSION

This study was designed to characterize and evaluate the productive and reproductive performance of hill goat reared under farmers' condition in Rolpa district of Nepal during December, 2015 to July 2016. The other objectives were to study the effect of non-genetic factors like altitude, colour, age, sex, parity etc in relation to the existing goat production system. Periodic observations on morphological traits, productive and reproductive performance were measured and recorded. The high altitudes VDCS were (Jhamksala and Pakhapani) followed by mid altitude VDCS (Gairigaun and Libang) and finally to low altitude VDCS (Masina and Jhenam). The elevation of selected high, mid and low altitude from the sea level in this research is at the range of 3639, 1375, 701 m respectively. The elevation of selected goat pocket areas for this research at low, mid,

high altitudes were 800-1000, 1200-1400, 1500-1800 m respectively from the sea level. The two wards were selected from each VDCS. There were three recognized breeds Khari, Khapari (Khari*Jamunapari) and Khabari (Khari*Barbari). However, Chyangra, Boers and Terai crosses were also observed in a few numbers. The data of 43 bucks were collected from different altitudes of Rolpa districts. The numbers of kids above five months' age were found in less number during data collection. So, they were not analyzed in this research. Farmers and technicians claimed the selected goats of being pure Khari, (Khari and Jamunapari) 50% cross breed and (Khari and Barbari) 50% cross breed. The bucks were also found in cross type genetically. However, characterization of such breeds at molecular level was not carried at Rolpa district. So, it could not be understood so far their DNA level.

Morphological attributes, productive, and reproductive performance were collected based on field monitoring and measurements. Least square analysis was performed using Harvey (1990) computer software package. Information on goat production systems were collected by employing a semi-structured questionnaire and analyzed using SPSS.

The data of bucks were collected from (1-4) years age. The overall mean body weights of bucks were 38.76 ± 0.35 kg. Body weight of buck differ significantly ($P < 0.001$) within ages and also differ significantly ($P < 0.01$) within breed. Khabari buck body weight (45.32 ± 0.63 kg) was significantly higher ($P < 0.01$) than that of Khari buck (35.68 ± 0.55 kg) and Khapari (37.29 ± 1.04 kg) bucks.

The mean age of first service of buck was 336.93 ± 1.04 days The overall mean for wither height of bucks were 71.88 ± 2.13 cm. Wither height of buck differed significantly ($P < 0.01$) within breed. The overall mean for body length of bucks were 78.01 ± 0.35 cm. Body length of bucks differed significantly ($P < 0.001$) with respect to ages. The overall mean for heart girth of buck were 77.57 ± 1.65 cm. Heart girth of buck differed significantly ($P < 0.01$) with respect to ages, and also significant ($P < 0.05$) within breed. The overall mean for scrotal circumference of bucks were 24.38 ± 0.71 cm. Goat farming was practiced as a subsistence occupation with three distinct systems of feeding i.e. extensive grazing, stall feeding, and grazing plus stall feeding supplementing little maize grains and flour as per availability. Majority of the farmers depended on the community forest as well as on their own farmland for collecting fodder and forages. Major problems of goat farming in Rolpa district were occurrence of epidemic disease, lack of veterinary and technical advice, and attack of wild animal. Thus the results of this study suggest that the performance of low altitude goat flocks was better than mid and high altitude goat flocks in Rolpa district in terms of production and reproduction traits. The selection of the best performance of bucks could be done to obtain higher weight and production of offspring at later stages. This result could be attributed to superior genotype along with better management practices followed by the farmers in Rolpa district.

CONCLUSION

The selection of the best performance of male kids could be done on the basis of both birth and weaning weights to obtain higher weight at later stages. The best kid could be selected as buck for breeding. This result could be attributed to superior genotype along with better management practices followed by the farmers in Rolpa district.

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REFERENCES

1. Akpa, G.N., M.S. Abdulkareem, I.R. Muhammed and C. Alphonsus. 2010. Effect of breed and age on measures of growth and scrotal size in Rams. *Nigeria Journal of Animal Science* 12: 1-6pp.
2. Bezerra, F.Q.G., C.R. Aguiar Filho, L.M. Freitas, E.R. Santos Junior, R.M. Chaves, E.M.P. Azevedo, M.H.B Santos, P.F. Lima and M.A.L Oliveira. 2009. Body weight, scrotal circumference and testosterone concentration in young Boer goat males born during the dry or rainy seasons. *South African Journal of Animal Science*.39pp
3. Central Bureau of Statistics (CBS). 2016. National Population and Housing Census 2011 Volume 03. Kathmandu: Government of Nepal, National Planning Secretariat. 28pp.
4. Coulter GH and RH Foote. 1979. Bovine testicular measurements as indicators of reproductive performance and their relationships to productive traits in cattle. *Theriogenology*.297p.
5. Darwish, R. A. and H.D.H Mahboub. 2011. Breed and experience effect on the sexual behaviors of Damascus and Egyptian-Nubian goat bucks, *Theriogenology* (76): 1386-1392pp.
6. Harvey W.R. 1990. Least-square analysis of data with unequal subclass numbers. United States Department of Agriculture, Agricultural Research Service, ARS 20, USA.
7. HMG/N.1993. Livestock Master plan. His Majesty's Government of Nepal Livestock development. Asian development bank. ANZDEC Limited, Agricultural Project Service Center.
8. Islam, M. R., Saadullah, M., Howlider, M. A. R. and Huq, M. A. 1991.Estimation of live weight and dressed carcass weight from the different body measurements of goats. *Indian Journal of Animal Science* (61): 460-461pp.
9. Joshi, B. R and B.S Shrestha.2003. The Goats. Their Production and Health Management. 11-18pp. Central Bureau of Statistics (CBS). 2016. National Population and Housing Census 2011 Volume 03. Kathmandu: Government of Nepal, National Planning Secretariat. 28pp.
10. Kabiraj, S.K., S.A M.H, M.Y Khandoker and S.S Hussain. 2011. *Journal of cell Animal Biology* (5): 27-32pp.
11. Keith, L., C. Okere, S. Solaimanand O.2009. Tille. Accuracy of Predicting Body Weights from Body Conformation and Testicular Morphometry in Pubertal Boer Goats. *Research Journal of Animal Sciences* 3(2): 26-31pp.
12. Mekasha, Y., A. Tegegne and A. Abera, H. Rodriguez.2008. Body size and testicular traits of tropically adapted bucks raised under extensive husbandry in Ethiopia. *Reproduction of Domestic Animal* (43): 196-206pp.

13. MOAD. 2018. Selected indicators of Nepalese agriculture and population. Government of Nepal. Ministry of Agriculture and Cooperatives. Agribusiness promotion and statistical division. Singha Darbar, Kathmandu, Nepal
14. Noble, R.C. 2004. Effect of Condition Scores on Late Pregnancy Does Subsequent Performances. Reproduction Management of Beef Cattle and Goats. Goat Workshop. Florida Journal of Animal Science 73(10): 1141-1143pp.
15. Pokharel PK and SP Neopane.2008. Indigenous Goats of Nepal. National Animal Science Research Institute.Nepal Agricultural Research Council. Khumaltar, Lalitpur. 3-8pp.
16. Raji, AO., J.U. Igwebuike, J. Aliyu.2008. Testicular biometry and its relationship with body weight of indigenous goats in a Semi-Arid region of Nigeria. Journal of Agricultural and Biological Science 3(4): 6-9pp.
17. Statoids. 2014. Districts of Nepal.Gwillim Law. Land administration and management mapping, surveying and aerial photopgraphy, Major refrences projects. Finn map.
18. Raji, AO., J.U. Igwebuike, J. Aliyu.2008. Testicular biometry and its relationship with body weight of indigenous goats in a Semi-Arid region of Nigeria. Journal of Agricultural and Biological Science 3(4): 6-9pp.
19. Ugwu, S.C.2009. Relationship between scrotal circumference, in situ testicular measurements and sperm reserves in the West African dwarf bucks. African Journal of Biotechnology 8(7): 1354-1357pp.

Study on Management Status and Disease aspects of Pheasant (*Phasianus colchicus*) of Kathmandu Valley, Nepal

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ABSTRACT

Even though Nepal has several wild pheasant species, captive pheasant rearing for human consumption is a relatively new farming practice in Nepal. Pheasant farming is a growing practice among Nepalese farmers. However, farmers lack understanding and background in pheasant management techniques and associated diseases. The purpose of this study was to investigate the distribution, location, management status, and disease characteristics of commercial pheasant farming in the Kathmandu valley. A retrospective cross-sectional study was conducted in Kathmandu valley and the Central Veterinary Laboratory (CVL), from July to September 2021. The case records from 2017 to 2021 were analyzed at CVL and the farms were mapped based on their locations. The top ten pheasant diseases reported in CVL were identified and the disease outbreak farms were followed to get additional information on management aspects. A total of 42 pheasant farms were visited to collect data, and dead birds from these pheasant farms were submitted to CVL for postmortem and bacterial culture. MS-EXCEL and the online software "Open Epi" were used for statistical analysis. Sixty percent of the surveyed farms exclusively raised pheasants, while 40 percent practiced mixed farming. The source of hatching eggs was from abroad (12%), local breeding center (70%) and inter-district farms (18%). Ninety percent of the farms practiced some extent of biosecurity such as clothes changes and visitors log maintained. The temporal analysis identified that pheasant mortality was highest in July, followed by June and August. Of the total cases reported in retrospective study urolithiasis was responsible for 20.27 percent of deaths, Newcastle disease and Mycotoxicosis each were responsible for 13.51 percent of deaths of birds. Though urolithiasis is one of the leading causes of death there was no significant statistical associations ($p > 0.05$) with ages and the flock size. The study collected the baseline data on management practices and disease distributions among the commercial pheasant farming in Kathmandu valley. We suggest, further studies on morbidity, mortality, seasonal stress, and economic aspects of commercial pheasant farming. Prior knowledge and expertise on management and health aspects related to farming is needed for sustainable pheasant farming in Nepal.

Keywords: Pheasant farming, Kalij, disease distribution, management practices.

INTRODUCTION

Nepal's unique geography with drastic elevation changes, and the accompanying wide range of eco-climatic conditions, has resulted in a very diverse range of flora and wildlife. Nepal hosts 118 ecosystems and is located at the crossroads of two bio-geographic domains: the Palearctic in the north and the indo-malayan realms in south (Udvardy 1975). Pheasants are birds of several genera within the subfamily phasianinae, of the family Phasianidae in the order Galliformes. Despite pheasants are introduced worldwide, they are native to Asia. There are 49 species native to Asia out of 181 species of pheasants (Ashraf, 2015). Among them 8 species of pheasant; Himalayan monal, (*Lophophorus impejanus*), satyr tragopan (*Tragopan satyra*), blood pheasant (*Ithaginis cruentus*), koklass pheasant (*Pucrasiamacrolopha*) and kalij pheasant (*Lophura leucomenalos*) and two lowland pheasants red junglefowl (*Gallus gallus*) and blue peafowl (*Pavo cristatus*) are recorded in Nepal (Poudyal et al., 2011). Himalayan monal (*Lophophorus impejanus*) is a national bird of Nepal and natively called as Danfe.

The ring-necked pheasant (*Phasianus colchicus*) is a game bird native to South Asia which first made successful introduction from Eastern China (Geaumont & Field, 2016). Pheasants are primarily used as game birds for hunters in Europe and eastern continent but in case of South Asia the native pheasants are listed in International Union for Conservation of Nature (IUCN). They are very popular for their nutritious meat with low fat and high essential amino acids (Ashraf, 2015). Pheasants are polygamous species (Baxter & Wolfe, 1973). Pheasant are easily distinguishable, male are large and have colorful plumage whereas females are simple (Ashraf, 2015; Lelliot & Yonzon, 1980). Females choose their mates on the basis of size, color, intensity of sexual ornaments to obtain good genes for their off-spring (Ashraf, 2015). In developing world, pheasant production and shooting is one of the most productive businesses (Fulton, 2021). They are eye catching and most impressive species in nature, therefore they are often put on display in aviaries and on posters for conservation and educational purposes and they are widely used as a source of food (McGowan & Garson 1995; Fuller & Garson 2000).

Pheasant rearing system consist of primary two methods: intensive and semi-intensive rearing system while semi-extensive system is also applied in Europe. Semi-intensive system is also known as semi-natural system of rearing (Gheřa et al., 2020). Breeding the wild birds in captivity gives rise to a problem of feather picking and cannibalism which causes heavy economic losses to the farmers. There are two main factors: extrinsic and intrinsic. Extrinsic factors include nutritional factors, environmental factors and condition of breeding while intrinsic factors depend on the birds such as social behavior, sex, age, stress, nervous, hereditary, and immunology (Nikolov & Kanakov, 2020).

Bacterial septicemia with parasitic infestation are more common in pheasants species similarly salmonellosis followed by enteric bacterial infections and then infection with *Mycoplasma gallisepticum*; also, infections with *Pasteurella multocida* and *Erysipelothrix rhusiopathiae* were common on ring-necked pheasant. (Fulton, 2021). With parasitic infection dominating the pheasant death bacterial disease and viral infection such as rotaviral enteritis; Marek's disease; lymphoid leucosis; were evident in the retrospective study, where the necropsy was submitted in Michigan State University Veterinary Diagnostic Laboratory (MSU VDL) for disease surveillance and diagnostic investigation (Liebhart et al., 2023).

MATERIALS AND METHODS

Study site and design

This study was carried out from July 2021 to September 2021 in the Kathmandu valley. The dead pheasants were brought to Central Veterinary Laboratory (CVL), Tripureshwor, Kathmandu for necropsy examination. After necropsy, the tissue samples such as liver and lungs were collected for the bacteriological study and virological investigations by rapid antigen tests. The study consists of two approaches; first, the farmers who submitted the dead birds were contacted by telephone and arranged onsite visits for the face-to-face interview using the questionnaire. On farm visit, the farmers were asked about the farm characteristics such as farm size, rearing system, shed type and biosecurity status. In addition, bird characteristics such as flock size, breed types, and population diversity were assessed. Farmers were requested to submit dead birds to CVL if they suspected disease and if unusual mortality was found. Second, the historical data of the postmortem and bacteriology unit of year 2017 to 2021 were analyzed.

Sample size

Actual locations and the numbers of farms were not able to estimate initially, as there have not been official records and kalij farming is relatively new profession in Nepal. Snowball sampling technique (Snowball Sampling on JSTOR, n.d.) was used to locate the farms of the pheasant. Therefore, with this technique, altogether, 42 pheasant farms were visited for the survey. 75 farms with the historic record of CVL were included in the analysis making total 117 farms in the study. The farms that submitted birds more than once within a week were considered duplicates and recorded each as a single farm.

Study area and profile

The survey was conducted in the farms of Kathmandu valley, which included the farms in Kathmandu, Bhaktapur and Lalitpur district. Yet, the historical data also includes farms from Nuwakot and Kavre.

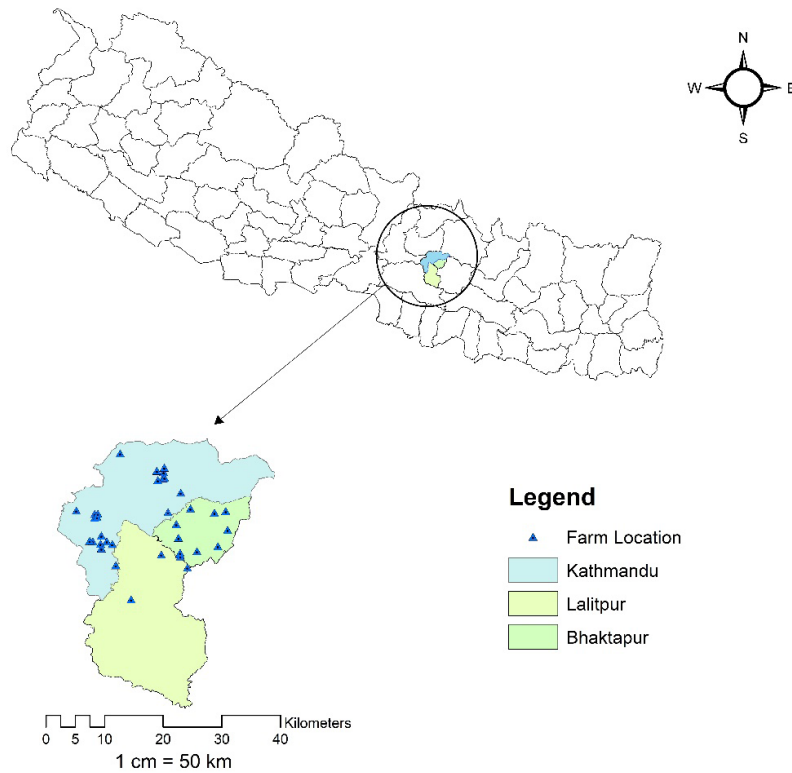


Figure 1 Study area and locations of pheasant farms (generated using QGIS 2.18)

Survey tools and data sources

A semi-structured questionnaire survey was prepared with the open and closed ended questions, which included the socio-economic aspect, management aspect, and disease occurrence history. The answers were collected by individual interview in very amicable environment with owner's consent. All the information was transferred into digital format using MS-Excel 2013.

Similarly, for the retrospective study primary and secondary data of post-mortem unit, bacteriological unit, and virology unit of 2018 AD to 2021 AD of the Central Veterinary Laboratory (CVL) was used for the study of frequency of disease occurrence.

Statistical analysis

All the raw data was recorded in MS Excel. Descriptive analysis was performed for the farm characteristics and disease distribution. Similarly, the association of health outcomes with the important factors were tested by chi-square test and their corresponding p-values calculated to test statistical significance. An online statistical tool "Open Epi" was used for calculating the p-value.

RESULTS

Descriptive study of pheasant populations

The survey involved 42 pheasant farms; from which, 60% farms were rearing pheasants only while 40% of farms were mixed farms. The median flock size of the pheasant farm was 1200 while mean flock size of pheasant farm was 1656.58. Out of the total farm surveyed, the average land covered for pheasant farming was 30943 sq feet, the maximum land covered was 76664 sq feet and the minimum land covered by the farm was 2738 sq feet. Average number of feeders used in the farms were 19, while the average number of drinkers used were 18. Average egg laying period for the pheasants was 4.4 months primarily during February to May. Rearing system adopted in pheasant farm was dominantly semi-intensive (92%), which includes the brooding pen and flight pen. Similarly, despite pheasant being game bird 7% farm owners adapted the intensive rearing system. Out of total farm visited 88% farms reared only ring-necked species while remaining 12% farms introduced melanistic mutant in their farms. The characteristics of pheasant farms and population is depicted in the Table 1.

Table 1 Descriptive characteristics of pheasant population in the farm

	Mean $\pm$ SE	Min	Median	Max
Total pheasant population	1656 $\pm$ 310	100	1200	12000
Total land covered (Sq. feet)	30943.2 $\pm$ 2965.60	2738	27380	76664
Total no of feeders	19 $\pm$ 1.82	5	15	50
Total no of drinkers	18 $\pm$ 1.90	3	15	50
Egg laying period (months)	4.4 $\pm$ 0.13	3	4	6
Note: Sq. feet is Square feet; Min is Minimum; SE is Standard Error; Max is Maximum				

From the survey, 12% of pheasants' eggs were either purchased from nearby farms or brought from abroad, while 17% were brought from abroad and 70% of the pheasant's eggs origin was from the farms locally. Interestingly, the community mainly residing in the edges of Kathmandu valley maintained the farms. The surveyed data showed that 90% of the farm applied biosecurity measures, although all the elements of biosecurity method was not strictly applied in the farms.

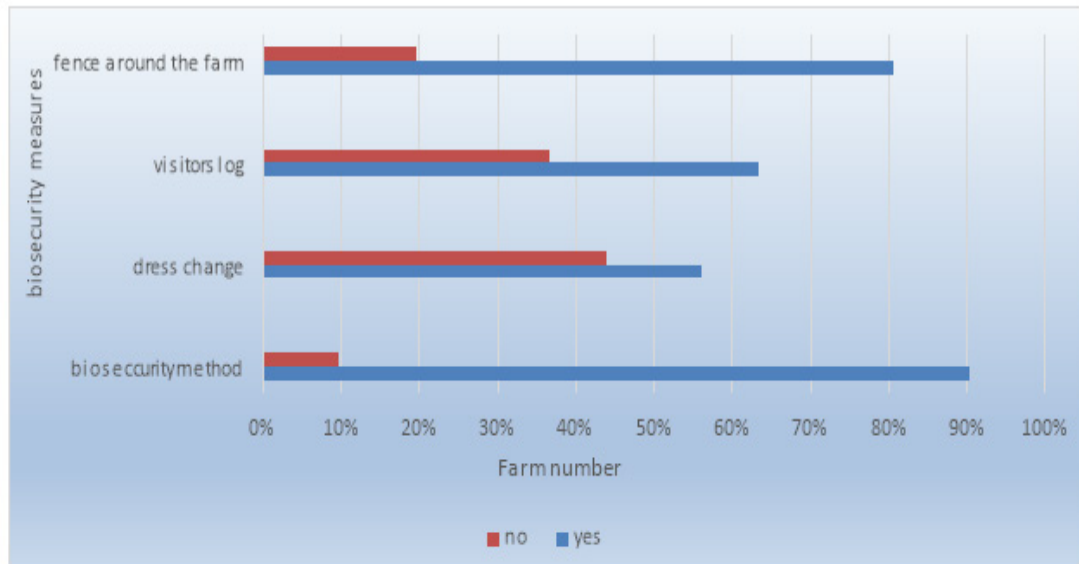


Figure 2 Graph showing the biosecurity status in pheasant farm.

Retrospective study of the pheasant populations

Frequency of disease

From the retrospective data from CVL, we found out that the frequency of disease was highest during the month of July while least during the month of December (Figure 3).

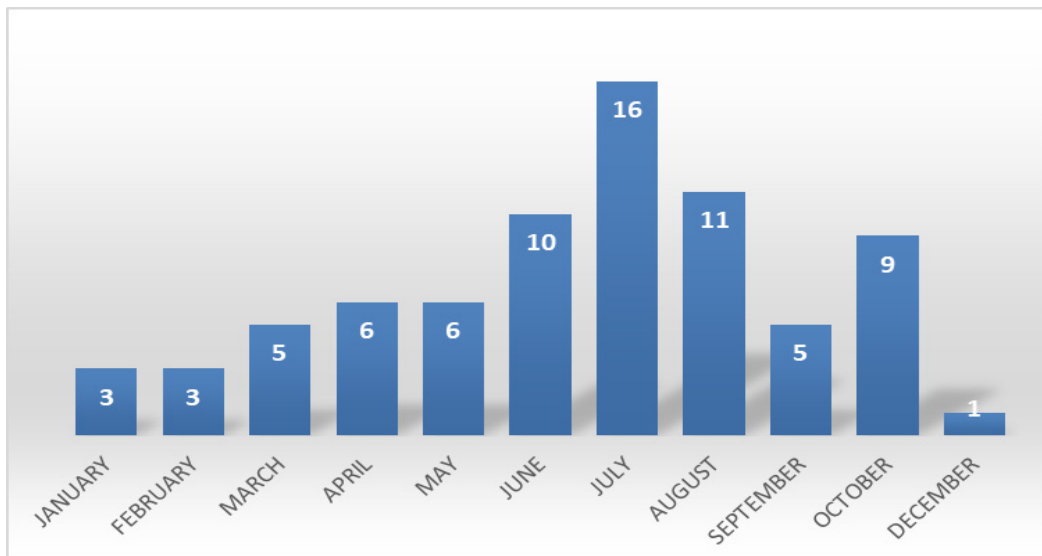


Figure 3 : Graph showing frequency of diseases occurrence according to months

Top 10 diseases of Pheasants

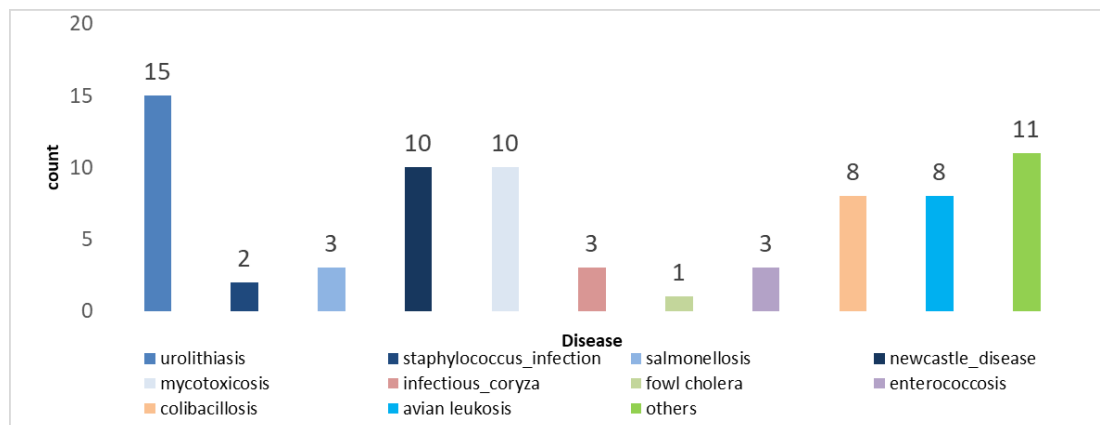


Figure 4: Graph showing frequency of common diseases reported in CVL

Out of the total samples tested in the CVL, 20.27% of the samples tested confirmed urolithiasis as a cause. Newcastle disease and mycotoxicosis accounted for the 13.51% each. Similarly, colibacillosis and avian leukosis accounted for 10.81% each of the total disease. Subsequently, infectious coryza, salmonellosis, and enterococcal infection were diagnosed in the bacterial culture accounting for 4.05 % each of the total disease reported. Lastly, fowl cholera was also reckoned in 1.35% of the cases presented.

All the factors like age in weeks and flock size had no significant difference in the occurrence of the urolithiasis in pheasants. There was no significant association of age (weeks) with the risk of development of urolithiasis in pheasant farm, (Chi-square value (Odds Ratio= 1.9, $p=0.3604$). There was no significant association of flock size with risk of development of urolithiasis in pheasant farm (Odds Ratio = $1.7\chi^2 = 0.1625$, $p=0.4607$) as shown in Table 2.

Table 2. Statistical analysis of bird's factor-“ Age (weeks) and flock size” with urolithiasis

Bird related factors	Urolithiasis		Odds Ratio (95%CI)	P value
	Yes	No		
Age in weeks				
<12	10	39	1.9 (0.49, 7.88)	0.3604
>12	3	23		
Flock Size				
>1000	4	13	1.7 (0.44, 6.32)	0.4607
<1000	9	49		
Grand Total	13	62		

CI: Confidence interval

DISCUSSION

This research finding discovered 42 functional farms in Kathmandu valley and estimated the total population of the pheasants as 67,020 until September 9, 2021. The ring-necked pheasant was reared in most of the farms while portion of the farms began introducing melanistic mutant sub-species too. 93% of the pheasant farm were semi-intensive while 7% of these were intensively reared as semi-intensive farm provided the trees and hedges for the cover and protect them from natural predators (Liebing et al., 2020). The biosecurity measures on most of the farm were below average/good. An awareness of the level of biosecurity on the premises and information on production techniques that are related to the risk of disease transmission were both goals of the survey questions. Due to the infeasibility of all-in and all-out production strategy, maintaining adequate biosecurity on game bird breeding farms poses intrinsic difficulties (Dwight et al., 2021). There had been outbreak of ND recently and not application of the strict biosecurity measures might be responsible for disease outbreak. The reasons of disease outbreaks could be multispecies rearing and poultry farm nearby the pheasant farm. Every farm dealt with the problem of cannibalism and feather pecking, which might be due to high stocking density, composition of feed, environmental factors (light, sound, temperature and air) and bad management practice (Nikolov & Kanakov, 2020).

In our analysis, there was no significant relationship between flock size and age in weeks with the risk of development of urolithiasis in pheasant farm. However (Pennycott, 2000) has mentioned the mortality of the pheasants with the deposition of urates in heart and ureters which were associated with the pheasant coronavirus-associated nephritis.

While the sporadic feeds and not appropriate constituent in the feed and deficient water accessible as per flock size may have been a reason for urolithiasis in the pheasants of Kathmandu valley. The goal of the retrospective study provided a summary of the management status, with particular attention to common diseases affecting the health and welfare of pheasants.

CONCLUSION

The study has provided information on current farming status and major diseases dominant on pheasant farms in Kathmandu valley, Nepal. To our knowledge, our study is the first to quantify the pheasant rearing and their management in Kathmandu Nepal. The management status of the pheasant/kalij in Kathmandu valley were informative to our study suggesting the practice of farming with little knowledge. The study has shown that well recognized cause of high mortality, such as “pheasant urolithiasis” and Newcastle disease affected the pheasant farm in Kathmandu valley. Additional research that utilizes virus isolation techniques can potentially link pathogens found on farms

to those detected in birds from pheasant farms, which allows for tracing of a pathogen from farm to the other commercial poultry farms. This research has made a road map for the further investigation of the diseases causing mortality in pheasants and summarized the prevalent diseases in pheasant rearing. There is a requirement for specialists in the infectious disease determination and to address the management issue in building up pheasant farm and breeding center in Nepal.

ETHICS STATEMENT

The author acknowledges that the ethical policies, as noted on the journal's author guidelines page, have been cohered to and the appropriate review has been received. Since no physical intervention was done in live birds and all the studies were conducted in dead birds during the study, no any ethical permission was needed. However, a verbal consent was obtained during collecting the data from the farmers and use of the retrospective data from the CVL.

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CONFLICTS OF INTEREST

Authors declared that there is no conflict of interest in publishing this research paper.

DATA AVAILABILITY STATEMENT

On request, the corresponding authors could provide the findings that support the study's conclusions. Due to privacy or ethical constraints, the data are not available to the public.

REFERENCES

1. Ashraf, S. (2015). Studies on Growth Performance, Morphology, Reproductive Traits and Behavioral Aspects of Ring Necked Pheasants in Captivity a Thesis Submitted in the Partial Fulfilment of the Requirement for the Degree of Doctor of Philosophy in Wildlife and Ecology Univ. 41–185.

2. Baxter, W. L., & Wolfe, C. W. (1973). The Ring-necked Pheasant in Nebraska.
3. Dwight, I. A., Coates, P. S., Stoute, S. T., & Pitesky, M. E. (2021). Health surveillance of a potential bridge host: Pathogen exposure risks posed to avian populations augmented with captive-bred pheasants. *Transboundary and Emerging Diseases*, October 2020, 1–13. <https://doi.org/10.1111/tbed.14068>
4. Fulton, R. M. (2021). Common Diseases of Michigan Gamebirds: A Retrospective Study. *Avian Diseases*, 65(1), 26–29. <https://doi.org/10.1637/aviandiseases-D-20-00069>
5. Geaumont, B., & Field, A. (2016). Pheasants Raising. May.
6. Gheța, M., Maftei, M., Bordei, I., & Nicolae, C. G. (2020). STUDY REGARDING THE DIFFERENT REARING SYSTEMS FOR PHEASANT. *LXIII*(2), 516–520.
7. Lelliot, A. D., & Yonzon, P. B. (1980). Studies of Himalayan Pheasants in Nepal. *Journal of World Pheasant Association*, 5, 11–30.
8. Liebhart, D., Bilic, I., Grafl, B., Hess, C., & Hess, M. (2023). Diagnosing Infectious Diseases in Poultry Requires a Holistic Approach : A Review. 252–280.
9. Liebing, J., Völker, I., Curland, N., Wohlsein, P., Baumgärtner, W., Braune, S., Runge, M., Moss, A., Rautenschlein, S., Jung, A., Ryll, M., Raue, K., Strube, C., Schulz, J., Heffels-Redmann, U., Fischer, L., Gethöffer, F., Voigt, U., Lierz, M., & Siebert, U. (2020). Health status of free-ranging ring-necked pheasant chicks (*Phasianus colchicus*) in NorthWestern Germany. *PLoS ONE*, 15(6), 1–17. <https://doi.org/10.1371/journal.pone.0234044>
10. Nikolov, S., & Kanakov, D. (2020). Influencing factors leading to damaging behavior - feather pecking and cannibalism in game birds. *Trakia Journal of Sciences*, 18(4), 377–387. <https://doi.org/10.15547/tjs.2020.04.012>
11. Pennycott, T. W. (2000). Causes of mortality and culling in adult pheasants. *Veterinary Record*, 146(10), 273–278. <https://doi.org/10.1136/vr.146.10.273>
12. Poudyal, L. P., Outhwaite, W., Poulton, S., & ... (2011). Monitoring pheasants in the Pipar area of Nepal: The 2011 expedition. ... and Norwich, UK, October. <https://doi.org/10.13140/RG.2.2.19043.55847>
13. Snowball Sampling on JSTOR. (n.d.). Retrieved November 20, 2021, from <https://www.jstor.org/stable/2237615>

Seroprevalence of Contagious Caprine Pleuropneumonia (CCPP) in Bharatpur, Chitwan, Nepal

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ABSTRACT

A study was conducted to determine the seroprevalence of Contagious Caprine Pleuropneumonia (CCPP) in 106 goats of Bharatpur Municipality of Chitwan, Nepal from July to October 2021. A questionnaire was used to determine the potential risk factors. Collected samples were subjected to a competitive enzyme-linked immunosorbent assay (cELISA) for the detection of antibodies. The seroprevalence of Contagious Caprine Pleuropneumonia in goats was 4.71% (5/106). Although seroprevalence was high among females and goats below or equal to 5 years, there was no statistically significant. This study was carried out in the Chitwan district for the first time, applying the (Mccp) Antibody test technique, considering the need for control of this economically important disease for poor livestock farmers in Chitwan and it can play important role in the epidemiological study of CCPP in Nepal.

Keywords: Seroprevalence, Goats, CCPP, cELISA, Nepal

INTRODUCTION

The goat population in Nepal is estimated at 12.28 million and goat meat ranks third in terms of national meat output (13.58 percent), behind chicken and buffalo meat (Krishi Diary, 2020). Small ruminants provide meat, milk, manure, leather, and packaging material to Nepalese rural households, contributing greatly to their livelihoods. Even though goats represent plenty of national resource in the country, their productivity is hampered by a variety of reasons, including a lack of feed, a lack of genetic potential, and infectious diseases (Boyazoglu et al., 2005; Ahuya et al., 2005).

Among the infectious diseases, Contagious Caprine Pleuropneumonia (CCPP) which is

caused by *Mycoplasma capricolum subspecies capripneumonia* is a highly contagious severe respiratory disease affecting goats in over 40 countries throughout the globe, particularly in Asia, Africa, and the Middle East (Iqbal et al., 2019; Parray et al., 2019). It is an OIE-listed notifiable devastating disease in goats (Bascunana et al., 1994; Manso-Silván et al., 2011). The major transmission route is direct contact between sick and healthy animals, with the predominant route of infection being the inhalation of infective droplets from the carrier or active animals (Shahzad et al., 2016). Anorexia, dyspnea, coughing, thoracic pain lying down, nasal discharge, depression, high temperature (41–44 °C), lagging, loss of body condition score, and substantial morbidity (up to 100%) and mortality leading to 80–100% are the symptoms of acute and chronic stage of CCPP (MacOwan & Minette, 1976; Radostis et al., 2006; Ahaduzzaman, 2021). Age, sedentary agricultural systems, species, location, season, the introduction of new animals from markets, and the absence of therapeutic intervention are all common risk factors linked to higher CCPP seroprevalence (Parray et al., 2019).

Biochemical, microbiological, serological, and gene-based identification are used to confirm the diagnosis following a clinical tentative diagnosis. The competitive ELISA kit which is based on Mccp-specific monoclonal antibody has a high specificity (99.8–100%) and its applicability for mass-scale testing is an appropriate tool for epidemiological analysis of CCPP (Thiaucourt et al., 1994; Asmare et al., 2016). This study was carried out to determine the seroprevalence of CCPP and related risk factors in Bharatpur, Chitwan, Nepal. This study will help in the epidemiological study of CCPP in Nepal.

MATERIALS AND METHODS

A cross-sectional study was carried out in Bharatpur Metropolitan (Ward Number: 4, 5, 12, 14, 15, and 17) (27.6706° N, 84.4385° E) of Chitwan, Nepal (Figure 1) from 15 July 2021 to 21 October 2021 through multistage random sampling techniques among goat population. A questionnaire survey was done to collect general descriptions, herd history, and individual animal history.

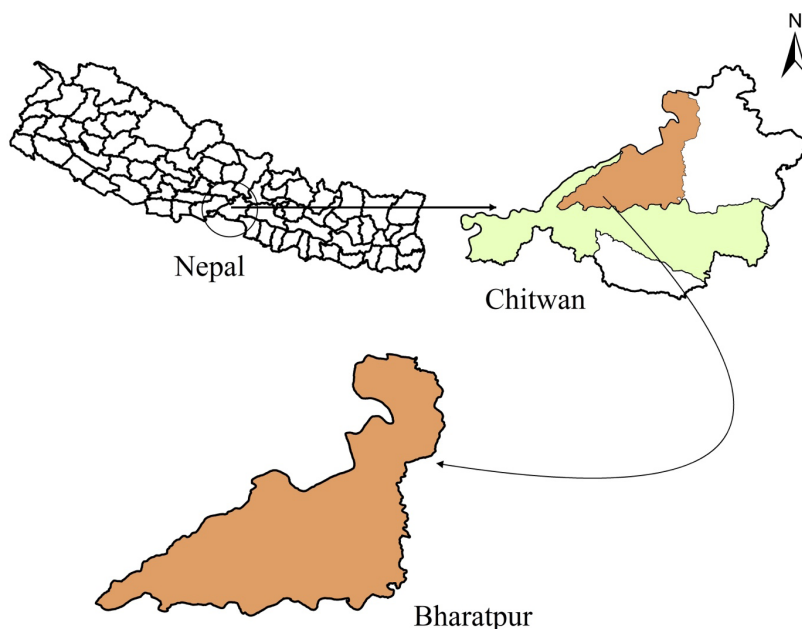


Figure1. Map of Nepal and Chitwan with study area i.e., Bharatpur

The total goat population in Bharatpur Metropolitan is 94,531 and in western Chitwan is 61,348 (Krishi Diary, 2020). There had been no previous reports on the prevalence of CCPP in Chitwan. According to the literature review, the prevalence of CCPP in neighboring state i.e., Uttar Pradesh of India was found to be 10.5 % (Jain et al., 2015). As Uttar Pradesh is very close to our study area, its prevalence was taken as a reference and the desired sample size was calculated with expected precision of 5 % at a confidence level of 95% (Thrusfield et al., 2017). The calculated minimum sample size required for the study was 145. In the study 106 samples were taken due to time and budget constraints.

$$n = \frac{1.96^2 \times P_{\text{exp}} (1 - P_{\text{ex}})}{d^2}$$

n = Required sample size,

d = Desired absolute precision (0.05)

P_{exp} = Expected prevalence (0.05)

A semi-structured questionnaire was administered to collect data from the goat farmers by the purposive sampling method. The closed and open-ended questionnaire was pretested to ensure its validity and verbal consent was obtained from the respondent by explaining the objectives of the survey.

One hundred six blood samples were taken from goats with a history of nasal discharge, and cough from the herd of healthy animals. Selected animals were restrained by two people and the site was prepared around the mid-jugular vein by using 70% ethanol.

Using a 10 ml sterile disposable syringe and needle, five ml of blood was taken from the jugular vein aseptically.

To prevent blood hemolysis and extract the serum fluid, the syringe with blood was left undisturbed at room temperature in a 45-degree position overnight. The serum was extracted, transferred to serum vials, and maintained in a cool box. An icebox containing ice packs was used to transfer the sample to the Central Veterinary Laboratory (CVL) in Tripureswor, Kathmandu.

Collected goat serum samples were examined by the competitive enzyme-linked immunosorbent assay (cELISA) (Thiaucourt et al., 1994) by IDEXX CCPP Ab Test which is based on the monoclonal antibody Mab 4.52 recommended by the International Organization for Animal Health and CIRAD -EMVT (France). The test was performed according to the manufacturer's guidelines. All reagents were allowed to come to 18-26°C before use and reagents were mixed by gentle inverting or swirling. Controls were dispensed anywhere on the microplate. The serum samples were diluted and mixed in an uncoated plate with a specific monoclonal anti-Mccp antibody (Mab 4.52), then the homogenized components of the uncoated plate were transferred to the Mccp antigen-coated microplates and incubated for 1 hour at 37°C with gentle agitation to avoid desiccation of the plates. After removing the solution, each well was washed twice with the washing solution. Each well was filled with a diluted Conjugate solution of Anti-mouse IgG horseradish peroxidase, and the microplate was covered at 37°C for 30 minutes with gentle agitation. The technique was repeated 3 times, after which TMB Substrate was added to each well and incubated at + 37 °C, for 20 minutes. Finally, a Stop Solution was added in each well, and the absorbance values (OD) of controls and samples were measured and recorded at 450 nm using an ELISA plate reader. The result was interpreted by a percentage of inhibition (PI)= $((\text{OD Mab} - \text{test serum}) / (\text{OD Mab} - \text{OD conjugate})) \times 100$ and Serum with a $\text{PI} \geq 55\%$ was judged positive for Mccp infection, whereas a $\text{PI} < 55\%$ were considered negative.

The data entry of the serological test was done using Microsoft office excel and statical analysis was done by SPSS V 20.0. Chi-square was utilized as a statistical test. In all of the analyses, the confidence level was set at 95%, and the significance level was fixed at $p < 0.05$.

Ethical approval of the study was sought from the Ethical Approval Committee of Agriculture and Forestry University, Nepal. Verbal consent was obtained from the respondent by explaining the objectives of the survey. Blood was collected by a technical expert with minimal pain to the animals.

RESULTS

In this study, the seroprevalence of CCPP in the goat population was found to be 4.71% (5 out of 106 goats). Among the tested population, 81 were females and 25 were males. The seroprevalence of CCPP was higher in the female (4.93%) than in male (4%) goats, although the relationship was not significant at a 5% level of significance ($p=0.663$). The goats below or equal to 5 months of age were found to have higher seroprevalence (7.69%) of CCPP than above 5 months of age (3.75%). The age-wise prevalence was not significant at a 5% level of significance ($p=0.357$) (Table 1).

Table 1: Sex and age-wise seroprevalence of CCPP in Bharatpur, Chitwan, Nepal.

SN	Risk Factors		Total	Positive (Percentage)	p-value
1	Sex	Male	25	1 (4%)	0.663
		Female	81	4 (4.93%)	
2	Age	Below or equal 5 months	26	2 (3.75%)	0.357
		Above 5 months	80	3 (7.69%)	

DISCUSSION

In this study, the seroprevalence of CCPP was found to be 4.71% (5 out of 106 goats) which is higher than a study conducted in the Rupandehi and Palpa districts of Nepal (3.40%) (Adhikari et al., 2022) and lower than serological research conducted in different parts of South Asia, where the prevalence rates of 32.5 to 45.7 % in Pakistan (Awan et al., 2010), 9.9 to 33.7 % in India (Suryawanshi et al., 2015), 8.52 % in Pakistan (Shahzad et al., 2016), and 10.65% in India (Gupta et al., 2016) were reported from the goats. The differences in seroprevalence might be due to the low sample size, goat management and production systems, agroecological systems, variances in research areas, population density, and the procedures employed to quantify seropositivity.

The seroprevalence of CCPP was found to be higher in female goats (4.93 percent) than in male goats (4%) in our investigation. Similarly, Abegunde (1981) discovered a greater frequency of CCPP in female goats (16.1%) than in male goats (10.7%). A study by Bekele et al., (2011), also found a higher prevalence in females (14%) than in males (11.6%). The female seroprevalence of CCPP was somewhat higher (10.67%) than the male seroprevalence (10.61%) reported by Gupta et al., (2016). However, Suryawanshi et al., (2015) reported a higher prevalence in males (35.71%) than in females (17.14%). This result could be attributed to a lack of CCPP data in the Nepalese context for comparison and a lack of CCPP tendency for either gender.

Furthermore, goats younger than or equal to 5 months of age had a higher seroprevalence of CCPP (7.69%) than goats older than or equal to 5 months of age (3.75%). However, the study of Bekele et al., (2011) found a significant seroprevalence of 9.2 percent, 11.0

percent, and 34.8 percent in young (2 years), adult (2-5 years), and old (>5 years) age groups. A study by Suryawanshi et al., (2015) discovered a higher frequency (23.08%) in goats aged 2-3 years. Also, Gupta et al., (2016) found the highest prevalence in goats over 12 months of age (10.67%) followed by 10.64% in goats under 6 months of age, and 10.59% in goats 6-12 months of age, although the result was insignificant with each other. Goats become more susceptible to illness as they age. They're also prone to being infected time and again. These results were in contrast to our findings, which were based on a separate comparative age group, with younger goats (<5 months) being more susceptible to CCPP infection than the goats (>5 months). This could be related to differences in sample size, past infections, predisposing factors, and due to the weak immune system of the kids.

CONCLUSION

Our results conclude a seroprevalence (4.71%) of contagious caprine pleuropneumonia in the goat of Bharatpur Metropolitan, Chitwan conducted in 106 goats. There was a variation in the seroprevalence level among the age and sex. Female goats were found to be more susceptible to CCPP than the male goats and young goats below or equal to 5 months were more susceptible to CCPP than the goats above 5 months in this study. Since there is no vaccination practice for CCPP in Nepal, seropositivity results might be from natural infection of the causative agent. As this is the first research conducted in Chitwan, Nepal for CCPP, it will provide a base for further surveillance and monitoring in the future.

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AUTHOR'S CONTRIBUTION

LBR: Conceptualized and designed the study, LBR, SP: collected data and samples, SP and DS: drafted the manuscript, DS and RA: Revised the manuscript, RA: Supervised the study. All authors read and approved the final manuscript.

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were carried out as part of routine tests in the Central Veterinary Laboratory (CVL), Kathmandu, Nepal.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Abegunde, T.A., 1981. A serological survey of *Mycoplasma putrefaciens* infection in goats. *American J. Vet. Res.*, 42(10): 1798-1801.
2. Adhikari, B. K., Subedi, D., Jyoti, S., Kaphle, K., Kharel, C. N., & Khanal, D. R. (2022). Seroprevalence of contagious caprine pleuropneumonia (CCPP) in Rupandehi and Palpa Districts of Nepal. *Veterinary Sciences: Research and Reviews*, 8(1), 23-29. <https://dx.doi.org/10.17582/journal.vsr/2022.8.1.23.29>
3. Ahaduzzaman, M.D., 2021. Contagious caprine pleuropneumonia (CCPP): A systematic review and meta-analysis of the prevalence in sheep and goats. *Transbound Emerg Dis.*, 68(3): 1332-1344. <https://doi.org/10.1111/tbed.13794>
4. Ahuya, C.O., Okeyo, A.M., Mwangi-Njuru, Peacock, C., 2005. Developmental challenges and opportunities in the goat industry: The Kenyan experience. *Small Rumin. Res.*, 60(1-2): 197-206. <https://doi.org/10.1016/j.smallrumres.2005.06.013>
5. Asmare, K., Abayneh, T., Mekuria, S., Ayelet, G., Sibhat, B., Skjerve, E., Szonyi, B., Wieland, B., 2016. A meta-analysis of contagious caprine pleuropneumonia (CCPP) in Ethiopia. *Acta Tropica.*, 158: 231-239. <https://doi.org/10.1016/j.actatropica.2016.02.023>
6. Awan, M.A., Abbas, F., Yasinzai, M., Nicholas, R.A.J., Babar, S., Ayling, R.D., Attique, M. A., Ahmed, Z., Wadood, A., Khan, F. A., 2010. First report on the molecular prevalence of *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) in goats the cause of contagious caprine pleuropneumonia (CCPP) in Balochistan province of Pakistan. *Mol. Biol. Rep.*, 37(7): 3401–3406. <https://doi.org/10.1007/s11033-009-9929-0>
7. Bascunana, C.R., Mattsson, J.G., Bolske, G., Johansson, K.E., 1994. Characterization of the 16S rRNA genes from *Mycoplasma* sp. strain F38 and development of an identification system based on PCR. *J. Bacteriol. Res.*, 176(9). <https://doi.org/10.1128/jb.176.9.2577-2586.1994>
8. Bekele, T., Asfaw, Y., Gebre-Egziabeher, B., Abebe, G., 2011. Seroprevalence of contagious caprine pleuropneumonia in Borana and Guji lowlands, Southern Ethiopia. *Ethiop. vet. j.*, 15(2): 69-76. <https://doi.org/10.4314/evj.v15i2.67695>
9. Boyazoglu, J., Hatziminaoglou, I., Morand-Fehr, P., 2005. The role of the goat in society: Past, present and perspectives for the future. *Small Rumin. Res.*, 60(1-2):13-23. <https://doi.org/10.1016/j.smallrumres.2005.06.003>
10. Gupta, D.K., Shukla, P.C., Tiwari, A., Baghel, R.P.S., Sharma, V., Shivhare, J., Gupta, N., 2016. Seroprevalence Study on Goat Contagious Caprine Pleuropneumonia in Jabalpur, Madhya Pradesh. *J Anim Res.*, 6(4): 743-746. <https://doi.org/10.5958/2277-940x.2016.00092.9>
11. Iqbal Y.M., Raffiq P.O., Tauseef B.S., Muheet, A.B.R., Gopalakrishnan, A., Karthik, K., Dhama, K., Vir Singh, S., 2019. Contagious caprine pleuropneumonia—a comprehensive review. *Vet Q.*, 39(1): 1-25. <https://doi.org/10.1080/01652176.2019.1580826>
12. Jain, U. D. I. T., Verma, A. K., & Pal, B. C. (2015). Occurrence of mycoplasma infection in Barbari goats of Uttar Pradesh, India. *Haryana Vet*, 54(1), 53-55.
13. MacOwan, K.J., Minette, J.E., 1976. A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. *Trop. Anim. Health Prod.*, 8(1). <https://doi.org/10.1007/BF02383376>

14. Manso-Silván, L., Dupuy, V., Chu, Y., Thiaucourt, F., 2011. Multi-locus sequence analysis of mycoplasma capricolum subsp. capripneumoniae for the molecular epidemiology of contagious caprine pleuropneumonia. *Vet. Res.*, 42(1): 1-10. <https://doi.org/10.1186/1297-9716-42-86>
15. Parray, O.R., Yattoo, M.I., Bhat, R.A., Malik, H.U., Bashir, S.T., Magray, S.N., 2019. Seroepidemiology and risk factor analysis of contagious caprine pleuropneumonia in Himalayan Pashmina Goats. *Small Rumin. Res.*, 171: 23-36. <https://doi.org/10.1016/j.smallrumres.2018.12.004>
16. Radostits, O. M., Gay, C. C., Blood, D. C., Hinchcliff, K.W., 2000. A textbook of the diseases of cattle, sheep, pigs, goats and horses. *Vet. Med.*, 9: 603-700.
17. Shahzad, W., Yaqoob, T., Mukhtar, N., Munir, R., Ahmad, R., Khan, M.S., Hussain, A. 2016. Seroprevalence of mycoplasma capricolum subsp. Capripneumoniae in goats through celisa in different districts of Punjab, Pakistan. *J Anim. Plant Sci.*, 26(4).
18. Suryawanshi, S.N., Tembhurne, P.A., Gohain, S., Kesharkar, J.A., Tumlam, U. M., Ingle, V.C., 2015. Seroprevalence of contagious caprine pleuropneumonia in small ruminants in Maharashtra. *The Ind. J. Vet. Sci. Biot.*, 10(4).
19. Thiaucourt, F., Bölske, G., Libeau, G., Le Goff, C., Lefèvre, P.C., 1994. The use of monoclonal antibodies in the diagnosis of contagious caprine pleuropneumonia (CCPP). *Vet. Microbiol.*, 41(3):191-203. [https://doi.org/10.1016/0378-1135\(94\)90100-7](https://doi.org/10.1016/0378-1135(94)90100-7)
20. Thrusfield, M., Christley, R., Brown, H., Diggle, P.J., French, N., Howe, K., Kelly, L., O'Connor, A., Sargeant, J., Wood, H., 2017. *Veterinary Epidemiology: Fourth Edition*. <https://doi.org/10.1002/9781118280249>

Sugarcane Derived Polyphenol Feed Additive Supplemented to the Wheat Diet Improved Broiler Performance

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ABSTRACT

This study examined effects of incremental levels of sugarcane derived polyphenol on most commonly used cereals in the poultry feed industry; wheat and maize. Both cereals rapidly digestible starch, which is usually digested completely than protein itself whereas polyphenol slows down glucose absorption in rat without compromising feed intake. Polyphenol may enhance depositing of muscle in broilers due to synchronous availability of the plasma glucose and amino acid. In this experiment, 240 Ross 308 male broilers (5 birds/cage; n=6) were given 8 different diets, wheat and maize; without or with polyphenol inclusion level at 0.5%, 2% and 4% respectively. Feeds were given ad-libitum in 2 phases as grower (day 11-24) and finisher (day 24-38). Parameters assessed were body weight (BW), average daily feed intake (ADFI), feed conversion ratio (FCR), drip loss%, shear force and meat color. Birds were euthanized at day 38 for meat quality inspection using Pectoralis muscle. The research revealed that 2% polyphenol in wheat diets significantly reduced ADFI (day 24-38) as compared to unsupplemented wheat diets ($p<0.0001$). At the same level, polyphenol numerically improved FCR (day 24-38) in broilers fed wheat diets (FCR 1.93) and maize diets (FCR 1.63) as compared to unsupplemented wheat diets (FCR 1.98) and maize diets (FCR 1.70) respectively. However, 4% polyphenol inclusion level had adverse effect on BW (day 17 and 38) and ADG (day 10-38, day 24-38) in broilers fed wheat diets as compared to unsupplemented wheat diets. On other hand, maize diets supplemented with different level of polyphenol did not have any significant effect on BW, ADG, ADFI and FCR in broilers as compared to unsupplemented maize diets. Also, there was significantly higher ADFI (day 24-38) in broilers fed wheat diets as compared to maize diets ($p<0.0001$). Regarding meat qualities, there were not any effect of polyphenol on drip loss%, shear force and meat color. However, cereal type itself had significant effect on meat color appearance as maize fed broilers had more yellowness value of meat (b^ ; $p<0.0001$) than broilers fed wheat diets. In conclusion, polyphenol supplementation found to be useful for broilers mainly on wheat diets at 2% inclusion level.*

Keywords: FCR, Drip loss%, Meat color, Polyphenol, Ross 308

INTRODUCTION

Wheat and maize are most commonly used cereals in the poultry feed (Selle et al., 2003). Both of them have rapidly digestible starch which is usually digested completely (Weurding. R et al., 2003, Liu et al., 2013b) than protein. It is suggested that feed efficiency can be improved by slowing down the starch digestibility and accelerating protein digestibility due to higher protein and energy utilization in broilers at the site of muscle synthesis (Giuberti et al., 2013, Liu et al., 2013b, Selle et al., 2015). Polyphenol, on other hand, has shown to slow down glucose absorption in rat without affecting the feed intake. Polyphenol can play a role for synchronous availability of plasma glucose and amino acid for depositing muscle protein efficiently in broilers as suggested by Geiger (et al., 1950), Weurding. R (et al., 2003), and Liu (et al., 2013).

Besides, there are evidences from animal and human studies that dietary polyphenols have useful gut modulatory effects. It has been observed that polyphenol improves villus height: crypt depth ratio in the duodenum in piglet (Sehm et al., 2006). This is in agreement with broiler's research by Viveros (et al., 2010) where there was an improvement of villus height in the small intestine due to polyphenol rich products. Moreover, Caspary (et al., 1992) had demonstrated that increased villus height leads to an improvement of gut function as a result of increased absorption surface, expression of brush border enzyme and nutrient transport mechanism. Additionally, polyphenol has shown to inhibit proliferation of pathogens in gastrointestinal tract of human (Duda. A et al., 2012).

At molecular level, dietary polyphenol can reduce inflammation (Blanch. C et al., 2012) through modulating nuclear factor kappa (NF- κ B). In several studies, flavonoid-rich products have shown anti-inflammatory effect both in vivo and in vitro (Gessner et al., 2013). In addition, polyphenol can improve growth performance and beneficial fatty acids level whereas it can also reduce lipid oxidation and cholesterol value in broilers as mentioned by Starcević. K (et al., 2014). Polyphenols have shown to minimize the adverse reaction of lipid peroxidation by decreasing the level of malondialdehyde (MDA) and improving antioxidant status in blood and tissue (Labban, L et al., 2014) in human. Jung (et al., 2010) reported that gallic acid which is a polyphenol found in grape, wine and tea improved nutritional value of poultry meat by increasing arachidonic acid and docosahexanoic acid level and also, water-holding capacity of breast meat in broilers. Likewise, dry rosemary leaves and rosemary oil; rich in polyphenol found to improve sensory value of meat and significantly decreased the malondialdehyde concentration (Yesilbag et al., 2011) in chicken breast muscle. In another experiment, hesperidin and genistein supplemented on broiler ration enhanced the fatty acid profile (PUFA, n-6:n-3 PUFA ratio) of breast muscle (Kamboh and Zhu et al., 2013) in poultry.

However, Voljc (et al., 2013) suggested that addition of only 3 gm per kg of a product containing polyphenol was not sufficient to prevent the adverse effect of lipid peroxidation

in the broiler meat. Furthermore, Viveros (et al., 2011) had observed negative effect of polyphenol on body weight in 21-days old chickens whose diets were supplemented with grape seed extract at the level of 7.2 gm per kg feed as compared to birds from other level of supplementation. Besides, significant drop in FCR (1.43 vs 1.51) was also reported in chicken fed grape pomace at the rate of 60 g per kg of feed.

It was speculated that sugarcane derived polyphenol supplementation on wheat diet improves growth performance and meat quality in broilers. Wheat is rich in viscous, non-starch polysaccharide (Xylan) which has shown to affect growth performance in poultry at higher degree as compared to less viscous diet like maize (Marquardt. et al., 1994, Jia et al., 2009, Rodriguez et al., 2012). The higher transit time and lower intestinal passage rate in birds due to viscous digesta ascribed to wheat diets may provide polyphenol advantage to act as antioxidant efficiently, and may even improve function of intestinal villi and enzymatic action, thus enhancing the feed efficiency. The objective of this research was to find out optimum inclusion level of sugarcane derived polyphenol supplement to improve broiler's performance and meat quality.

MATERIALS AND METHODS

This study was carried out from June 2019 to September 2019. The experiment was conducted at the University of Sydney, Australia on behalf of Poultry Research Foundation. Later, the raw data was analyzed at the Department of Bioscience at the University of Nottingham, UK. Meanwhile, all birds were kept in battery cage system throughout research period at the Poultry Research Foundation. All procedures used in this research were approved by the Animal Ethics Committee of The University of Sydney and were in accordance with Australian Code for the care and use of animals for scientific purpose.

Experimental design and dietary treatments

The research was conducted as a completely randomized design; comprising two different cereals (wheat and maize) as a source of energy along with four inclusion level of polyphenol (0%, 0.5%, 2% and 4%) making a total 8 treatment diets as shown in Table 1. Thus, it was 2×4 factorial array of dietary treatments offered to 240 Ross 308 male broilers (5 birds/cage; n=6). Chicks had free access to water and feed. Chicks were provided common wheat-soybean based starter diet until day 10. Afterwards, broilers were offered experimental grower diets from day 11 to 24, and finisher diets from day 24 to 38 as shown in Table 2. The sugarcane derived polyphenol product had a syrup like consistency and contained 3.5 g polyphenol per kg product. All diets were formulated to satisfy nutrients requirement as per the recommendation of Ross 308 guidelines. Exogenous enzymes were not added, and energy value were adjusted with soybean oil. Digestible amino acid level was adjusted with canola meal, soybean meal and synthetic amino acids. Average body weight (BW), average daily gain (ADG), average daily feed

intake (ADFI) and feed conversion ratio (FCR) were recorded on a weekly interval basis. The FCR values were adjusted for body weight of bird that died during research period. Table 1. Experimental diets based on growth stage, cereal type and polyphenol inclusion level

Diets	Stage	Cereal Type	Polyphenol %
1	Grower / Finisher	Wheat-based diet	0%
2	Grower / Finisher	Wheat -based diet	0.5%
3	Grower / Finisher	Wheat- based diet	2%
4	Grower / Finisher	Wheat-bdased diet	4%
5	Grower / Finisher	Maize-based diet	0%
6	Grower / Finisher	Maize-based diet	0.5%
7	Grower / Finisher	Maize-based diet	2%
8	Grower / Finisher	Maize-based diet	4%

Table 2. Dietary formulation, calculated and analyzed nutrient composition of the diets based on maize or wheat for broiler chicken from 11 to 38 days' post- hatch

Ingredient	Wheat grower diet (11-24 days post hatch)	Wheat finisher diet (24-38 days post hatch)	Maize grower diet (11-24 days post hatch)	Maize finisher diet (24-38 days post hatch)
Wheat/Maize	590	689.5	558	605
Soyabean meal	272	152	281	240
Canola meal	37	45	66.5	40
Vegetable oil	36	54	32	56
Limestone	9	8.45	8	8.35
Dicalcium Phosphate	21	16	20	16
Salt	1.5	1.3	1.5	1.3
Sodium Bicarbonate	5.25	4.6	5.25	4.6
Lysine Hcl	2.75	3.25	2.45	3.25
Methionine	2.7	2.4	2.5	2
Celite	20	20	20	20
Threonine	0.8	1.5	0.8	1.5
TMV premix	2	2	2	2
TOTAL	1000	1000	1000	1000
ME (MJ/Kg)	12.44	12.99	12.44	12.99
CP%	22.2	18.5	22.2	18.5

Meat quality assessment

Birds were sacrificed when they reached day 38 following the recommendation of euthanasia of experiment animals. Meat quality was determined taking one bird per cage. To measure drip loss%, approximately 25 g of samples were taken, trimmed off any noticeable fats and weighted and then calculated as weight of the fresh meat sample (g) minus weight of the sample stored at 4°C at 7 days' post-slaughter which was later expressed into percentage. Meat color was determined on day 0 and day 7 post-slaughter using CIELAB method (International Commission on Illumination, 1976) where triplicate color measurement for Lightness (L^*), redness (a^*) and yellowness (b^*) of the Pectoralis muscle were assessed using a Minolta Lab CR-10 colorimeter. Using a Warner Bratzler Shear attachment on a Stable Micro Systems TAXT2 Texture Analyzer, shear force value was also calculated.

Statistics

Data were tested for normality using the univariate procedure of SAS (SAS Inst. Inc., Cary, NC) and was analysed as a completely randomized design incorporating 8 dietary treatments. The cage served as the experimental unit for growth performance data and one bird per cage for the meat quality variables. Data were analysed using the generalized linear model procedure of SAS. Preplanned contrasts for growth performance and a meat quality variables were evaluated between cereal types (unsupplemented Wheat vs Maize) and polyphenol level (0.5%, 2% or 4% versus 0% polyphenol). Finally, data are presented as least square means $\pm$ standard error of the mean (sem). Differences were considered significant at $P < 0.05$.

RESULTS

Growth performance

Wheat fed broilers had higher FCR than maize fed birds ($p < 0.001$) in last week (day 24-38) which was due to higher ADFI (day 24-38) in wheat fed birds. Both wheat and maize fed birds gained similar BW at day 38 ($p = 0.018$) (Table 3). It has been observed that higher ADFI (day 24-38) was improved by polyphenol at 2% level of inclusion in wheat diets as compared to unsupplemented wheat diets ($p < 0.0001$). However, maximum supplement level (4% polyphenol) on wheat diets had adverse effect on BW (day 17 and 38) and ADG (day 10-38, day 24-38) as compared to unsupplemented wheat diets. Contrary to this, maize diets supplemented with different level of polyphenols didn't have any significant effect on BW, ADG, ADFI and FCR in broilers as compared to unsupplemented maize diets. Overall, 2% polyphenol inclusion level numerically improved FCR at day 24-38 ($p < 0.001$) in both wheat and maize fed broilers as compared to the unsupplemented wheat and unsupplemented maize diets respectively.

Table 3. Effect of polyphenol on body weight (BW), average daily gain (ADG) and feed conversion ratio (FCR) in broilers from 10- 38 days post-hatch fed wheat and maize diets

Parameter	Wheat diet 0% Pp	Wheat diet 0.5% Pp	Wheat diet 2% Pp	Wheat diet 4% Pp	Maize diet 0% Pp	Maize diet 0.5% Pp	Maize diet 2% Pp	Maize diet 4% Pp	SEM	P-value
BW d10	396	385	366	365	380	363	374	372	10	0.238
ADG d10-17	68bc	65b	64b	67bc	71ac	74a	70ac	69bc	2	0.003
ADFI d10-17	73	74	74	72	74	75	74	73	1	0.791
FCR d10-17	1.06b	1.14a	1.14a	1.06b	1.03b	1.01b	1.04b	1.06b	0.02	0.002
BW d17	875cd	838abc	817a	831ab	877cd	883d	866bcd	852abcd	15	0.019
ADG d17-24	99	97	100	94	99	98	98	97	2	0.451
ADFI d17-24	163	161	156	153	165	160	157	158	6	0.850
FCR d17-24	1.63	1.66	1.56	1.62	1.65	1.62	1.59	1.62	0.05	0.908
BW d24	1571	1518	1514	1490	1573	1570	1554	1533	23	0.103
BW d32	2461	2418	2354	2295	2507	2541	2537	2496	62	0.071
BW d38	3184bc	3074ab	3045ab	2983a	3155bc	3162bc	3239c	3166bc	50	0.018
ADG d32-38	120	109	1155	115	108	103	117	112	8	0.871
ADG d10-38	100bc	96ab	96ab	94a	99bc	100bc	102c	100bc	2	0.029
ADG d24-38	115bc	111ab	109ab	107a	113abc	114abc	120c	117bc	3	0.040
ADFI d24-38	229a	224ab	211bc	217ab	193d	194d	196d	199cd	4	<.0001
FCR d24-38	1.98a	2.01a	1.93a	2.04a	1.70b	1.70b	1.63b	1.71b	0.03	<0.001

a, b, c, d: means within columns not sharing common letters are significantly different ($P < 0.05$)

Meat quality

As shown in Table 4, there were not effect of polyphenol on drip loss%, shear force, and meat color. However, dietary cereal type itself had significant effect on the meat color. At day 0 post-slaughter, the unsupplemented wheat fed chicken had more L*value than unsupplemented maize diets ($p=0.04$). In addition, at day 0 ($p<0.0001$) and day 7 ($p<0.001$) post-slaughter, cereal type had significant effect on the yellow color trait of the meat where maize fed broilers had higher b* value (more yellowness color of meat) than wheat fed broilers.

Table 4. Effect of cereals and polyphenol (Pp) inclusion level on drip loss (%), shear force (N) and meat color (day 0 and day 7) in broilers post-slaughter

Parameter	Wheat diet 0% Pp	Wheat diet 0.5% Pp	Wheat diet 2% Pp	Wheat diet 4% Pp	Maize diet 0% Pp	Maize diet 0.5% Pp	Maize diet 2% Pp	Maize diet 4% Pp	SEM	P-value
Drip loss (%)	5.01	5.37	7.58	4.75	6.67	6.98	5.97	6.22	0.91	0.345
Shear force (N)	35.8	36.4	32.3	47.3	35.3	36.5	35.5	34.7	4.34	0.412
Meat color (Day 0)										
L*	54.56ab	52.4bc	56.3a	54.63ab	50.85c	50.99c	52.14bc	52.71abc	1.27	0.04
a*	2.49	2.67	1.78	3.38	2.24	2.74	2.13	1.90	0.37	0.08
b*	3.61a	3.47a	4.29a	4.55a	9.32b	8.24b	9.28b	9.39b	0.73	<0.001
Meat color (Day 7)										
L*	54.06	51.12	54.73	53.29	51.78	52.40	51.27	50.61	1.3	0.28
a*	2.19	2.18	1.56	2.51	1.71	2.23	1.85	2.08	0.34	0.57
b*	6.04a	5.83a	6.88a	6.95a	11.01b	10.87b	10.48b	10.69b	0.68	<0.0001

a, b, c: means within columns not sharing common letters are significantly different ($P < 0.05$) / L*-Lightness, a*-redness, b*-yellowness

DISCUSSION

It is well documented that feed ingredients rich in soluble non-starch polysaccharide (NSP) like wheat diet results in poor performance in broilers as compared to less viscous diet like maize. Moreover, such adverse effect has been reported higher on young species (Kalmendal et al., 2011). In this research, there was no effect of cereal type (unsupplemented maize and wheat) during early growing phase of broilers from day 10 to 17 in terms of ADG and FCR. This contrasts to the findings by Marquardt (et al., 1994), Jia (et al., 2009) and Rodriguez (et al., 2012). This outcome on early growth performance might appeared due to the fact that all broilers were given same basal diets, wheat-soybean meal starter, at the beginning of trial. It was only after day 10 that maize and wheat diets were given separately to the experimental groups. Therefore, it is likely that experimental diets might have taken time to have impact on birds.

There was increased ADFI (day 24-38) in broilers fed unsupplemented wheat diets which in turn had increased FCR as compared to unsupplemented maize fed broilers. Similar observation was reported by Peng (et al., 2003) where birds fed wheat diets had higher

FCR than maize fed birds and it was reported due to higher NSP content of the wheat diets. On day 38, both wheat and maize fed broilers attained similar BW ($p=0.018$). Therefore, it may be concluded that broilers on wheat diets were compensating nutrients requirement by increasing feed intake to meet its genetic potential.

Wheat diets supplemented with maximum level of polyphenol i.e. 4% had negative effect on BW (day 17, day 38) and ADG (day 10-38, day 24-38) as compared to unsupplemented wheat diets. Similar outcomes have been reported by Jansman (et al., 1989) and Nyachotti (et al., 1997) stating relatively higher dietary concentration of polyphenol may reduce performance. On other hand, wheat diets containing 2% polyphenol level had beneficial effect on broilers. At this level, there was decreased ADFI (day 24-38); without getting affected ADG (day 24-38) and final BW (day 38) as compared to unsupplemented wheat diets. At this level, polyphenol might have increased villus height leading to an improvement of gut function as a result of increased absorption surface, expression of brush border enzyme and nutrient transport mechanism as suggested by Duda. A (et al., 2012).

It is also concluded that 4% polyphenol inclusion level on wheat diets had most detrimental effect on broilers. Furthermore, it might be concluded that polyphenol gets tightly regulated when supplemented to wheat diet ascribed to the viscosity of digesta due to soluble NSP. As suggested by Jansman (et al., 1989), Ortiz (et al., 1993) and Surai. P.F (et al., 2014) excess polyphenol in wheat diets may have been poorly absorbed, quickly transformed into range of metabolites which may have formed complexes with lipoprotein due to its highly reactive hydroxyl group, ultimately reducing nutrient digestibility in presence of viscous intestinal digesta. Further research is required to understand the relationship in between polyphenol, cereal type and its effect in terms of dose.

In addition, maize diets supplemented with different levels of polyphenol did not have any effect in broilers as compared to unsupplemented maize diets. However, maize diets supplemented with 2% polyphenol inclusion level numerically improved FCR (1.63 vs 1.7) at day 24-38 as compared to unsupplemented maize diets. Broilers fed maize diets may have higher nutrients absorption in intestine, thus increasing opportunities for nutrient utilization as maize is comparatively less viscous and lower in fiber to digest (Jacob. J et al., 2015).

Overall, supplementation of polyphenol had multiple effects on broilers. Similar outcomes have been reported by Rohn (et al., 2006), Brenes (et al., 2010), and Chamorro (et al., 2013). It has been also stated (Hodek et al., 2002) that the physiological effects of polyphenol not only depend on one but various factors like type/subtype, concentration, absorption, metabolic transformation of polyphenol. The average FCR of commercial male Ross 308 (day 24-38) according to the Ross Performance Objective Guideline (2019)

is 1.74. As sugarcane derived polyphenol, at 2% inclusion level, numerically lowered the FCR in both diets, and in maize diets it is even lower than Ross Guideline, there is need for research whether it is achievable so in farm condition.

Regarding meat qualities, there were no effect of polyphenol and cereal types on drip loss% and shear force. This contrast with finding by Ao and Choct (et al., 2004) who reported that wheat diets fed group of birds had lower drip loss% value than maize fed birds. As suggested by Mir. N (et al., 2017) broiler chicken meat quality is dependent on multiple factors, thus a very complex process. Absence of effect on drip loss% and shear force when compared in between wheat and maize fed birds in this study might have happened due to muscle pH, temperature, glycogen, stress level to which chickens were exposed were similar for all treatment groups before and after slaughter.

Lastly, cereal type itself had significant effect on color trait of the meat in this study where b^* (yellowness) value was higher in broilers fed maize diets as compared to the wheat diets. This outcome is supported by Smith (et al., 2002) who stated that wheat fed birds produce paler meat than from the maize fed birds. This result may be due to pigments (xanthophylls and carotene) present in maize diets. Color of meat is a valuable attribute as consumers think yellow meat is reasonably healthy (Sunde et al., 1992). Consumers prefer broilers meat with more yellow color (Mateo and Carandang et al., 2006) and hence this can affect broiler meat purchase decision (Garcia et al., 2013). However, limited studies have been conducted correlating the color and nutritional value of the poultry meat.

CONCLUSION

Phytogenic feed additives like sugarcane derived polyphenol supplementation in the poultry feed have drawn interest of animal nutritionists; with more companies manufacturing such products globally. In this research, polyphenol supplementation has not only shown to be beneficial, but also proven to be detrimental with higher inclusion rate. On other hand, interaction of typical polyphenol with various feed ingredients to be used in feed formulation is largely unknow. Their metabolites and consequent effect on microflora with various farm mangement condition is yet to be explore as future research directive. Lastly, the cost-benefit or return on investment analysis should be major criteria for incorporating such exogenous products.

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REFERENCES

1. Hafsa. A. (2016). Effect of dietary polyphenol-rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. *Journal of Animal Physiology and Animal Nutrition* 2018, 102, pp 268-275. 2017 Blackwell Verlag GmbH. doi: 10.1111/jpn.12688
2. Liu. SY. (2011). Modelling starch digestion in sweet potato with biphasic digestograms. *Journal of Food Engineering* 2011, 104, 307–315.
3. Lipinski. K. (2017). Polyphenols in monogastric nutrition- a review. *Journal of Annual Animal Science* 2017,1, pp 41-48.
4. Liu. SY. (2013a). Protease supplementation of sorghum-based broiler diets enhance amino acid digestibility coefficients in four small intestinal sites and accelerates their rate of digestion. *Journal of Animal Feed Science and Technology* 183, 175-183.
5. Akter. Y. (2019). Optimization and investigations into the effect of a phosphorylated tocopherol mixture on growth performance, meat quality and plasma inflammatory biomarkers in broilers. *Journal of Animal Feed Science and Technology* 2019, 253, 181-190.
6. Liu. SY. (2013b). Strategies to enhance the performance of pigs and poultry on sorghum-based diets. *Journal of Animal Feed Science and Technology*, 181, 1-14.
7. Liu. SY. (2014). A combination of xylanase, amylase and protease influences growth performance, nutrition utilization, starch and protein digestive dynamics in broiler chickens offered maize-sorghum- and wheat-based diets. *Animal Production Science* 2015, 55, 1255-1263.
8. Yang. JY. (2016). Effects of dietary grape proanthocyanidins on the growth performance, jejunum morphology and plasma biochemical indices of broiler chicks. *Animal* (2017), 11, 5 pp 762-770, *The Animal Consortium* 2016. doi: 10.1017/S1751731116002056.
9. Liu. SY. (2013c). The kinetics of starch and nitrogen digestion regulate growth performance and nutrient utilization in coarsely- ground, sorghum-based broiler diets. *Animal Production Science* 53, 1033–1040.
10. Ahmad. A. (2013). Effect of black tea extract (polyphenol) on performance of broilers. *International Journal of Advanced Research* (2013),1, 7, 563-566.
11. Gessner. D.K. (2013). Supplementation of a grape seed and grape marc meal extract decreases activities of the oxidative stress-responsive transcription factors NF- κ B and Nrf2 in the duodenal mucosa of pigs. *Acta Vet Scand* 55, 18 (2013). doi: 10.1186/1751-0147-55-18
12. Surai. P.F. (2013). Polyphenol compounds in the chicken/animal diets: from the past to the future. *Journal of Animal Physiology and Animal Nutrition* (2014), 98, pp 19-31, 2013 Blackwell Verlag GmbH. doi: 10.1111/jpn.12070
13. Kim. Y. (2016). Polyphenols and glycemic control. *Nutrients* 2016, 8(1): 17. doi:10.3390/nu8010017
14. Karunaweera. N. (2015). Plant polyphenols as inhibitors of NF- κ B induced cytokine production—a potential anti-inflammatory treatment for Alzheimer’s disease? *frontier in Molecular Neuroscience* 2015, 8, 24. doi:10.3389/fnmol.2015.00024
15. Liu. SY. (2013d). Influence of white- and red-sorghum varieties and hydrothermal component of steam-pelleting on digestibility coefficients of amino acids and kinetics of amino acids, nitrogen and starch digestion in diets for broiler chickens. *Animal Feed Science and Technology* 186, 53–63.
16. Sehm. J. (2006). The influence of polyphenol rich apple pomace or red-wine pomace diets on the gut morphology in weaning piglets. *Journal of Animal Physiology & Animal Nutrition* 91, (7-8):289-96. doi: 10.1111/j.1439-0396.2006.00650.x
17. Viveros. A. (2010). Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science* 90 (3): 566-578.
18. Caspary. W. (1992). Physiology and pathophysiology of intestinal absorption. *American Journal of Clinical Nutrition* 55 (1 Suppl):299S-308S.
19. Duda. A. (2012). Inhibitory effect of polyphenol on human gut microbiota. *Journal of Physiology and*

- Pharmacology 2012, 63, 5, 497-503.
20. Giuberti. (2013). Factors affecting starch utilization in large animal food production system: A review. *Starch/Stärke* 2014, 66, 72–90.
 21. Liu. SY, Selle. P. (2015). A consideration of starch and protein digestive dynamics in chicken-meat production. *World's Poultry Science Journal* 71(2), 297-310.
 22. Gutierrez. A. (2009) . Wheat starch digestion rate affects broiler performance. *Poultry Science* 88, 1666–1675.
 23. Nian. F. (2011). Effect of exogenous xylanase supplementation on the performance, net energy and gut microflora of broiler chickens fed wheat-based diets. *Asian-Australasian Journal of Animal Sciences* 24, 400–406.
 24. Weurding. R.E. (2003). The relation between starch digestion rate and amino acid level for broiler chickens. *Poultry Science* 82, 279–284.
 25. Svihus. B. (2011). The gizzard: function, influence of diets structure and effects on nutrient availability. *World's Poultry Science Journal* 67, 207–223.
 26. Siriwan. P. (1993). Measurement of endogenous amino-acid losses in poultry. *British Poultry Science* 34, 939–949.
 27. Selle. PH. (2011). The protein quality of sorghum. *Proceedings of Australian Poultry Science Symposium* 22, 147–160.
 28. Cant. J.P. (1996). The regulation of intestinal metabolism and its impact on whole animal energetics. *Journal of Animal Science* 74, 2541–2553. doi: 10.2527/1996.74102541x

Evaluation of Condition of Meat Shop on Prospective of Meat Act in Madi Municipality, Chitwan, Nepal

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ABSTRACT

There are many meat shop around the country and only few are maintaining standard according to animal slaughter house and meat inspection act, 2057. We are trying to evaluate standard of 122 meat shop of Madi Municipality. We have prepared a questionnaire and surveyed all the meat shop to know the condition of shop and their standard with reference to animal slaughter house and meat inspection act, 2057. Meat shop in Madi is dominated by chicken, 85.71%. Which are equipped with D-freeze (60.65%) for freezing meat for a day with 91.22% having backup support for freezers. 70.08% of the shops are registered as mentioned by act. Similarly, only few (7.09%) of the shop have area met the requirement given by act. Ventilation in 59.02% of the shops are perfectly in line with act whereas tiles in the floor was absent in 83.61% of the shops. Good water supply and Slab in butchering area is in minimal with 8.20% and 4.10% respectively. Good condition mesh wire is present in order to prevent flies in 19.67% of the shops. Furthermore, only 1.64% of the butcher are aware about rules and regulations and 11.48% of the shop uses proper disinfection. Similarly, waste management is mostly done by making manure (28.69%) followed by burning (27.05%). Additionally, consumer's preference is in fresh meat (79.51%) among which demand of chest meat is higher (52.46%) in chicken meat. In conclusion, most of the butcher are unaware of act and are doing their business without any knowledge of act and are not in line with the instructions given by the act.

Keywords: Act, Madi, Meat inspection, Meat shop

INTRODUCTION

Animal means castrated or castrated goat, sheep, Himalayan goat (*Chyangra*), pig, wild pig, he buffaloes or rabbit the females of buffalo, goat sheep, Himalayan goat, pig, or rabbit which are fit for meat other than a cow, an ox a bull, and this word also includes poultry, ducks, pigeon or other species of beasts and birds kept for meat purpose. "Meat" means meat of animals which is fit for human consumption (SMIA-2001). In developing

countries Food Borne Disease and Zoonosis are very common due to poor handling and practices of hygiene, poor food safety law and regulatory bodies, lack of financial resources to invest on well safety equipment and lack of education for food handler (WHO, 2004).

Meat shop and slaughter house is one of the major parts of meat value chain which determines the hygiene of meat products and their quality. According to fish, meat and dairy shop planning and management standard, 2077, there are several criteria which indicate that there must be at least 100 square feet area for small retailer meat shop (MLMAC, 2020).

The animal slaughter house and meat inspection act-2057 requires below conditions to fulfilled: nobody shall establish a slaughterhouse or become a meat seller without obtaining license under this act; there shall be at least 100 sq. meter area for meat shop; there shall be at least 6 feet wall covered with white tiles and floor shall be non-slippery, easy to clean tiles or marble; for culling purpose, there shall be refrigerator with digital display or the D-freeze; equipment/tools which are used in shop shall be of stainless steel; well ventilated shop with fly and other animal preventable mesh wire/window/door shall be present; shop shall have enough clean water, good waste management system and no leakage of water on shop; alternative electricity resource shall be present such as generator or solar; employee who worked in the shop shall wore an apron, gloves, mask, head cover and boot; different meat shall be stored and sold separately and meat cutting slab shall be clean and covered when not in use.

However, this act is yet to be effectively implemented in Nepal. Unhygienic practices of meat shops, inadequate sanitation, unclean water, unskilled manpower, lack for tools for preservation meats, lack of separate areas for different types of meat are major problems of meat shops of Nepal (Bhattaria et.al., 2017). This study was carried out to find condition of meat retailer prevails in Madi valley.

MATERIALS AND METHODS

This study was carried out from February 2022 to May 2022 in retail meat shops in Madi, Chitwan, Nepal. Purposive sampling was done in all retail meat shops (n=122) of Madi municipality. In order to understand the state of the meat shops and their standards in relation to the animal slaughter house and meat inspection act-2057, we devised a questionnaire and assessed every meat shop.

This survey was done to know the status of meat shops. Madi municipality was chooses for the survey because it is a potential area for the production of livestock, because it is surrounded by Chitwan national park and there is larger community forest for the grazing and there is large grassland for the production of livestock. Data were entered

and analyzed into Microsoft Excel 2016.

RESULTS

This study revealed that only 29.92% (Figure 1) of the people have registered their shop among 122 shops and maximum of the total shop i.e., 91.22% (Figure 2) have backup facility for keeping meat in managed cool condition.

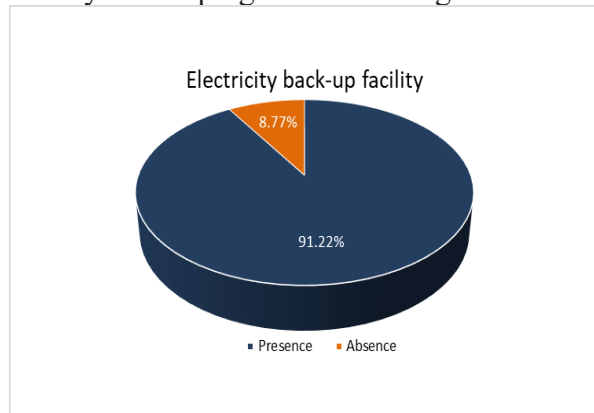


Figure 1. Registration of Shop

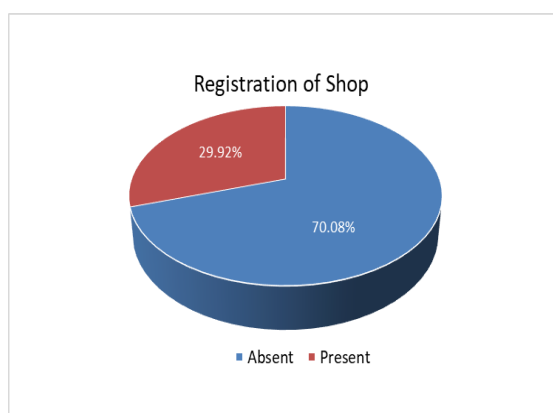


Figure 2. Back-up facility

Figure 3 indicated that the meat shop in Madi is dominated by chicken (85.71%) followed by Mixed type (4.51%) of shop and only 0.75% was occupied by pork meat shop.

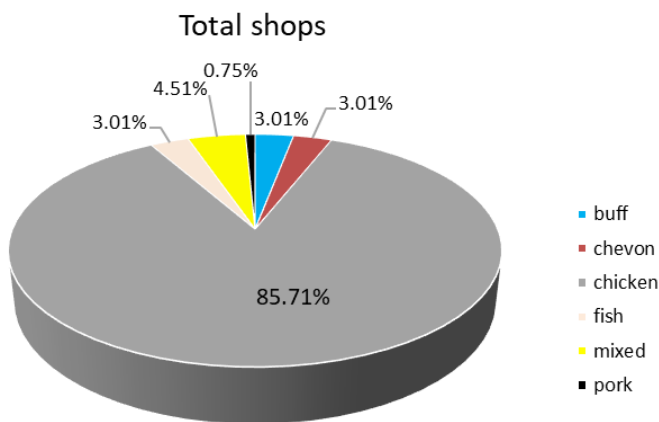


Figure 3. Different types of meat shop in Madi

Table 1 indicates that about 83.61% of the meat retailer shop do not have tiles on their wall and only 3.28% of the shops have tiles all over the area of wall. Similarly, for storage of meat 60.65% have D-freeze with them and only 10% have both D-freeze and normal

freeze.

Table 1. Area covered with marble and equipment for storage

Marble covered area		Equipment for storage	
No tiles	102 (83.61)	D-freeze	74 (60.65)
2 feet tall tiles	15 (12.29)	Freeze	35 (28.69)
3 feet tall tiles	1 (0.82)	Both	13 (10.66)
6 feet tall tiles all over the area	4 (3.28)		

Note: numbers in parenthesis are in percentage

Water supply in the meat cutting area was absent in 91.80% of the shop. Additionally, there were only 4.10% of the shop having marble or tiles slab on the table. However, as indicated by act, 59.02% have a good ventilation in the shop and rest are with bad condition of ventilation. Furthermore, use of mesh wire to prevent flies and other insect was pitiful in 80.33% of the shops (table 2).

Table 2. Management and condition of meat shop

Water supply		Presence of slab		Condition of ventilation		Use of mesh wire	
Yes	10(8.20)	Yes	5(4.10)	Good	72(59.02)	Good	24(19.67)
No	112(91.80)	No	117(95.90)	Bad	50(40.98)	Bad	98(80.33)

Note: numbers in parenthesis are in percentage

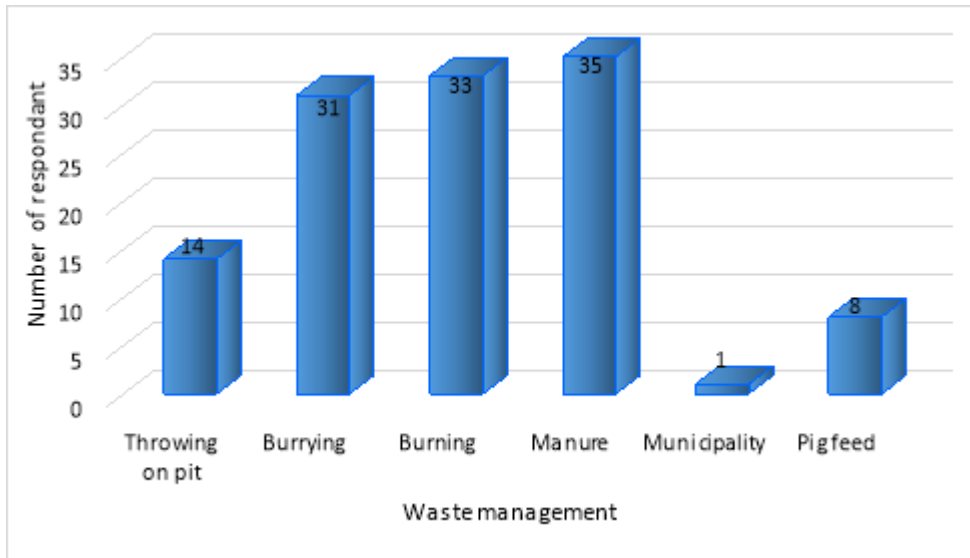
During the survey it is found that the most of the shopkeeper (98.36%) are unaware about the rules and regulation given by animal slaughter house and meat inspection act-2057. Similarly, only 11.48% of the shopkeeper use chemical (mostly phenol) for cleaning the shop and rest do not use any type of chemical. Area of the shop (including butchering area, cutting and selling area) is less than 100 sq. feet in 92.62% of the shop (Table 3).

Aware about Rules and Regulations		Use of Disinfectants		Area	
Yes	2(1.64)	Yes	14(11.48)	≥100 sq feet	9(7.38)
No	120(98.36)	No	108(88.52)	<100 sq feet	113(92.62)

Table 3. rules and regulation, use of disinfection and area of shop.

Note: numbers in parenthesis are in percentage

In this study, it is found that most of the respondents (28.69%) make manure from the waste of meat shop followed by burning (27.05%) and burying (25.41%) and only 0.82% of respondents use waste management vehicle implemented by municipality.



Consumers preference in this study was higher (79.51%) for fresh meat and only 20.49% for the frozen meat. Similarly, preference was different for different part of meat, higher for chest meat (52.46%) followed by leg meat (28.69%).

Table 4. Consumers preference for different type of meat.

Consumers preference			
Fresh meat	97(79.51)	Chest meat	64 (52.46)
Frozen meat	25(20.49)	Leg meat	35(28.69)
		Mixed	23(18.85)

DISCUSSION

Zoonotic and meat-borne diseases are preventable in developing countries, but lack of awareness of zoonotic and meat-borne diseases and poor implementation of SIMA make this a major public health problem in developing countries. In this study, most butcher shops did not meet the minimum requirements of the Animal Slaughterhouses and Meat Inspection Regulations Act (SMIA) of 2057 BS. The legal provision for individuals who do not meet the criteria are a fine of 5,000 (NPR) for the first time, 10,000 for subsequent offenses, or a month's imprisonment, or both. However, there was no evidence of such punishment and no shop met all criteria.

Contamination from dirt, dirty water, intestinal contents, or dirty knives, hands, or clothing from butchers during slaughter operations in slaughterhouses with poor hygiene and unskilled staff. All these lead to meat, bacterial infections (Upadhayaya et al., 2017). Potential for proliferation and toxin production. Lack of basic facilities, cross-

contamination with insects and flies, and many other unhygienic factors all contribute to the risk of consumer infection. There is strong evidence that slaughterhouse conditions and current meat processing practices are a major contributor to the spread of zoonotic diseases in many developing countries (Bhandari et al., 2018). However, the magnitude of public health concern and the importance of meat-borne diseases in developing countries are often underestimated. The frequency, intensity and prevalence of such diseases are increasing dramatically in developing countries (Mann et al., 1983).

We are comparing the condition with respect to animal slaughter house and meat inspection act-2057, according to the study only few have registered according to the act and most of the criteria are not met by shops. However, very few have met (7.09%) the criteria of Act and most of the shop have met only few criteria but not all.

CONCLUSION

This study concluded that condition of meat retailer is not good in madi municipality. Most of the shops do not meet the basic criteria of meat shops criteria although certain shops meet some provision of SIMA. Concern authority should address to uplift the condition of meat shops to provide quality meat for consumer.

REFERENCES

1. Bhandari, R., Singh, A. K., Bhatt, P. R., Timalsina, A., Bhandari, R., Thapa, P., ... & Adhikari, N. (2022). Factors associated with meat hygiene-practices among meat-handlers in Metropolitan City of Kathmandu, Nepal. *PLOS Global Public Health*, 2(11), e0001181.
2. Bhattarai J, Badhu A, Shah T, Niraula SR. Meat Hygiene Practices among Meat Sellers in Dharan Municipality of Eastern Nepal. *BJHS* 2017;2 (2)3: 184-190
3. Haileselassie, M., Taddele, H., Adhana, K., & Kalayou, S. (2013). Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. *Asian Pacific journal of tropical biomedicine*, 3(5), 407-412.
4. LGOA(2074). Local Government Operation act 2074. Government of Nepal. www.lawcommission.gov.np
5. Mann, I, Koulikovskii, A, Matyas, Z & World Health Organization. Veterinary Public Health Unit. (1983). Guidelines on small slaughterhouses and meat hygiene for developing countries / by I. Mann; edited by A. Koulikovskii and Z. Matyas. World Health Organization. <https://apps.who.int/iris/handle/10665/66404>
6. MLMAC (2020). Ministry of Land Management, Agriculture and Cooperatives, Gandaki Province. Fish, Meat and Dairy shop planning and management standard, 2077. Ministerial decision on 27th September, 2020.
7. SMIA (1999). Slaughterhouses and Meat Inspection Act-1999. Government of Nepal. www.lawcommission.gov.np
8. SMIA (2001). Slaughterhouses and Meat Inspection Regulation-2001. Government of Nepal. www.lawcommission.gov.np
9. Upadhayaya, M., & Ghimire, B. (2018). Survey on good hygiene practices in retail meat shops in Butwal Municipality, Nepal. *Nepalese Veterinary Journal*, 35, 110-121.
10. WHO (2004). World Health Organization, Regional Office for Africa "Developing and Maintaining Food Safety Control Systems for Africa Current Status and Prospects for Change", Second FAO/WHO Global Forum of Food Safety Regulators, Bangkok, Thailand, 12-14

A Retrospective Study of Parvovirus Infection in Dogs in Kathmandu

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ABSTRACT

Canine parvo virus infection (CPVI) is highly infectious disease of dogs causing acute gastrointestinal illness in dog with high morbidity. In Kathmandu valley, knowledge on the epidemiology of diseases of dogs is limited. To determine the prevalence of disease in owned dogs in the Kathmandu valley and its associated risk factors, a retrospective study of data from clinical records of the Mount Everest kennel club (old Baneshwor height) during a one-year period was conducted. Using an Excel line graph and pie chart, the prevalence of canine parvovirus infection was examined according to season, age, and vaccination status. A total of 504 cases were seen in the 735 clinical records reviewed, of which 145 cases—or 11.01 % of all cases—had CPVI-like symptoms. The prevalence of CPVI in dogs sold by Mount Everest Kennel club in the valley was estimated to be 11.01% where high cases were observed during the dry season (peaks at April and December) though it could also reach peak during the rainy season (July-August). High number (55.6%; 45/81) of outbreaks were noted among the age group below 3 months followed by age group 4 to 7 months (33.3%; 27/81) and least number of outbreaks were noted in the age group older than 7 months (11.1%;9/81). High number (55.6%; 45/81) of outbreaks were noted among the age group below 3 months followed by age group 4 to 7 months (33.3%; 27/81) and least number of outbreaks were noted in the age group older than 7 months (11.1%;9/81). Disease incidence was highest in German shepherd (21%; 17/81), Japanese spitz (18.51%; 15/81), Labrador (18.51%; 15/81) and mixed/cross (42%; 34/81). Few Vaccinated dogs (7.4%; 6/81) were also found to be tested positive with CPVI. Our finding showed that temperature, breed susceptibility and vaccination status can be the possible risk factors associated with CPVI infection among dog population in Kathmandu valley suggesting pet owners to regularly follow the vaccination schedule, vaccinate their dogs in a timely manner, and routinely check on the health of their canines. Additionally, owners should be aware of the risks and should keep their dogs away from stray dogs.

Keywords: Clinical, Epidemiology, Dogs, Kathmandu valley

INTRODUCTION

Canine parvovirus (CPV) is a global infection and highly contagious viral disease that affects both stray and owned dog population with high morbidity and mortality rate. Within three to seven days of infection, canines with CPV appear with acute gastroenteritis, lethargy, vomiting, fever, and diarrhea (usually bloody) (Nelson et al., 1979; Qi et al., 2020). The disease most often strikes in pups six and twenty weeks old, but older animals may sometimes also affect. A rare variant of the disease is well known to cause myocarditis in young puppies (Ford et al., 2017). Effective vaccination and disinfection are crucial for disease dissemination and prevention in dog household and animal shelters. Although the disease is vaccinated against, still there is chance of vaccine failure among pups. CPV can affect all the dogs, but unvaccinated dogs and puppies younger than four months old are at most risk.

The season has an impact on the prevalence of the Parvo virus, with cases peaking in the dry season months compared to wet (Sayed-Ahmed et al., 2020; Shima et al., 2015). In Dry season, more number of cases were found to occur in the month of May and June whereas in the wet season, the highest incidence of parvoviruses occurred in September. On the Other hand, breed also acts as a predisposition factor for the occurrence of Parvovirus infection in the dog. The breed-wise incidence rate over positive cases was found to be higher in local or non-descriptive breeds, followed by German shepherd and other exotic breeds (Sayed-Ahmed et al., 2020). Males were more likely to contract the virus than females were, with the largest frequency of the parvo virus occurring in dog under the age of six months (Behera et al., 2015). Young, unvaccinated pups and exotic breeds were particularly vulnerable to CPV infection among other risk factors. But puppies that have received a vaccination might also get recurrent parvovirus infections (Gamage et al., 2020).

Numerous studies have documented the prevalence of canine CPV infection and its high association with various risk factors. However, the epidemiological study in the study area is still scarce. Large Nepalese regions like Kathmandu have a significant large stray dog population (Díaz-Regañón et al., 2020). Due to the fact that most owned dogs are not kept inside of their owners' homes and that contact with stray dogs is unavoidable, owned dogs are thus more likely to get an infection through stay dogs. Therefore, this study aimed to collect data from clinical case records of dogs admitted in Everest kennel club to determine the prevalence and risk factors associated with Parvo outbreak in Kathmandu Valley.

MATERIALS AND METHODS

Study location

The capital of Nepal, Kathmandu, is located in the heart of the Kathmandu Valley, a popular tourist destination. Both in terms of human and canine populations, it is one of the most populous areas of the valley. The earliest and largest kennel club in the valley, Mount Everest Kennel Club (MEKC), with branches throughout the valley, has been providing veterinary services in the old Baneshwor area since 2055. The clinic of MEKC was chosen due to its establishment year and competent clinical record keeping. At MEKC, both native and exotic dog breeds—whether they were used as pets or security dogs—were brought for vaccination or medical care.

Data collection

An analysis of clinical data base records from the previous year was done retrospectively using information from the MEKC veterinary clinic in the old Baneshwor. With the exception of instances including routine castration, deworming, immunization, general checkups, tail and ear trimming, and ear notching, all clinical cases from January 2018 to December 2018 were taken into account. The selection of canine parvo viral infection (CPVI) cases was based on the patient's history, clinical condition, results of a test using a kit for canine parvo and canine corona viruses, and necropsy findings. Dogs showing signs of at least one of the following conditions were chosen: fever, hemorrhagic diarrhea, diarrhea and vomiting, and foul-smelling diarrhea. At the Mount Everest Kennel Club, every instance of CPVI was meticulously examined taking into account the dog's history from the initial day of registration at the MEKC clinic until the day of recovery from or death from CPVI. Additionally, the day of the presentation as well as the breed, sex, quantity, and timing of the Distemper, Hepatitis, parvo, para influenza, and leptospirosis (DHPPiL) immunization doses were noted.

Statistical analysis

For descriptive analysis of variables including breed, dog's household number of CPVI incidence, cases result for positive, cases died of parvo, treatment success, vaccination status, and age, data were input into an MS Excel sheet. Following that, the data were examined using statistical techniques such as line graphs, bar graphs, pie charts, and pivot table fields with a 95 percent confidence level. Analysis of the prevalence is done to identify any relevant risk factors for CPVI.

RESULTS AND DISCUSSION

A total of 504 cases were seen in the 735 clinical records reviewed, of which 145 cases—or 11.01 % of all cases—had CPVI-like symptoms. The highest occurrence of CPVI positive cases was recorded in April and August (14.81 %) while the lowest occurrence was

recorded in June (7.40%) which is shown in Fig (1). This result was consistent with that of a retrospective research by (Khan et al., 2017), which revealed an 11.81 % prevalence in the months of April and August demonstrating a considerable increase in the occurrence of CPVI cases as the temperature rises.

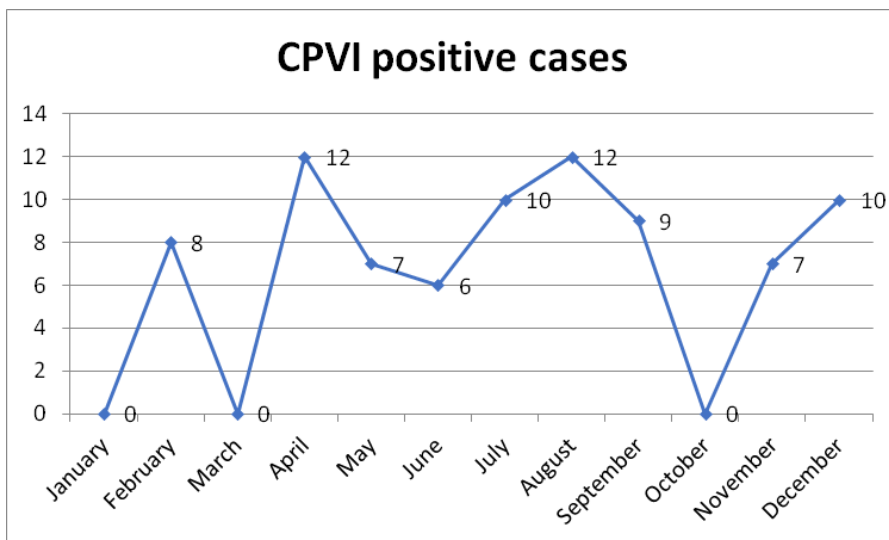


Figure 1: Graph showing month wise distribution of CPVI positive cases

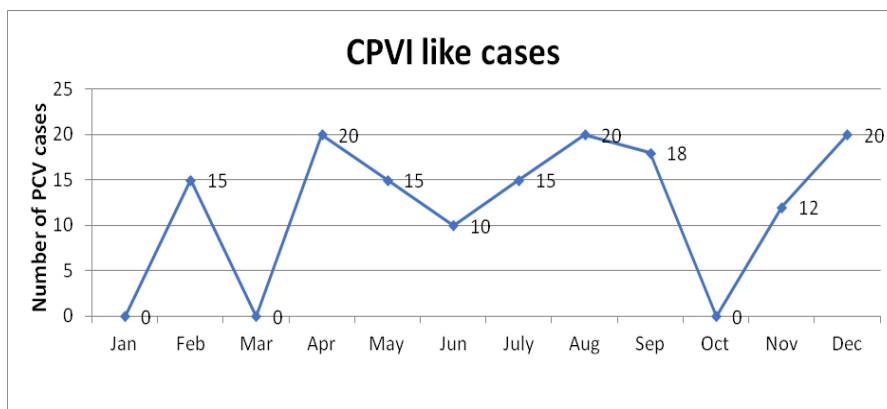


Figure 2: Line graph showing month-wise distribution of CPVI like cases

Based on age category, highest prevalence (55.6 %) was reported in puppies (below the age 3 months) and lower prevalence (11.1 %) was observed in adult dog (above 7 months). Similar age wise prevalence was also shown in a study conducted in India which showed highest prevalence of CPVI in the age group of 1-3 month (27.59%) and lowest prevalence in the adult dog (3.45%)(Behera et al., 2015). Similar data were also reported

by (Khare et al., 2019), who found that dogs aged 0 to 3 months had a prevalence of 11.9 %, followed by those aged 3-6 months by 7.09 %, and those aged 6 to 12 months by 5.31 %. The virus' attraction for quickly proliferating intestinal crypt cells in weaning pups with higher mitotic index due to changes in bacterial ecology as well as in the food related to weaning may account for the highest prevalence of CPVI in puppies under 3 months (Stepita et al., 2013).

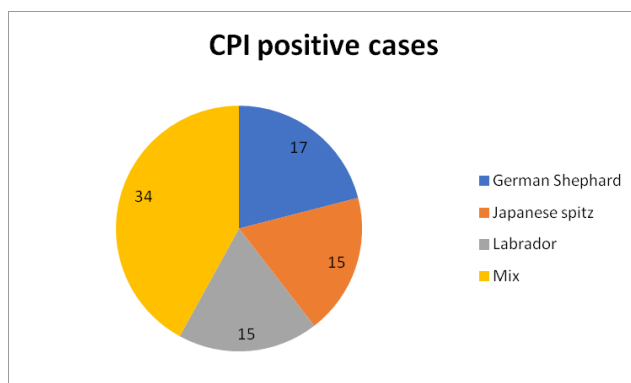


Figure 3: Pie chart showing breed-wise prevalence of CPVI

According to Breed, mix breed showed highest prevalence (42 %) followed by German shepherd (21%), and equal incidence was observed in Japanese spitz and Labrador (18.5%) which is shown fig (3). This result was in contrast to an epidemiological research of canine parvovirus infection carried out in India, which showed that the local breed had the highest frequency followed by the German shepherd and an equal incidence in mixed breed and Labrador retrievers. However, both results showed that the German shepherd came in second, suggesting that breed of the dog might be predisposing factor for occurring of the disease. Our findings are reasonably similar to the research of (Glickman et al., 1985), which revealed the greatest incidence of CPVI in mix breed, followed by German shepherd and other exotic breed. This suggests that there may be breed-related susceptibility to CPVI.

Based on vaccination status, a lower incidence (7.4%) was recorded among dogs that had received vaccinations as compared to dogs that had not received (92.59%). This shows that dogs that have not had vaccines have a higher chance of developing CPVI than those who have, and this is corroborated by a number of studies (Francis et al., 2020; Khan et al., 2017; Sayed-Ahmed et al., 2020). Due to vaccine failure, dogs continue to get CPVI after receiving extensive vaccinations, and it remains a significant source of infection among dogs (Decaro et al., 2020).

CONCLUSION

Our findings indicated that CPVI was more prevalent in the warmer months of the year

and in pups (below 3 months) respectively. Additionally, vaccinated dogs are less likely to get the infection. We recommend pet owners to regularly vaccinate their dogs in a timely manner, and routinely check the health of their canines. Also, we suggest owners to take precautions to keep their dogs away from stray dogs.

REFERENCES

1. Behera, M., Panda, S. K., Sahoo, P. K., Acharya, A. P., Patra, R. C., Das, S., & Pati, S. (2015). Epidemiological study of canine parvovirus infection in and around Bhubaneswar, Odisha, India. *Vet World*, 8(1), 33-37. <https://doi.org/10.14202/vetworld.2015.33-37>
2. Decaro, N., Buonavoglia, C., & Barrs, V. R. (2020). Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? *Vet Microbiol*, 247, 108760. <https://doi.org/10.1016/j.vetmic.2020.108760>
3. Díaz-Regañón, D., Agulla, B., Piya, B., Fernández-Ruiz, N., Villaescusa, A., García-Sancho, M., Rodríguez-Franco, F., & Sainz, Á. (2020). Stray dogs in Nepal have high prevalence of vector-borne pathogens: a molecular survey. *Parasites & Vectors*, 13(1), 174. <https://doi.org/10.1186/s13071-020-04057-7>
4. Ford, J., McEndaffer, L., Renshaw, R., Molesan, A., & Kelly, K. (2017). Parvovirus Infection Is Associated With Myocarditis and Myocardial Fibrosis in Young Dogs. *Vet Pathol*, 54(6), 964-971. <https://doi.org/10.1177/0300985817725387>
5. Francis, M. I., Kalang, J. J., Liba, J. W., Taluvwa, A. B., Tillo, I. M., Zakari, C., & Abdulrahman, R. B. (2020). Prevalence of canine parvoviral enteritis in Yola metropolitan region of Adamawa State, Nigeria. *Sokoto Journal of Veterinary Sciences*, 17(3). <https://doi.org/10.4314/sokjvs.v17i3.4>
6. Gamage, B. G. S. S., Dissanayake, D. R. A., Prasada, D. V. P., & Silva, I. D. (2020). Risk, prognosis and causality of parvo viral enteritis in dogs in Sri Lanka. *Comparative Immunology, Microbiology and Infectious Diseases*, 72, 101496. <https://doi.org/https://doi.org/10.1016/j.cimid.2020.101496>
7. Glickman, L., Domanski, L., Patronek, G., & Visintainer, F. (1985). Breed related risk factors for canine parvovirus enteritis. *Journal of the American Veterinary Medical Association*, 187, 589-594.
8. Khan, A., Ud, M., Ahmad, D., Muhammad Hassan, M., Ijaz, M., Khan, A., & Farooqi, S. (2017). A Retrospective Study on Canine Parvovirus in Lahore, Pakistan. *Pakistan journal of zoology*, 49. <https://doi.org/10.17582/journal.pjz/2017.4>
9. Khare, D., Gupta, D., Shukla, P., Das, G., Tiwari, A., Meena, S., & Khare, R. (2019). Prevalence of canine parvovirus infection in dogs in Jabalpur (M.P.). *JOURNAL OF ENTOMOLOGY AND ZOOLOGY STUDIES*, 1495-1498.
10. Nelson, D. T., Eustis, S. L., McAdaragh, J. P., & Stotz, I. (1979). Lesions of spontaneous canine viral enteritis. *Vet Pathol*, 16(6), 680-686. <https://doi.org/10.1177/030098587901600606>
11. Qi, S., Zhao, J., Guo, D., & Sun, D. (2020). A Mini-Review on the Epidemiology of Canine Parvovirus in China. *Front Vet Sci*, 7, 5. <https://doi.org/10.3389/fvets.2020.00005>
12. Sayed-Ahmed, M. Z., Elbaz, E., Younis, E., & Khodier, M. (2020). Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt. *World's Veterinary Journal*, 571-577. <https://doi.org/10.54203/scil.2020.wvj68>
13. Shima, K., Apaa, T., & Mosugu, J. (2015). Epidemiology of Canine Parvovirus Enteritis among Hospitalized Dogs in Effurun/Warri Metropolitan Region of Delta State, Nigeria. *OALib*, 02, 1-7. <https://doi.org/10.4236/oalib.1101208>
14. Stepita, M. E., Bain, M. J., & Kass, P. H. (2013). Frequency of CPV Infection in Vaccinated Puppies that Attended Puppy Socialization Classes. *Journal of the American Animal Hospital Association*, 49(2), 95-100. <https://doi.org/10.5326/jaaha-ms-5825>

Assess the quality of Murrah Buffalo (*Bubalus Bubalus*) Bull Semen regarding seasonal and geographical variability in Nepal

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ABSTRACT

We carried out this study to assess the quality of semen regarding the seasonal and geographical variability of Murrah buffalo breeding bull in Nepal. We aimed to access the quality of semen of murrah buffalo bull considering the seasonal and geographical variability to help standardize quality semen producing protocol and concern. A total of eight Murrah buffalo bulls were used. Semen collection was done at once week and two ejaculations were considered at an interval of 30 minutes during the Summer (June to August), Autumn (September to November), Winter (December to February), and Spring (March to May) from 2017 to 2020 AD. Immediately after collection, initial motility, sperm concentration, pre-filling motility, post-thaw motility, live sperm, and abnormality of sperms were evaluated. Findings revealed that the season had affect ($p < 0.005$) to the semen volume, individual motility, and post-thawing motility except for semen volume and sperm abnormality ($p > 0.05$). However, initial motility, sperm concentration, and pre-filling motility did not vary in months ($p > 0.05$). It is thus well reflected that several traits related to quality and quantity of semen Murrah buffalo bulls may vary depending on the season of collection, location and age ($p < 0.05$) with the semen traits of initial motility, sperm concentration, and pre-filling motility. Accordingly the semen collected during the autumn and spring season is revealed the percentage of average post thaw motility above the 52% that considered the success of artificial insemination is good for buffaloes.

Keywords: Murrah buffalo, location, Season, Semen quality, months

INTRODUCTION

Buffalo is called the 'Black Gold', and farmers rearing all parts of Nepal with varying population densities, but the majority (70%) of the milking buffaloes are found in Terai

and Mid-hill. The higher progress rate of buffalo in the Terai and mid-hill because of the increasing demand for buffalo milk, meat, and superior buffalo germplasm. It offers a daily source of income and is well recognized as a means for poverty reduction and food security. Milk and milk product demand is increasing day by day in Nepal. There is a need for high-yielding breeds of dairy buffaloes to meet the increased demand for milk. Genetic improvement is one of the prioritized activities of the Nepal government to improve the productivity of the buffalo herd (S. et al., 2018). Therefore, an enormous scope is to upgrade the nondescript buffaloes using superior germplasm to improve productivity. Artificial insemination has played an important role in the improvement in productivity through the use of cryopreserving semen of superior bulls. The importance of artificial insemination in buffaloes increasing at a faster rate, as a result, the demand for buffalo bull semen by the rural inseminator is as well high. So far the maximum number of females been served with the quality of frozen semen of superior genetic make-up (Singh and Pant, 2000).

Bull could produce superior quality semen with a high breeding value for the milk-producing ability. Quality semen production is vital for assessing the bull's fertility to enhance the artificial insemination programs (Lemma & Shemsu, 2015). However, semen traits are often influenced by environmental effects than genetic effects. There are very limited studies for the scientific research carried out so far including appropriate records of the semen on different buffalo bull breeds (Bhakat et al. 2015, Ramajayan 2016, Pathak et al. 2018) in Nepal.

Buffaloes are the main source of milk production in Nepal producing about 65.3% of the total annual milk. Daily milk production is more in Murrah buffaloes. On an average Murrah Buffaloes give about 8-16 liters per day. The other big advantages these animals are, they are more disease resistant when compared to cross-bred cows. They can thrive on any crop residue in absence of concentrates during drought. The demand for milk and meat is large, and these animals are also important for religious purposes.

Semen quality parameters are important indicators of bull fertility, whereas, poor quality semen results in female impregnation failure, affecting the production and reproductive life of buffaloes. Due to natural limitations, the breeding potential may not be reached at the maximum level also due to the fact that the area behind the size of the testes is relatively smaller, the daily rate of sperm production is low, and the epididymal sperm reserve is smaller in the buffalo bull (Suryaprakasa, 1993; Sudheer, Xavier 2000).

Semen quality in bulls reflects the normality of the function of their testicles, epididymal ducts, and genital tract. The reproductive system depends on the hormonal balance of the father, which is sensitive to changes in health, nutrition, and management. Variations in these situations affect sperm yield, accessory gonadal secretion, and epididymal function, all of which are reflected in ejaculate volume and sperm characteristics (Seri et al., 2007). Semen volume, sperm concentration, sperm motility, mass activity, sperm morphology, and semen pH are common criteria for evaluating sperm quality (Den Daas, 1992). Breed of the reproductive health status of bulls, technical, skills, and age (Mandal et al. 2010)

influence the quality of semen and genetic constitution of bulls (Koivisto et al. 2009) for a long time not to be reliable because it is not under one hand of management. (Seri et al., 2007).

Artificial insemination is an important tool to improve production and productivity in buffalo by breeding animals with high genetic potential (Baruselli and Carvalho 2005). Different seasons significantly affect the quality of sperm production (Snoj et al. 2013, Bhakat et al. 2015). Research findings revealed the fact that post-thaw plasma membrane integrity, stability, and DNA fragmentation index (DFI) could significantly better in ejaculates processed during winter than in other seasons (Koonjaenak et al. 2007). In bulls, differences in sperm quality have been attributed to seasonal variation in the expression of low-density lipid receptors on the sperm surface that influence the utilization of seminal plasma components between seasons (Argov et al. 2007).

However, little information is available about the scientific facts on the study of sperm quality of Murrah buffalo bull in the Nepalese context with sperm production performance. Under such context quality of sperm is largely very important to consider as it could be well influenced by several environmental factors. The main objective of the research was to evaluate semen quality traits and parameters in terms of seasonal and geographic variability in frozen semen of Murrah buffalo breeding bulls.

MATERIALS AND METHODS

Study area

This research was done at the National Livestock Breeding Office Pokhara, which was located at 28°18'19.08" North and 84°04'37.20" East. According to the rainfall data, we divided the climatic condition into the summer (June to August), autumn (September to November), Winter (December to February) and Spring (March to May).

Animals

8 Murrah buffalo bulls were used included in this study in the fiscal year 2017/2020. Five to seven years old buffalo bulls having an average body weight of 450 to 550 kg were used to collect semen.

Semen collection

Buffalo bulls were trained for semen collection through an Artificial Vagina (AV) set before starting the experiment. Semen was collected twice/week regularly in the early morning (6-6.30 AM) with an AV set (Walton, 1945) and considering two ejaculations at an interval of 30 minutes at each collection.

The AV comprises of outer rubber cylinder, inner rubber line, rubber band, cone and collecting tube. Semen was collected with the help of a teaser animal. All the apparatus

used for semen collection was sterilized in autoclave machine before collection of semen. The inner liner temperature of AV was conserved at 42-43°C water packing at 52-54°C two thirds of its volume. The rest of one-third area of water jacket was filled with air. Some non spermicidal gel was applied into the inner side of artificial vagina before semen collection. The semen collected tube was labeled with aluminium foil and kept in water bath at 37°C for five minutes. Collected semen from each bull was transferred to the laboratory for evaluation and further processing.

Motility, morphology and concentration were measured using a Computer Assisted Sperm Analyzer (CASA) (Miraz et al. 2022). The CASA settings for analyzing sperm motility were set as; Frame rate 60Hz, Frames acquired 30, Minimum contrast 35, Minimum cell size, 5 pixels, Cell size, 9 pixels, Cell intensity 110 pixels, Path velocity (VAP) 50 μ m/s, Straightness (STR) 70%, VAP cut-off, 30 μ /s and VSL cut-off 15 μ /s. Prepared semen (1 μ l) sample was loaded on a prewarmed Leja® slide and analyzed for sperm motility.

Seasonally available green fodder (maize, teosinte in the summer rainy season and oats during the winter) were fed to each bull at the rate of 25-30 kg green herbage (8 to 10 kg DM on an average per day bull along with ad libitum feeding of paddy straw. Besides this, a concentrate of 3 to 4 kg/bull/day (CP 16%, energy 4000 kilo calorie fortified with mineral and vitamin mixture) planned in Pokhara Livestock farm was given along with an ad-libitum supply of fresh water.

Semen dilution and equilibration

The egg yolk-tris-fructose-citric acid-glycerol was used as an extender. Briefly, a stock solution was prepared by dissolving tris (24.20 gms), fructose (10.00 gms), citric acid (13.60 gms), penicillin G-sodium (1000 I.U/ml), and streptomycin sulfate (1 mg/ml) in glass distilled water. The fresh yolk was added with the buffer at a concentration of 20% (v/v). We divided the volume of extender into two equal parts. Thereafter, glycerol was added to one part of the extender at times of the desired concentration (12.8%). The other part of the diluents was used to make the initial dilution of semen that contained two times the desired concentration of spermatozoa. The initial dilution of semen was made at +37°C. Three equal parts of initially diluted semen and double concentration glycerol containing extender were mixed at four steps during a 2 to 3 hours cooling process, the dilution steps were at +18, +12, +8, and +4°C. Finally, the diluted semen contained 6.4% (v/v) glycerol and 20x10 motile spermatozoa per individual insemination doses were sucked in 0.25 ml French straws and the open ends of the straws were sealed by using an automatic filling, sealing and printing of straw by IS-4 (IMV Company, France), and quattro automatic filling, sealing and printing machine (Minitube Company, Germany). After loading, the straws were left for equilibration at +4°C for 4 hours. We did the cooling and equilibration operation in a cold handling cabinet. After equilibration, the freezing operation was conducted in a deep semen freezer to cool the semen from +4 to -140°C, and then the straws were directly plunged into liquid nitrogen (-196°C). Only semen that showed equal to or 50% post-thaw motility was preserved for artificial insemination.

Sealing, filling and printing of straw

Filling, sealing and printing of semen straws was done with the help of IS-4 (IMV Company, France), minitube automated machine (Minitube Company, Germany. This machine were used for this purpose. 0.25 ml of semen was filled in each straw. Bull number, breed, date of semen collection, batch number, address and date of prepared straw.

Cooling of straw

After printing, straws are arranged on freezing racks using ramp meant for Mini straws. These racks are kept in Cold Handling Cabinet at 4-6° C for 4 hours for equilibration. The cabinet should be maintained at the prescribed temperature during this period so that sperms are not exposed to temperature fluctuation.

Freezing of straw

Then racks are transferred into chamber of freezing unit (Bio-freezer for bovine semen) controlled by a computerized programmer. Pre-decided freezing protocol on the programmer takes care of freezing of semen doses. Freezing completes at -140 °C. Frozen semen doses are now taken from freezing unit and plunged into liquid nitrogen (LN) for cryo-preservation. It is uses the integration of SMILE software for data recording and the CASA system for semen evaluation. We used different quality parameters for the evaluation of the semen as mentioned in (Table 1).

Table 1: Quality parameters for the evaluation of bull semen

Parameters	Descriptions
Semen volume	Volume of Semen in ml ejaculated by bull/ejaculation
Initial motility of sperms	Initial motility % of individual sperms of fresh semen
Concentration of sperms	Billion (10^9 /ml) of sperms/ml of semen
Pre-filling motility %	Motility % of sperms after mixing with diluters and before filling into the straws.
Post thaw motility %	Motility % of sperms after freezing of semen by post thawing process of it.

Thawing of Semen

Post-thaw evaluation of cryopreserved semen was conducted after 24 hours of storage at +37° of warm water for 20 sec. Frozen semen samples of these genotypes were evaluated by CASA for motility and morphology of fresh semen. Artificial insemination (AI) was conducted with frozen semen in naturally estrous crossbred murrah buffalo at their different parity. After 60 days post-AI, inseminated animals were checked for pregnancy with rectal palpation and the non-return rate was calculated. Artificial insemination and pregnancy diagnosis was conducted by the same technician to avoid the variation in

conception rate

Data Analysis Techniques

All the concerned data were tabulated, and it carried statistical analysis out using Harvey's (1990) software. We presented data as the mean followed by the standard error (SE). We considered the data to be statistically significant at $P < 0.05$.

The following fixed effect model was used to express each observation of semen quality produced from breeding buffalo bulls.

$$Y_{ijkno} = \mu + a_i + b_j + c_k + g_n + h_o + e_{ijkno}$$

Where, μ is the overall mean

a_i is the effect of i th year ($i = 1, 2, 3, 4$)

b_j is the effect of j th bull ($j = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11$)

c_k is the effect of k th interval of semen collection ($k = 1, 2$)

g_n is the effect of n th density of fresh semen ($n = 1, 2, 3$)

e_{ijkno} is the random element (error mean) assumed to be normally and independently distributed among the sampled population.

RESULTS AND DISCUSSION

Quality Parameters of Buffalo Bull Semen Production

Physical quality parameters of bull semen include semen volume. In this study, microscopic quality of bull semen were semen volume, prefilling motility, the concentration of sperm, and post-thaw motility (Table 2 and 3).

Table 2: The mean values ($\pm$ S.E.) for traits of semen of Murrah buffaloes bull

Factors		N	Initial Volume of Semen (%)	Premotility (%)	Sperm Concentration (10^9 /ml)
			SSM $\pm$ SEM	SSM $\pm$ SEM	SSM $\pm$ SEM
Overall Mean		532	6.15 $\pm$ 0.13	74.99 $\pm$ 0.26	1599.33 $\pm$ 27.95
Location			$P < 0.001$	$P < 0.001$	$P < 0.001$
	Hill	394	5.42 $\pm$ 0.10	75.94 $\pm$ 0.21	1489.86 $\pm$ 22.61
	Terai	138	6.87 $\pm$ 0.20	74.04 $\pm$ 0.42	1708.80 $\pm$ 44.06
Year			$P < 0.002$	$P < 0.001$	$P < 0.001$
	2017	44	5.93 $\pm$ 0.27	75.19 $\pm$ 0.57	1663.76 $\pm$ 60.61
	2018	66	6.09 $\pm$ 0.23	76.93 $\pm$ 0.49	1565.86 $\pm$ 51.40
	2019	348	6.56 $\pm$ 0.10	75.52 $\pm$ 0.20	1399.73 $\pm$ 21.14
	2020	74	6.00 $\pm$ 0.22	72.32 $\pm$ 0.46	1767.97 $\pm$ 49.03

Season			P<0.033	P<0.001	P<0.001
	Summer	133	5.53±0.33	74.00±0.70	1655.34±73.61
	Autumn	126	6.44±0.31	76.01±0.66	1681.94±69.23
	Winter	143	5.97±0.32	75.06±0.67	1612.92±70.44
	Spring	130	6.65±0.34	74.88±0.71	1447.12±74.63
Months			NS	NS	NS
	January	54	6.02±0.38	75.90±0.79	1530.93±83.47
	February	44	6.34±0.39	75.48±0.82	1501.43±86.91
	March	51	5.88±0.34	75.32±0.71	1631.91±75.29
	April	29	5.73±0.46	76.01±0.97	1797.06±102.39
	May	56	5.97±0.39	75.21±0.83	1830.92±87.18
	June	39	6.40±0.37	75.42±0.77	1727.79±81.41
	July	44	6.89±0.42	75.41±0.88	1578.53±92.89
	August	43	6.89±0.43	75.89±0.89	1606.87±94.23
	September	44	5.93±0.34	73.74±0.71	1598.99±75.04
	October	30	5.90±0.43	73.40±0.91	1511.98±95.61
	November	48	5.72±0.39	73.28±0.81	1491.31±86.00
	December	50	6.07±0.32	74.80±0.66	1384.25±69.76
CV			28.20	4.69	26.07
R squared			0.19	0.17	0.16

Initial volume of semen

The overall least squares mean of the initial ejaculate volume was 6.15±0.13 ml (Table 2) at two times of the same day at an interval of one hour. It was differ significantly ($p<0.05$) between location, year, season, and month.

Tomar et al. (1966) reported ejaculate volume ranging 2-12 ml (5.11±0.17 ml), Shukla and Mishra (2005) in Murrah bulls and Javed et al. (2000) also reported similar findings in Nili-Ravi bulls. Several authers (Bhakat et al., 2011; Pant et al., 2003; Koonjaenak et al., 2007; Rehman et al., 2012) also reported lower ejaculate volume compared to Jaffrabadi in Murrah, Swamp, and Kundhi breeds, respectively. Differences in semen volume among different buffalo breeds may be due to differences in genetics, the reproductive health status of bulls, age of bulls, collection frequency, pooled volume, nutrition, season, and management (Nazir, 1988). Differences may also be due to the skill of the semen collector and the temperature of the artificial vagina. Andrabi (2014) reported that the sperm volume

and sperm concentration of Murrah bulls ranged from 2.0 to 8.0 mL and 320 million to 5.9 billion, respectively. Mahmoud et al. (2013) observed that the concentration of buffalo bull sperm varied from 1.02 to 1.17 billion/mL, while their motility varied from 65.00 to 68.30% (fresh semen) and from 39.33 to 45, 87% (thawed sperm). Therefore, variation in frozen sperm production between two bulls could not prevent both bulls from being used for artificial insemination. In addition, Bull had a similar result in post-thaw sperm motility, which was previously categorized as the best single predictor for buffalo field fertility (Ahmed et al. (2016) & Ahmed et al. (2017).

Sperm motility

During the study, the initial progressive sperm motility ranged from 73.28 ± 0.81 to 76.01 ± 0.97 and the overall average sperm motility was 74.99 ± 0.26 (Table 2). It reports a similar finding from 65 to 95%, with an average of 79.41 ± 0.60 (Ghodasara et al. 2016). Sahu and Pandit (1997) and Shukla and Mishra (2005) reported higher initial progressive motility in Murrah bulls. Bhakat et al. (2011) also reported a lower percentage of initial motility than the present findings in Murrah bulls.

Sperm motility of fresh semen of buffalo bull varied significantly ($p < 0.001$) concerning location, season, and, months, while the year is not significantly ($p > 0.001$) affected. The results of this study indicate that the total average of the thawed sperm was 52.17 ± 0.28 . And this study may cause higher field fertility as noted by Mahmoud et al. (2013) that buffalo bull semen with post-thaw sperm motility between 39.33 and 43.84% had a similar result in pregnancy rate, which has the potential to be used as a male sire for artificial insemination. The difference in motility in different reports could be due to changes in motility assessment, the few bulls studied, or differences in seasons. However, Mohan et al. (1977) and Gill et al. (1974) reported higher motility in winter (75 and 65%) in buffaloes, while Saeed et al. (1990) reported no effect of seasons on sperm motility.

Sperm concentration

The total average sperm concentration in the semen of the Murrah bull was $1599.33 \pm 27.95 \times 10^9/\text{ml}$ (Table 2). Sperm concentration per unit of sperm volume is perhaps one of the most studied seminal attributes of seasonal changes in sperm quality. The results showed that sperm concentration per ml (SPC) varied significantly ($P < 0.01$) between site, year, and season, with a maximum in autumn followed by summer winter, and spring (1681.94 ± 69.3 , 1655.34 ± 73.61 , 1612.92 ± 70.44 and $1447.12 \pm 74.63 \times 10^9/\text{ml}$), which is support with the statement of Mandal et al. (2000) and Ravimurugan et al. (2003) at Murrah Bulls.

Bhosrekar (1980) and Manik & Mudgal (1984) reported the highest SPC during summer at Murrah, while Prajapati (1995) and Dhami et al. (1998) obtained the highest value in the rainy season; however, in Egyptian buffalo bulls, Oloufa et al. (1959) recorded the

highest value in spring. On the contrary, Zafar et al. (1988) in Nili Ravi buffalo bulls and Koonjaenak et al. (2007) found no significant seasonal differences in sperm concentration in swamp buffalo.

Table 3: The mean values ($\pm$ S.E.) for various characteristics of semen of Murrah buffaloes bull of National Livestock Breeding Office(NLBO) Pokhara and Lahan

Quality parameters	Location	N	Final Volume (ml)	Post thaw mortality (%)	Live sperm (%)	Sperm abnormality (%)
			SSM $\pm$ SEM	SSM $\pm$ SEM	SSM $\pm$ SEM	SSM $\pm$ SEM
Overall Mean		532	84.54 $\pm$ 1.78	52.17 $\pm$ 0.28	66.98 $\pm$ 0.08	4.21 $\pm$ 0.05
Location			P<0.001	P<0.001	P<0.001	NS
	Hill	394	78.04 $\pm$ 1.44	50.58 $\pm$ 0.22	68.42 $\pm$ 0.06	4.26 $\pm$ 0.04
	Teraï	138	91.05 $\pm$ 2.81	53.77 $\pm$ 0.44	65.54 $\pm$ 0.12	4.16 $\pm$ 0.08
Year			P<0.0326	NS	P<0.001	P<0.001
	2017	44	86.84 $\pm$ 3.86	53.02 $\pm$ 0.60	68.56 $\pm$ 0.17	4.37 $\pm$ 0.11
	2018	66	84.98 $\pm$ 3.27	52.11 $\pm$ 0.51	68.56 $\pm$ 0.14	5.30 $\pm$ 0.09
	2019	348	79.19 $\pm$ 1.35	52.23 $\pm$ 0.21	65.66 $\pm$ 0.06	3.55 $\pm$ 0.04
	2020	74	87.16 $\pm$ 3.12	51.34 $\pm$ 0.48	65.14 $\pm$ 0.14	3.62 $\pm$ 0.09
Seasons			NS	P<0.011	NS	P<0.001
	Summer	133	87.47 $\pm$ 4.69	51.23 $\pm$ 0.73	67.14 $\pm$ 0.20	4.85 $\pm$ 0.14
	Autumn	126	91.04 $\pm$ 4.41	51.25 $\pm$ 0.68	67.10 $\pm$ 0.19	4.21 $\pm$ 0.13
	Winter	143	82.09 $\pm$ 4.49	51.82 $\pm$ 0.70	66.96 $\pm$ 0.19	4.03 $\pm$ 0.13
	Spring	130	77.57 $\pm$ 4.75	54.41 $\pm$ 0.74	66.72 $\pm$ 0.21	3.75 $\pm$ 0.14
Months			NS	P<0.0002	NS	P<0.0002
	January	54	83.45 $\pm$ 5.32	54.20 $\pm$ 0.82	66.82 $\pm$ 0.23	4.59 $\pm$ 0.15
	February	44	80.88 $\pm$ 5.54	54.25 $\pm$ 0.86	67.11 $\pm$ 0.24	4.53 $\pm$ 0.16
	March	51	90.69 $\pm$ 4.80	50.94 $\pm$ 0.74	67.09 $\pm$ 0.21	4.74 $\pm$ 0.14
	April	29	95.02 $\pm$ 6.52	49.55 $\pm$ 1.01	67.30 $\pm$ 0.28	4.48 $\pm$ 0.19
	May	56	102.57 $\pm$ 5.55	48.85 $\pm$ 0.86	67.46 $\pm$ 0.24	4.30 $\pm$ 0.16
	June	39	90.77 $\pm$ 5.19	50.25 $\pm$ 0.80	67.02 $\pm$ 0.23	3.77 $\pm$ 0.15
	July	44	84.58 $\pm$ 5.92	51.38 $\pm$ 0.92	67.19 $\pm$ 0.26	3.53 $\pm$ 0.17
	August	43	83.43 $\pm$ 6.00	52.41 $\pm$ 0.93	66.91 $\pm$ 0.26	3.67 $\pm$ 0.17
	September	44	78.92 $\pm$ 4.78	52.84 $\pm$ 0.74	66.85 $\pm$ 0.21	3.99 $\pm$ 0.14

	October	30	76.14±6.09	52.42±0.94	66.72±0.26	4.28±0.18
	November	48	75.66±5.48	54.91±0.85	66.60±0.24	4.40±0.16
	December	50	72.40±4.44	54.10±0.69	66.68±0.19	4.27±0.13
CV			30.39	7.19	1.55	17.75
R squared			0.11	0.24	0.79	0.48

Final Volume and Post thaw mortality

Results of this study revealed that the overall mean final volume of semen was 84.54 ± 1.78 ml in the present study (Table 3). In line with the findings of the present study, According to NLBO, Poudel et al. (2021), we reported this finding lower than the present study $57.9.2 \pm 1.79$.

Post-thaw sperm motility: Results of this study showed that the overall mean of post-thaw motility was 52.17 ± 0.28 . The post-thaw motility ranged from 48.85% to 54% with an average mean of 52.17 ± 0.28 during the entire study period. According to NLBO, Poudel et al. (2022), reported this finding was lower than 48.2 ± 0.25 which is similar to the present study. Maurya et al. (2003) emphasized that the success of AI depends upon the effective prolongation of fertile life of spermatozoa under in-vitro conditions and he reported that the acceptable percentage (40%) of post-thaw sperm motility considered fit for artificial insemination in buffaloes was maintained up to 60 minutes of incubation in semen, which was frozen in winter and rainy seasons, whereas semen frozen in spring and summer seasons could maintain it only up to less than 30 min of post-thaw incubation. No significant difference between progressive and rapid sperm motility was observed at different time intervals of incubation across the seasons. Argaman et al. (2007) also reported a significant reduction in sperm motility during summer. Majic-Balic et al. (2012) stated that it may be due to more oxidative stress during summer to seminal plasma and spermatozoa that lead to a decrease in sperm progressive motility, which further leads to semen quality deterioration.

Live and sperm abnormalities

The overall least square means of live and abnormal spermatozoa percentages were 66.98 ± 0.08 and 4.21 ± 0.05 respectively (Table 3). Effect of location and year was significant ($p < 0.05$) in live spermatozoa but effect of seasons and months were nonsignificant ($p > 0.05$) in the case of status of live sperm. The lowest live sperm percentages were observed during the spring season (Table 3), followed by the summer, autumn, and winter seasons. Salem et al. (1973) documented lowest live sperm percentage and greatest sperm abnormality during the spring season which is partly in agreement with the present results. Values of live sperm were lowest in November and then increased up to April (Table 3). According to the semen quality control at the NLBO lab, the standard of average live sperms has been recorded 55% and lowest at 45%, and best at 75% (Annual Technical Report, 2021). Percentages of abnormal sperm were significantly ($p < 0.05$) in different seasons (Table 3). The sperm abnormalities of 4.85 ± 0.14 , 4.21 ± 0.13 , 4.03 ± 0.13 and 3.75 ± 0.14 percent

observed in summer, autumn, winter and spring, respectively. The highest percent of abnormal spermatozoa were noted in March, January, February, April, October, November, and December, and the lowest in June-September (Table 3), respectively. Singh et al. (1992) and Igboeli et al. (1987) also reported higher sperm abnormalities in winter. Heuer et al. (1987), Bhavsar et al. (1990) and Younis (1996) observed higher percentage of sperm abnormalities in buffalo bulls during summer season, while Ahmad et al. (1984) reported it autumn in Nili-Ravi buffalo bulls. The higher sperm abnormalities during the winter have been related to effect of cold or improper handling during semen collection (Javed, 2019). Ahmed et al. (1984) observed total abnormalities of 12.68 ± 7.06 , 9.83 ± 3.64 , 11.48 ± 6.57 and 15.04 ± 6.59 percent in winter, spring, summer and autumn, respectively (lowest in spring and highest in autumn). Koonjaenak et al. (2007) reported overall total mean percentages of sperm abnormalities of buffalo bull spermatozoa ($13.7 \pm 0.5\%$) in the rainy season, ($12.4 \pm 0.5\%$) in winter and ($10.7 \pm 0.5\%$) in summer. The rate and pattern of sperm removal seemed to depend particularly on the quality of spermatozoa (Rao et al., 1980). The maximum allowed limit for bulls is 10 percent for young and 20 percent for older bulls with 18 percent upper limit in all bulls (Lagerlof 1934).

It was also observed that abnormalities of the spermatozoa could occur due to disorder of the seminiferous tubules or germinal epithelium (Hossain et al., 1990). However, the sperm concentration did not differ significantly between the groups.

CONCLUSION

The present study provides evidence that the seasons of a collection influenced the semen volume of Murrah buffalo bulls. During the autumn and spring semen collection is more profitable than in the other two seasons. The quality of product semen used in Nepal is good and meets international standards and requirements. The existing breed of buffaloes bulls for semen production traits would help to scale up the genetic make-up in the hill and terai buffaloes to produce good progeny.

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REFERENCES

1. Ahmad, M., Latif, M., Ahmad, M., Qazi, M.H., Sahir, M. and Arslan, M. 1984. Age-related changes in body weight, scrotal size and plasma testosterone levels in buffalo bulls (*Bubalus bubalis*). *Theriogenology*, 22: 651-656.
2. Argaman N, Sklan D, Zeron Y and Roth Z. 2007. Association between seasonal changes in fatty-acid composition, expression of VLDL receptor, and bovine sperm quality. *Theriogenology* 67: 878–85
3. Argov-Argaman N, Sklan D, Zeron Y and Roth Z. 2007. Association between seasonal changes in fatty-acid composition, expression of VLDL receptor, and bovine sperm quality. *Theriogenology* 67: 878–85.
4. Baruselli P S and Carvalho N A T. 2005. Biotechnology of reproduction in buffaloes (*Bubalus bubalis*). *Brazilian Journal of Animal Breeding* 29(2): 4–17
5. Bhakat M, Mohanty T K, Gupta A K, Prasad S, Chakravarty A K and Khan H M. 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bulletin* 34: 100-12
6. Bhakat M, Mohanty T K, Gupta A K, Prasad S, Chakravarty A K and Khan H M. 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bulletin* 34: 100–12
7. Bhakat, M., T.K. Mohanty, V.S. Raina, A.K. Gupta, and H.M. Khan. 2011. frozen semen production performance of Murrah buffalo bulls. *Buffalo Bull.*, 30: 157-162.
8. Bhaskar, M.R. 1980. Studies on buffalo semen. Seasonal variation in seminal characteristics. *Indian Vet. J.*, 57: 806-810
9. Bhavsar, B.K., Dharni, A.J., Kodagali, S.B. 1990. Abnormal sperm content in Mehsana buffalo semen with regard to freezability, seasonality and fertility. *Indian Veterinary Journal*, 67: 233-237.
10. Den Daas N 1992: Laboratory measurement of semen characteristics. *Animal Reproduction Science* 28 87-94.
11. Dharni, A.J., M. Greesh and K.L. Sahni. 1998. Seasonal influences on the quality and freezability of semen of Friesian and Murrah buffalo bulls. *Indian J. Anim. Reprod.*, 19(1): 55-58.
12. Ghodasara, S.N., P.U. Gajbhiye, A.R. Ahlawat, and K.S. Murthy, Evaluation of fresh semen quality and predicting the number of frozen semen doses in jaffrabadi buffalo bull *Buffalo Bulletin* (March 2016) Vol.35 No.1
13. Gill, R. S., P. C. Gangwar, O. P. Takkar (1974): Seminal attributes in buffalo bulls as affected by different seasons. *Ind. J. Ani. Sci.* 44, 415-418.
14. H. Ahmed, S. M. H. Andrabi, and S. Jahan, "Semen quality parameters as fertility predictors of water buffalo bull spermatozoa during the low-breeding season," *Theriogenology*, vol. 86, Oct. 2016, pp. 1516-1522.
15. H. Ahmed, S. M. H. Andrabi, M. Anwar, and S. Jahan, "Use of post-thaw semen quality parameters to predict the fertility of water buffalo (*Bubalus bubalis*) bull during peak breeding season," *Andrologia*, vol. 49, May 2017, e12639.
16. Heuer, C., Tahir, M.N. and Amjad, H. 1987. Effect of season on fertility of frozen buffalo semen. *Animal Reproduction Science*, 13: 15-21.
17. Hossain MF, Alam MGS, Ahmed JU, Rahman MM and Rahman A. 1990. Bacteriological examination of semen as an aid to sexual health control in bulls. *Bangladesh Journal of Microbiology*. 7: 85-88.
18. Igboeli, G., Nwakalor, L.N., Orji, B.I. and Onuora, G.I., 1987. Seasonal variation in the semen characteristics of Muturu (*Bos bubalis*) bulls. *Animal Reproduction Science*, 14: 31-38.
19. Javed, M.T., A. Khan and R. Kausar. 2000. Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Vet. Arhiv*, 70: 83-94.
20. K. Mahmoud, A. A. E. El-Sokary, M. E. A. Abou El-Roos, A. D. Abdel Ghaffar, and M. Naito, "Sperm characteristics in cryopreserved buffalo bull semen and field fertility," *Iran. J. Appl. Anim. Sci.*, vol. 3, Dec. 2013, pp. 777-783.
21. K. Mahmoud, A. A. E. El-Sokary, M. E. A. Abou El-Roos, A. D. Abdul Ghaffar, and M. Naito,

- “Sperm characteristics in cryopreserved buffalo bull semen and field fertility,” Iran. J. Appl. Anim. Sci., vol. 3, Dec. 2013, pp. 777-783.
22. Koonjaenak, S., Chanatinart, V., Aiumlamai, S., Pinyopumimintr T. and Rodriguez-Martinez, H. 2007. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. Asian Journal of Andrology, 9: 92–101.
 23. Koonjaenak, S., V. Chanatinart, S. Aiumlamai, T. Pinyopumimintr and H. Rodriguez Martinez. 2007. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. Asian J. Andro., 9: 92-101.
 24. Lagerlof, N. 1938. Infertility in male domestic animals: In proceedings.12th International Veterinary Congress, 214-241.
 25. Lemma, A., & Shemsu, T. (2015). Effect of Age and Breed on Semen Quality and Breeding Soundness Evaluation of Pre-Service Young Bulls. Journal of Reproduction and Infertility , 6(2): 35–40.
 26. M. Tariq Javed¹ , Nazir Ahmad² , Gulbeen Saleem³ , Haleema Sadia⁴ , Farkhanda Manzoor⁵ and Ghulam Hussain Dilbar²., Effect of age and season on semen characteristics of buffalo bull-A REVIEW.J. INNOV.SCI.4(1):65-82.
 27. Majic-Balic I, Milinkoviæ-Tur S, Samardzija M and Vince S. 2012. Effect of age and environmental factors on semen quality, glutathione peroxidase activity, and oxidative parameters in Simmental bulls. Theriogenology 78(2): 423-43.
 28. Mandal D K, Nagpaul P K and Gupta A K. 2000. Seasonal variation in seminal attributes and sexual behavior of Murrah buffalo bulls. Indian Journal of Dairy Science 53: 278–83.
 29. Mandal, D.K., P.K. Nagpaul and A.K. Gupta. 2010. Seasonal variation in seminal attributes and sexual behavior of Murrah buffalo bulls. Indian J. Dairy Sci., 53: 278-283.
 30. Manik, R.S. and V.D. Mudgal. 1984. Monthly and seasonal variation in Physico-chemical and biochemical attributes of buffalo semen. World Rev. Anim. Prod., 4: 45-50.
 31. Maurya V P and Tuli R K. 2003. Post-thaw thermal resistance test on motility and acrosomal integrity of filtered and nonfiltered frozen semen of Murrah buffalo bulls. Asian Australasian Journal Animal Science 16(10): 1424–28.
 32. Miraz, M.F.H., Deb, G.K., Hossain, S.M.J., Akter, S., 2022. Motion characteristics and plasma integrity evaluation of Murrah buffalo semen. Asian Australasian Journal of Bioscience and Biotechnology, 7(2), 75-81.
 33. MoAD (2021) Nepal Portfolio Performance Review. Retrieved November 20, 2017, from MinistryofAgriculturalDevelopment:http://www.mof.gov.np/uploads/document/file/Agriculture_NPPR-2015_20150913011507.pd
 34. Mohan, G., M. L. Madan, M. N. Razdan (1977): Composition of Murrah buffalo bull semen during winter and summer months in India. Trop. Agri. 54, 21--28.
 35. Nazir, M. 1988. Semen evaluation and sperm morphology - monography on a reproductive pattern of Riverine buffaloes and recommendations to improve their reproductive performance at the small farmer level. PARC, Islamabad.
 36. Olfa, M.M., A.A. Sayed, and A.L. Bardin. 1959. Seasonal variation in reaction time of Egyptian buffalo- bulls and physiochemical characteristics of their semen. Indian J. Dairy Sci., 12: 10-17.
 37. Pant, H.C., R.K. Sharma, S.H. Patel, H.R. Shukla, A.K. Mittal and R. Kasiraj. 2003. Testicular development and its relationship to semen production in Murrah buffalo bulls. Theriogenology, 60: 27-34.
 38. Pathak P K, Dharni A J and Chaudhari D V. 2018. SeminalAttributes, Freezability and their Interrelationships in ZebuCattle and Buffalo Bulls from Central Gujarat. The Indian Journal of Veterinary Sciences and Biotechnology 14(2): 01–08
 39. Poudel R.P., Sharma S. & Sapkota S., 2021. Effects of green fodder feeding on semen quality of buffalo bulls maintained in National Livestock Breeding Center Pokhara, a review of annual semen production records. Annual Report 2021.

40. Prajapati, K.B. 1995. The effect of exercise and feeding bypass protein on sexual behavior and seminal attribute in Mehsana buffalo. Ph.D. Thesis, NDRI Deemed University, Karnal, India.
41. Ramajayan P. 2016. 'Genetic studies on semen production in Murrah buffalo bulls reared in Tamil Nadu'. M.V.Sc dissertation. Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.
42. Rao, A.R., Bane, A. and Gustafsson, B.K. 1980. Changes in the morphology of spermatozoa during their passage through the genital tract in dairy bulls with normal and impaired spermatogenesis. *Theriogenology*, 14: 1-12.
43. Ravimurugan, T., K.S. Raman and P. Thangaraju. 2003. Seasonal influences on the semen production traits of Murrah buffalo bulls. *Indian Buffalo J.*, 1(2): 85-87.
44. Rehman, Z.U., M.U. Samo, T.A. Qureshi, S. Khan, M.S. Qureshi, F.A. Khan and S. Bahadadr. 2012. Studies on the freezability of kundhi buffalo bull semen. *J. Anim. Plant Sci.*, 22: 18-23.
45. S. M. H. Andrabi, "Applied andrology in water buffalo," In *Animal Andrology: Theories and Applications*, P. J. Chenoweth and S. Lorton, Eds. Oxfordshire: CABI, 2014, pp. 380-403.
46. S. Sankhi¹, K. R. Sapkota² and B. Regmi, 2018. Effect of Age and Frequency of Collection in Quality of Jersey Bulls Semen at National Livestock Breeding Center, Nepal. *Nepalese Vet. J.* 35:9-22
47. Saeed, A., R. A. Chaudhry, I. H. Khan, N. U. Khan (1990): Morphology of semen of buffalo bulls of different age groups. *Proc. II World Buffalo Congr.*, Vol. 3. December 12-16, 1990. New Dehli, India.
48. Sahu, S.B. and R.K. Pandit. 1997. Grading of breeding Murrah bulls on sexual behavior and seminal characteristics including cryopreservation. *Indian Vet. J.*, 74: 557- 569.
49. Salem, H.M., Osman, S.A., Oloufa, M.M. and Ibrahim, S.T. (1973). Effect of seasonal variations on the physical characteristics of semen of Egyptian cattle and buffaloes. *Vet. Med. J. Cairo*. 21: 207-213.
50. Seri Koonjaenak^{1,2}, Vichai Chanatinart³, Suneerat Aiumlamai⁴, Tanu Pinyopumimint⁵, Heriberto Rodriguez-Martinez¹. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. Semen quality in swamp buffalo, DOI: 10.1111/j.1745-7262.2007.00230.
51. Shukla, M.S. and A.K. Mishra. 2005. Correlation between seminal characteristics in Murrah bulls. *Indian J. Anim. Sci.*, 75: 263-266.
52. Singh M and Pant HC. 2000. Effect of post-thaw incubation on semen quality of buffalo bull comparison with cattle. *Buffalo bulletin*. 19(3).
53. Singh R.S., Tomar, N.S., Sharma, K.C. and Sharma, K.B. 1992. Studies on acrosomal abnormalities of cattle and buffalo spermatozoa in relation to other semen characteristics and fertility. *Indian Veterinary Journal*, 69: 267-268.
54. Sudheer S, Xavier CJ. Disposal pattern of breeding bulls in Kerala. *Indian J. Anim. Reprod.* 2000; 211:72-73.
55. Suryaprakasam TB, Rao AVN. Studies on breeding life and disposal pattern of A. I sire in Andhra Pradesh. *Indian Vet. J.* 1993; 70:1022-1024.
56. Tomar, N.S., B.S. Mishra, and C.B. Johari. 1966. Seasonal variations in reaction time and semen production, and prediction of some semen attributes on initial motility of spermatozoa in Hariana and Murrah bulls. *Indian J. Dairy Sci.*, 19: 87-93. University, Chennai, Tamil Nadu, India.
57. Walton, A., 1945. *The Technique of Artificial Insemination*, 2nd ed. London: Holborn Surgical Instrument Co.
58. Younis, M., Samad, H.A., Ahmad, N. and Ahmad, I. 2003. Effects of age and season on the body weight, scrotal circumference and libido in Nili-Ravi buffalo bulls maintained at the semen production unit, Qadirabad. *Pakistan Veterinary Journal*, 23: 59-65.
59. Zafar, A.H., N. Ahmed, and S.K. Shah. 1988. Effect of seasonal variation on semen production of Nili-Ravi buffalo bulls. *Buffalo J.*, 4(1): 61-68.

Study of Reproductive Traits of Does in Different Altitudes of Rolpa District

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ABSTRACT

Goat is a domesticated small ruminant, primarily reared by small farmers for meat production, across the country. Productivity of goat is often low, which could be related to several genetic and non-genetic factors. A field study was carried out to characterize, evaluate and estimate the effect of non-genetic factors on the reproductive performance of hill goats during December, 2015 to August 2016 in different eco-zones at Rolpa district of mid-western Nepal. Altogether 482 dams of different age groups were identified for this study. Reproductive performance were collected based on field monitoring and measurements within different altitudes of Rolpa district. Least square analysis was performed using Harvey (1990) computer software package, and means were compared using DMRT. Results revealed that the mean age of first heat in does (299 days), age at first kidding (493 days), gestation length (150 days), kidding interval (251 days), postpartum estrus (91 days), and kidding rates were (1.54 per doe), respectively. Altitude significantly influenced ($P < 0.001$) along with parity ($P < 0.05$) with respect to age of first heat in does. Significance difference of kidding interval ($P < 0.05$) was significantly influenced within different altitude of goat farming. Similarly, colour and altitude also significantly differed ($P < 0.05$) with respect to kidding rates of does. Thus the results of this study suggest that the performance of low altitude goat flocks was better than mid and high altitude goat flocks in Rolpa district in terms of production and reproduction traits. This result could be attributed to superior genotype along with better management practices followed by the farmers in Rolpa district.

Keywords: Altitude, Doe, Weaning, Kidding interval, Parity

INTRODUCTION

Nepal is an agricultural country where about 66 percent of its population is involved in agricultural occupation. Agriculture contributes to around 27.1 percent of the gross domestic product (GDP) of Nepal, of which, the livestock sector contributes about 11.5

percent of the total GDP and 25.7 percent of the agricultural GDP (AGDP) as reported by MOAD (2018). Goat farming is being the most popular means of self-employment among the youths in the country. Current statistics regarding to goat population indicated that there are more than 11.64 million of goats in the country (MOAD, 2018). Among the agricultural commodities, livestock plays an important role in agricultural development and economic upliftment of the country. Goat farming has been practiced by a large section of population in rural areas of Nepal. The recent population of goat is about 11.64 million and total meat contribution was 6.9 thousand metric tonnes per year (MOAD, 2018). The rate of increment in goat population during last 15 years (2008 to 2018) was reported 3.74 percent per year contributing about 20.1% to the total meat production in the country (MOAD, 2018). Goats breed in Nepal are quite different with locational difference. There are gradients of topography, environment and climatic conditions vary from South to North, and each breed evolved is acclimatized corresponding to each topographical zone (Pradhan and Gurung1985). In Nepal there are four commonly documented breed of goats as Chyangra, Sinhal, Khari and Terai goats. Chyangra goats are found in 2400 meters in high Himalayans, while Sinhal found in high hill ranging 1500-3000 meters from sea level. Khari are available across the hills of Nepal while Terai goats are available in Terai region of Nepal. Chyangra (1%), Sinhal (16%), Khari/hill goat (56%) and remaining 27% are of Terai and other breeds (Pokharel and Neopane, 2008).

MATERIALS AND METHODS

This chapter deals with the site of study, data collection and recording procedures, data analysis techniques, description of data sets and models used for analyzing the recorded traits.

Time and Location of the study

This study was carried out from September, 2015 to August 2016 in Rolpa district of mid-western Nepal. Rolpa covers an area of 1,879 km² with population (2016) of 221,177. Rolpa is drained southward by the Madi River from a complex of 3,000 to 4,000 meter ridges about 50 kilometers south of the Dhaulagiri Himalaya (Statoids, 2014). The Rolpa district lies at the height of 701m to 3639 meters above the sea level. The total area is 189385 hectares out of which 59854.5 hectares of land is used for crop farming, forest consists 84474 hectares, pasture consists 32698.8 hectare, wild plants and forages 9620.8 hectare and rivers and rocks 1251.9 hectares. The average temperature in Rolpa district is maximum (31.2 Celsius), minimum (3.6 Celsius) and annual rainfall is 441mm (CBS, 2016).

Sampling procedure and sample size

The datas were collected on the basis of Pocket areas of goats distributed within different altitude at Rolpa district. The selected high altitude VDCS were (Jaimakshala and

Pakhapani) followed by mid altitude VDCS (Gairigaun and Libang) and low altitude VDCS (Masina and Jhenam). The elevations of high, mid and low altitude from the sea level in Rolpa district are at the range of 3639, 1375 and 701 m respectively. The elevation of selected areas for this research low, mid and high altitudes were 800-1000, 1200-1400 and 1500-1800 m respectively from the sea level. The two wards were selected from each VDCS. From each ward at least 40 does with reproductive parameters were recorded. The reproductive parameters of does were parity, age at first heat (puberty), age at first kidding, gestation length, kidding interval and post-partum estrus. Within the population, in each selected site, sampling goats were identified randomly. A data recording format was developed to collect data and information related to growth performance, reproductive performance and litter traits of hill goats reared in the study area.

Statistical analysis

Collected data were entered in the computer using MS- Excel and converted into text documents i.e. Text (MS-DOS). To study the main causes of variation and effects of non-genetic factors on productive and reproductive traits, as well as to overcome the difficulty of disproportionate subclass numbers, data were analyzed by least squares procedure using Harvey, (1990) which is based on least squares technique of variance analysis. The pair wise comparison of the least square mean comparison was made using DMRT (Duncan's Multiple Range Test) (Duncan, 1955) as modified by Kramer (1957).

Models used to analyze the collected data

A fixed effect model given by Handerson (1953) was used to analyze the body weights of kids at different stages of growth.

Model I (fixed effect model) for dam weight and reproductive traits

$$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + e_{ijklmn}$$

Where, μ is the overall mean

a_i is the effect of i^{th} altitude ($i=1,2$ and 3)

b_j is the effect of j^{th} type of breed ($j = 1, 2$ and 3)

c_k is the effect of k^{th} type of colour ($l= 1,2,3$ and 4)

d_l is the effect of parity ($k= 1,2,3,4,5,6,7$ and 8)

e_{ijk} is the random element assumed (error mean) to be normally and independently distributed among the sampled population.

RESULTS AND DISCUSSION

This chapter describes the study results focusing to the growth performance, morphological traits, reproductive traits, and litter traits as well as production systems of goats.

Body weight of does

The mean body weight of doe was 38.26 ± 0.61 as mentioned in below Table (1). The data were collected and analyzed from one to seven-year-old does. Joshi *et al.* (2003) reported that the average body weight of Khari, Khapari and Khabari does were 20-40, 34.4 ± 10.2 and 27.4 ± 6.6 kg respectively.

Table 1. Least square means for body weight (kg) of does in Rolpa district, Nepal, 2015/16

Factors	LS $\pm$ SE	NO	Significant Level
Overall	38.26 ± 0.61	482	
Altitude			***
LA	39.40 ± 0.72^a	162	
MA	37.55 ± 0.65^{ab}	160	
HA	36.81 ± 0.77^b	160	
Breed			**
Khari	34.73 ± 0.63^b	223	
Khapari	39.23 ± 0.67^a	180	
Khabari	37.80 ± 0.82^a	79	
Colour			NS
Black	38.80 ± 0.66	192	
Brown	38.02 ± 0.64	183	
White	38.87 ± 0.10	41	
Mixed	37.33 ± 0.87	66	
Parity			***
1	35.14 ± 0.45^b	189	
2	36.27 ± 0.37^b	282	
3	43.36 ± 0.16^a	11	

Note: **significant at 1% ($P < 0.01$) ***significant at 0.1% ($P < 0.001$), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Teat length of does

The overall average teat length of goats was 6.19 ± 1.39 cm. The various factors effecting the teat length of does are presented in Table (2). Hashan *et al.* (2010) reported jamunapari $8.5\text{cm} \pm 1.5\text{cm}$ long teat length. Fasulkov *et al.* (2014) local Bulgarian breed, 2 to 8 years of age, weighing 45-60 kg. Sixty teats were examined by ultrasonography using a 7.5 MHz linear transducer and the “water bath” technique. Ultrasound measured parameters were teat canal length and diameter, diameter in the region of Furstenberg’s rosette, widest teat cistern diameter and teat wall thickness. Ultrasound scans were done during the 1st, 3rd and 5th months of lactation. The experiment revealed the mean teat length of goat to be 6.75 cm.

Table 2. Least square means for teat length (cm) of does in Rolpa district, Nepal, 2015/16

Factors	LS±SE	NO	Significant Level
Overall	6.19 ±1.39	482	
Altitude			*
LA	5.76 ±1.67 ^b	162	
MA	7.03 ±1.47 ^a	160	
HA	5.79 ±1.80 ^b	160	
Breed			NS
Khari	5.96±1.52	223	
Khapari	6.37±1.47	180	
Khabari	6.22±1.93	79	
Colour			**
Black	5.30 ±1.52 ^b	192	
Brown	5.43 ±1.47 ^b	183	
White	8.10 ±2.31 ^a	41	
Mixed	5.89 ± 1.77 ^{ab}	66	
Parity			NS
1	6.37±1.01	189	
2	6.03±0.83	282	
3	5.13±1.4	11	

Note: * significant at 5% ($P<0.05$), **significant at 1% ($P<0.01$), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Udder circumferences of does

The following section describes the detail about the udder circumferences of does representing Rolpa district. The average udder circumference from one to seven-year-old does in this study was 29.46 ± 1.43 cm. The various factors affecting the udder circumference of does are presented in below Table (3). The udder is a very important gland in reproducing animals and for milk production. Several studies have confirmed that udder and teat characteristics are important determinants of milk yield and ease of milking or milking ability in dairy animals (Akpa *et al.*, 2013). Udder and teat characteristics have been shown to be influenced by several factors such as genotype, breeding and management systems (Milerski *et al.*, 2006). Abu *et al.* (2013) also reported udder circumference of West African dwarf goats on average 22.60 ± 0.78 cm reared under semi-intensive and 22.69 ± 0.82 cm reared under extensive systems of management. This result is similar finding with this study.

Table 3. Least square means for udder circumference (cm) of doe in Rolpa district, Nepal, 2015/16

Factors	LS±SE	NO	Significant Level
Overall	29.46 ±1.43	482	
Altitude			***
LA	31.90 ±1.80 ^a	162	
MA	32.76 ±1.60 ^a	160	
HA	23.74 ±1.93 ^b	160	
Breed			NS
Khari	29.33±1.52	223	
Khapari	29.79±1.67	180	
Khabari	29.28±2.05	79	
Colour			***
Black	29.92 ±1.67 ^a	192	
Brown	30.86 ±1.60 ^a	183	
White	30.88 ±2.51 ^a	41	
Mixed	26.21 ± 2.18 ^b	66	
Parity			NS
1	29.64±1.09	189	
2	29.03±0.88	282	
3	29.71±1.60	11	

Note: ***significant at 0.1% ($P<0.001$), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Age at first heat on does

This is an important economic trait of goats that determines their productive lifespan. The overall age at first heat of does was 299.19±0.83 days. Fauque *et al.* (2010) studied reproductive traits of black bengal goats at bangladesh. The good management system might be the cause to show the early signs of heat in does. The study revealed that the age at first heat range between 123-294 days. He studied age at first heat in three consecutive seasons. On first, second and third season the age at first heat in does were recorded as 152.29, 216.16 and 193.2 days respectively. The effects of season and feeding level on age at first heat were significant ($P<0.01$) but rearing system did not affect ($P>0.05$) the age at first heat. Amin *et al.* (2000) also observed the age of first heat in randomly bred Black Bengal goat to be 241.23±15.18 days. The factors effecting age at first heat on does are presented in below Table (4).

Table 4. Least square analysis of means (days) of first heat in does at Rolpa district, Nepal 2015/ 2016

Factors	LS±SE	NO	Significant Level
Overall	299.19 ±0.83	482	

Altitude			***
LA	288.62±0.39 ^b	162	
MA	331.76 ±0.35 ^a	160	
HA	289.15 ±0.42 ^b	160	
Breed			***
Khari	298.54±0.34 ^b	223	
Khapari	323.71 ±0.37 ^a	180	
Khabari	300.01±0.45 ^b	79	
Colour			NS
Black	297.36±0.36	192	
Brown	300.10±0.15	183	
White	319.28±0.54	41	
Mixed	279.71± 0.47	66	
Parity			*
1	316.8 ±0.24 ^a	189	
2	294.62±0.20 ^b	282	
3	292.80±0.88 ^b	11	

Note: * significant at 5% (P<0.05), ***significant at 0.1% (P<0.001), NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Age at first kidding

The overall age of first kidding age of hill goats was 493.99±0.81 days as mentioned detail in below Table (5). This finding is somewhat closely resemblances with the findings of Joshi *et al.* (2003) where the author reported that the age at first kidding of Khari, Khapari and Khabari goats were 478, 577 and 564 days respectively. However, Pandey SR (2009) reported that the age at first kidding of Khari, Khapari and Khabari goats were 549, 696 and 588 days respectively. The age at first kidding was reported in between 387-693 days among the various production patterns and goat breeds (Zarkawi and Abu-Saker, 2013).

Table 5. Least square means for age at first kidding (days) of does in Rolpa district, Nepal, 2015/16

Factors	LS±SE	NO	Significant Level
Overall	493.99 ±0.81	482	
Altitude			***
LA	475.42 ±0.38 ^b	162	
MA	524.42 ±0.34 ^a	160	
HA	482.11 ±0.41 ^b	160	
Breed			**
Khari	506.20 ±0.33 ^b	223	

Khapari	509.64 ±0.36 ^a	180	
Khabari	505.55 ±0.43 ^b	79	
Colour			NS
Black	490.64±0.35	192	
Brown	499.46±0.34	183	
White	512.25±0.53	41	
Mixed	471.77± 0.46	66	
Parity			NS
1	506.46±0.24	189	
2	490.94±0.19	282	
3	483.94±0.86	11	

Note: **significant at 1% (P<0.01) ***significant at 0.1% (P<0.001), NS-non significant, LSD-Least significant difference, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Gestation length

The overall mean gestation length of hill goat as reflected in this study was 150.04±0.11 days, with the range of 147 to 154 days. This finding was also in agreement with the results of study conducted by Pokharel and Neopane (2008) in Hill-goats. The various factors affecting the gestation period of does are presented in below Table (6).

Table 6. Least square means for Gestation Length (days) of does in Rolpa district, Nepal, 2015/16

Factors	LS±SE	NO	Significant Level
Overall	150.04 ±0.11	482	
Altitude			NS
LA	150.12±0.12	162	
MA	149.99±0.11	160	
HA	150.00±0.14	160	
Breed			
Khari	149.98±0.11	223	NS
Khapari	150.14±0.12	180	
Khabari	149.99±0.14	79	
Colour			NS
Black	150.12±0.12	192	
Brown	149.95±0.15	183	
White	150.09±0.18	41	
Mixed	149.95± 0.15	66	
Parity			NS
1	150.09±0.82	189	
2	150±0.06	282	

3	150±0.29	11	
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Note: NS-non significant, LS mean- Least square mean, LSD-Least significant difference, SE- Standard error of mean, NO are the number of observations.

Kidding Interval of does

The overall mean kidding interval was 251.27±0.40 days mentioned detail in Table (7). However, Pokharel and Neopane (2008) also reported lower kidding interval of central Terai goats (218±5 days) which was in agreement with the findings of this study. According to Kamal and Nikhaila (2009) another factor which can lead to long open period is under nutrition which extends Kidding Interval. The high level of feeding after kidding shortens the interval from first breeding to conception and thus reduces Kidding Interval. Quality and quantity of feed have effect on Kidding Interval which seems to be shorter (250 days) for goats under plenty feed and longer (314 days) for goats under feed shortage. Rolpa farmers might have supplied good quality feed to does during breeding to conception. A study done by Alphonsus *et al.* (2010) in Nigeria found the KI to be 6.9 months (207 days), which is close to 204 days reported by Sodi (2014) in Indonesia on goat raised under traditional management.

Table 7. Least square means for kidding interval (days) of does in Rolpa district, Nepal, 2015/16

Factors	LS±SE	NO	Significant Level
Overall	251.27 ±0.40	394	
Altitude			*
LA	247.01 ±0.19 ^b	113	
MA	259.49 ±0.17 ^a	140	
HA	247.62 ±0.20 ^b	141	
Breed			NS
Khari	244.23±0.17	178	
Khapari	260.31±0.13	151	
Khabari	254.57±0.21	65	
Colour			NS
Black	248.23±0.17	159	
Brown	223.71±0.12	142	
White	226.45±0.26	37	
Mixed	217.01±0.23	56	
Parity			NS
1	230.40±0.13	106	
2	224.62±0.09	279	
3	209.10±0.42	9	

Note: * significant at 5% (P<0.05), NS-non significant, LS mean- Least square mean, LSD-Least significant difference, SE- Standard error of

mean. NO is the number of observations.

Postpartum estrus of does

The overall mean post-partum estrus interval of the hilly goat flock was 91.22 ± 0.94 days with the range of 88 to 96 days. The postpartum period is characterized by uterine involution and restoration of ovarian functions, since both should occur to establish a new pregnancy. The completion of uterine involution was defined as the day when the diameter of the uterus returned to the original non-pregnant size as observed during the normal estrous cycle. Postpartum period start from parturition and lasts until uterine involution is completed and female resumes ovarian activity (Garcia, *et al.* 2008). The interval between parturition and the first post-partum estrus is an important trait which contributes to the productive efficiency of a doe. The involution of the post-partum uterus was one of the economic important limitations in achieving the goal of suitable kidding interval. Postpartum estrus period was 77.00 ± 4.04 and 95.33 ± 2.60 days as reported by Sadat (2014) under semi-intensive production are in close with little difference to the present study in Kamohri goats (Greyling, 2004).

Table 8. Least square means for postpartum estrus (days) of does in Rolpa district, Nepal, 2015/16

Factors	LS $\pm$ SE	NO	Significant Level
Overall	91.22 $\pm$ 0.94	482	
Altitude			NS
LA	89.20 $\pm$ 0.44	162	
MA	96.44 $\pm$ 0.40	160	
HA	88.01 $\pm$ 0.48	160	
Breed			NS
Khari	92.83 $\pm$ 0.39	223	
Khapari	88.62 $\pm$ 0.42	180	
Khabari	92.19 $\pm$ 0.51	79	
Colour			NS
Black	90.54 $\pm$ 0.41	192	
Brown	91.35 $\pm$ 0.40	183	
White	91.27 $\pm$ 0.62	41	
Mixed	91.70 $\pm$ 0.54	66	
Parity			NS
1	89.71 $\pm$ 0.28	189	
2	95.12 $\pm$ 0.23	282	
3	88.82 $\pm$ 0.10	11	

Note: NS-non significant, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

Kidding rate of does

The average kidding rates is 1.54 ± 0.16 in this study. Average kidding rate of Rolpa goat was 1.54 ± 0.16 . HMG/N, (1993) had also reported similar value of kidding rate (i.e. 1.47) of local hill goats. The kidding rate of Khari, Khapari, Khabari breed were 1.41 ± 0.15 , 1.58 ± 0.17 and 1.59 ± 0.20 respectively. Pandey SR (2009) reported that the kidding rate of Khari, Khapari, Khabari goats were 1.70, 1.31, 1.44 respectively. However, Neopane (2000) reported the higher kidding rate of central Terai goat (1.60) and Tamrakar and Chapagain (2000) also reported similar value of kidding rate of Terai (1.60) and Barbari Goats (1.63) reared at RARS, Nepalgunj. The detail of kidding rates is presented in below Table (9).

Table 9. Least square means for kidding rate of does at Rolpa district, Nepal, 2015/16

Factors	LS $\pm$ SE	NO	Significant Level
Overall	1.54 ± 0.16	482	
Altitude			NS
LA	1.55 ± 0.18	162	
MA	1.52 ± 0.16	160	
HA	1.51 ± 0.17	160	
Breed			NS
Khari	1.41 ± 0.15	223	
Khapari	1.58 ± 0.17	180	
Khabari	1.59 ± 0.20	79	
Colour			*
Black	1.40 ± 0.17^{bc}	192	
Brown	1.55 ± 0.16^{ab}	183	
White	1.86 ± 0.23^a	41	
Mixed	1.29 ± 0.19^{bc}	66	
Parity			NS
1 Year	1.52 ± 0.15	56	
2 Year	1.55 ± 0.18	88	
3 Year	1.59 ± 0.25	6	

Note: * significant at 5% ($P < 0.05$), NS-non significant, LSD-Least significant difference, LS mean- Least square mean, SE- Standard error of mean. NO is the number of observations.

DISCUSSION

This study was designed to characterize and evaluate the productive and reproductive performance of hill goat reared under farmers' condition in Rolpa district of Nepal during December, 2015 to July 2016. The other objectives were to study the effect of non-genetic factors like altitude, colour, age, sex, parity etc in relation to the existing goat production

system. Periodic observations on morphological traits, productive and reproductive performance were measured and recorded. The high altitudes VDCS were (Jhamksala and Pakhapani) followed by mid altitude VDCS (Gairigaun and Libang) and finally to low altitude VDCS (Masina and Jhenam). The elevation of selected high, mid and low altitude from the sea level in this research is at the range of 3639, 1375, 701 m respectively. The elevation of selected goat pocket areas for this research at low, mid, high altitudes were 800-1000, 1200-1400, 1500-1800 m respectively from the sea level. The two wards were selected from each VDCS. There were three recognized breeds Khari, Khapari (Khari*Jamunapari) and Khabari (Khari*Barbari). However, Chyangra, Boers and Terai crosses were also observed in a few numbers. The data of 482 does were collected from different altitudes of Rolpa districts. Farmers and technicians claimed the selected goats of being pure Khari, (Khari and Jamunapari) 50% cross breed and (Khari and Barbari) 50% cross breed. However, characterization of such breeds at molecular level was not carried at Rolpa district. So, it could not be understood so far their DNA level.

Morphological attributes, productive, and reproductive performance were collected based on field monitoring and measurements. Least square analysis was performed using Harvey (1990) computer software package. Information on goat production systems were collected by employing a semi-structured questionnaire and analyzed using SPSS.

The non-genetic factors like altitude, color, sex, season and parity were considered for morphological and reproductive parameters of does. The overall mean for body weight of does were 38.26 ± 0.61 kg. Low altitude body weight of does 39.40 ± 0.72 kg were significantly heavier ($P < 0.001$) than that of mid altitude doe (37.55 ± 0.65 kg) and high altitude doe (36.81 ± 0.77 kg). Khapari does body weight (39.23 ± 0.67 kg) were significantly heavier ($P < 0.01$) than that of Khari (34.73 ± 0.63 kg) and Khabari (37.80 ± 0.82 kg). Parity of does also differed significantly ($P < 0.001$) to the body weight of does. The non-genetic factors like altitude, colour, age, were considered for morphological traits of goats. The non-genetic factors like altitude, color and parity were considered for reproductive performance of does.

The overall mean of age at first heat was 299.19 ± 0.83 days. The age of first heat in mid altitude (331.76 ± 0.35) days was significantly higher ($P < 0.001$) than that of high altitude (289.15 ± 0.42) days and low altitude (288.62 ± 0.39) days. The age of first heat in Khari (298.54 ± 0.34) days was significantly higher ($P < 0.001$) than that of Khapari (323.71 ± 0.37) days and Khabari (300.01 ± 0.45) days.

The overall mean for age at first kidding of does was 493.99 ± 0.81 days. The age at first kidding at mid altitude (524.42 ± 0.34) was significantly higher ($P < 0.001$) than that of low altitude (475.42 ± 0.38) and high altitude (482.11 ± 0.41) days respectively. The age at first kidding for Khari, Khapari and Khabari breeds were 506.20 ± 0.33 , 509.64 ± 0.36 and

505.55±0.43 days respectively. Age at first kidding differed significantly ($P<0.001$) with respect to breed.

The overall mean for gestation length was 150±0.11 days. The kidding interval of mid altitude (251.49±0.17) days was significantly ($P<0.05$) higher than that of low altitude (247.01±0.19) days and high altitude (247.62±0.20) days. The mean for post-partum estrus was 91.22±0.94 days. The overall mean for Kidding rate of does was 1.54±0.16. The kidding rates of Khapari, Khari and Khabari does were 1.58±0.17, 1.41±0.15 and 1.59±0.20 respectively. Kidding rates differed significantly ($P<0.5$) with respect to coat colour of does. The overall mean kidding interval was 251.27±0.40 days. The overall mean for body length of does were 74.29±0.99 cm. There was differed significantly ($P<0.001$) with respect to altitudes. The overall least square mean for heart girth of does were 74.21±0.66 cm. It differed significantly ($P<0.001$) within altitude, breed ($P<0.05$) and parity ($P<0.05$). The overall least square mean of wither height of does were 68.19±1.85 cm. The overall mean for teat length of does were 6.19±1.39 cm. Teat length differed significantly ($P<0.05$) with respect to altitude and ($P<0.01$) within Colour. The overall mean for udder circumference of does were 29.46±1.43 cm. It also differed significantly ($P<0.001$) within altitude and colour. Goat farming was practiced as a subsistence occupation with three distinct systems of feeding i.e. extensive grazing, stall feeding, and grazing plus stall feeding supplementing little maize grains and flour as per availability. Majority of the farmers depended on the community forest as well as on their own farmland for collecting fodder and forages. Major problems of goat farming in Rolpa district were occurrence of epidemic disease, lack of veterinary and technical advice, and attack of wild animal. Thus the results of this study suggest that the performance of low altitude goat flocks was better than mid and high altitude goat flocks in Rolpa district in terms of production and reproduction traits. This result could be attributed to superior genotype along with better management practices followed by the farmers in Rolpa district.

CONCLUSION

Results revealed that the mean age of first heat in does (299 days), age at first kidding (493 days), gestation length (150 days), kidding interval (251 days), postpartum estrus (91 days), and kidding rates were (1.54 per doe), respectively. Altitude significantly influenced ($P<0.001$) along with parity ($P<0.05$) with respect to age of first heat in does. Significance difference of kidding interval ($P<0.05$) was significantly influenced within different altitude of goat farming. Similarly, colour and altitude also significantly differed ($P<0.05$) with respect to kidding rates of does. Thus the results of this study suggest that the performance of low altitude goat flocks was better than mid and high altitude goat flocks in Rolpa district in terms of production and reproduction traits. This result could be attributed to superior genotype along with better management practices followed by the farmers in Rolpa district.

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REFERENCES

1. Abul A. H., L. I. Mhomga and Akogwu E. I. 2013. Assessment of udder characteristics of West African Dwarf (WAD) goats reared under different management systems in Makurdi, Benue State, Nigeria. *African Journal of Agricultural Research* 8(25): 3255-3258pp.
2. Akpa, G.N., M.S. Abdulkareem, I.R. Muhammed and C. Alphonsus. 2013. Effect of breed and age on measures of growth and scrotal size in Rams. *Nigeria Journal of Animal Science* 12: 1-6pp.
3. Alphonsus C., H. Finangwa, S.M. Yashim, O.C.P Agubosi and Sam I.M. 2010. Effect of dam parity on measures of growth in red sokoto goats 1, 3, 6 and month of age. *Journal Animal Veterinary Resources* (2): 9-13pp.
4. Amin, M.R., S.S. Husain and A.B. Islam. 2000. Reproductive peculiarities and litter weight in different genetic groups of Black Bengal does. *Asian-Australasian Journal of Animal Science* 14(3): 297-301pp.
5. Central Bureau of Statistics (CBS). 2016. National Population and Housing Census 2011 Volume 03. Kathmandu: Government of Nepal, National Planning Secretariat. 28pp.
6. Faruque, S., A. Chowdhury, N. U. Siddiquee and M. A. Afroz. 2010. Performance and genetic parameters of economically important traits of Black Bengal goat. *Journal of Bangladesh Agriculture* 8(1): 67-78pp.
7. Fasulkovi. R., P.I. Georgiev, A.L. Antonov and A.S. Atanasova. 2014. B-mode ultrasonography of mammary glands in goats during the lactation period. *Bulgarian Journal of Veterinary Medicine* (13): 245-251pp.
8. Garcia J.E., R Lopez and A. Rodriguez. 2008. Factors affecting the reproductive performance of goats under intensive conditions in a hot arid environment. *Small Ruminant Research* (63): 110-118pp.
9. Greyling, J. P. C. 2004. The Induction of puberty in female Boar goat kids. *South African Journal of Animal Science* (20): 193-200pp.
10. Hassan. M.R., M. A. I. Talukder and S. Sultana. 2010. Evaluation of the production characteristics of the Jamunapari goat and its adaptability to farm conditions in Bangladesh. *The Bangladesh Veterinarian* 27(1): 26 – 35pp.
11. Harvey W.R. 1990. Least-square analysis of data with unequal subclass numbers. United States Department of Agriculture, Agricultural Research Service, ARS 20, USA.
12. HMG/N.1993. Livestock Master plan. His Majesty's Government of Nepal Livestock development. Asian development bank. ANZDEC Limited, Agricultural Project Service Center.
13. Joshi, B. R and B.S Shrestha.2003. The Goats. Their Production and Health Management. 11-18pp.
14. Kamal, E. E. and Nikhaila, A. M. A.2009. A Study on some factors affecting mortality rates in Sudanese Nubian kids. *International Journal of Dairy Science* (4): 74 – 79pp.
15. Milerski M., M. Margetin, A. Capistrak, D. Apolen, J. Spanik and M. Oravcova. 2006. Relationships between external and internal udder measurements and the linear scores for udder morphology traits in dairy sheep. *Czech Journal of Animal Science* (51): 383-390pp.
16. MOAD. 2018. Selected indicators of Nepalese agriculture and population. Government of Nepal. Ministry of Agriculture and Cooperatives. Agribusiness promotion and statistical division. Singha Darbar, Kathmandu, Nepal

17. Neopane, S.P. 2000. Characterization of local breed of goat in the central Terai of Nepal. In: Proceedings of the fourth National Workshop on Livestock and Fisheries Research. 214-217pp.
18. Pandey SR, M.R. Kolacchapati, N.R. Devkota and S.P. Neopane. 2009. Effect of genetic groups on litter and dam traits of goat under the western hill condition of Nepal. *Journal of institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan* (30): 87-96pp.
19. Pokharel PK and SP Neopane. 2008. Indigenous Goats of Nepal. National Animal Science Research Institute. Nepal Agricultural Research Council. Khumaltar, Lalitpur. 3-8pp.
20. Sadat, S. 2014. Reproductive performances of Djallonke Sheep in the Northern region of Ghana. A master thesis submitted to University of Sciences and technology, Kumasi.
21. Sodiq, A. 2014. Doe production of Kacang and Peranakan Etawah goat and factors affecting them in the tropics and sub tropics in Indonesia. *Journal of Agricultural and Rural Development* (78): 1 – 11pp.
22. Statoids. 2014. Districts of Nepal. Gwillim Law. Land administration and management mapping, surveying and aerial photogrammetry, Major references projects. Finn map.
23. Tamarakar N.L. and P.B. Chapagain. 2000. Performance of Native Terai and Barbari Goats at RARS, Nepalgunj. In: Proceedings of the fourth National Workshop on Livestock and Fisheries Research, Kathmandu, Nepal. 193-196pp.
24. Zarkawi, H., K. KUTHU, K. Javed, M.E. Babar, A. Sattar and M Abdullaha. 2013. Environmental effects on growth traits of teddy goats. *The Journal of Animal and Plant Sciences* 23(3): 692p.

Association of Body Condition Score with Lactation and Reproductive Performance in Murrah Cross Buffaloes in Chitwan, Nepal

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ABSTRACT

Body condition score (BCS) is a precise method for determining the stored energy reserves of dairy animals, which are located in the form of subcutaneous fat tissue, indicated by the animal's degree of accomplished nutrition, and could be used as a management and selection tool to improve reproductive performance. The objective of this research was to investigate and quantify relationships between BCS and milk production, milk composition, and reproductive performance in Murrah cross buffalo. For this purpose, a semi-structured questionnaire was created and BCS was calculated using a visual technique based on a BCS chart. The sampling approach employed was a completely randomized design. A total of 165 Murrah cross buffaloes from 59 household farms were used in the study, where 130 milk samples were collected and used to assess the milk parameters by lacto-scan. Four BCS groups were namely 2.5-2.99, 3.0-3.49, 3.5-3.99, and 4.0-4.99 where the BCS score was found to grow during the dry period until calving, then decreased as milk production increased. BCS group (3.5-3.99) had higher performance during all the lactation periods with a peak milk yield of 11.4 ± 1.1 kg. At the late lactation stage BCS group (3.5-3.99) showed higher values of SNF (9.77), protein (4.01), and lactose (3.82), followed by BCS groups 4.0-4.49, 3.0-3.49, and 2.5-2.99. At the early, lactation stages, the BCS group (3.5-3.99) had the highest ($P < 0.01$) average milk yield of 10.28, 9.50, 9.37 kg, and the BCS group (4- 4.49) had highest ($P < 0.01$) average fat percent of 9.19, 10.20, and 10.32, respectively. The buffaloes of BCS group 3.5-3.99 had shorter post-partum estrus (61.14days) ($P < 0.01$), fewer services for conception (1.30) ($P < 0.01$) and higher first service conception rate (79.87%) ($P < 0.01$). Similarly, the incidence of associated reproductive disorders such as uterine prolapse, retained placenta, metritis, and mastitis are lowest in BCS group 3.5-3.99 with values of 7.69%, 3.44%, 3.44%, and 6.89% respectively. It can be concluded that the BCS group (3.5–3.99) provided the greatest production results in both milk and reproductive function. For optimal animal lactation and reproductive performance, the nutrition management of dairy cows should be necessarily monitored using the BCS score.

Keywords: BCS, milk parameters, Reproductive performance, Lactation stages, Chitwan

INTRODUCTION

The body condition score (BCS) system is a precise, instantaneous, inexpensive and universally accepted method for determining the stored energy reserves of dairy animals (Alapati et al., 2010). BCS is assessed in terms of subcutaneous fat cover, muscle appearance, and apparent skeletal features through subjective visual appraisal and (or) manual palpation, indicated by the animal's degree of accomplished nutrition (Momon & Pruitt, 2016). It gives the visual evaluation of the animal's body fat reserves thus influencing the energy balance. It provides the biological relationship between milk production, milk parameter, and reproductive performance. A computed relationship between BCS and other parameters facilitates the adoption of the most advantageous control practices to derive maximum production and maintain better health status (Obese & Tecku, 2019). The most widely used and traditional method of body condition scoring is by manual observation and or physical examination of the animal to assess overall body condition (Edmonson et al., 1989). Animals with higher BCS have early post-partum estrus, better breeding efficiency, and lower reproductive disturbances (Pryce et al., 2001). During early lactation, the animal undergoes a negative energy balance as a result of the utilization of energy reserves for the production of milk; as a result BCS goes on decreasing (Souissi & Bouraoui, 2020). Various body condition scoring systems have been developed by many scientists like (Jefferies, 1961) using 0 to 5 scale in ewes, (Lowman et al., 1976) using a 0 to 5 scale in beef cattle and (Earle, 1976) using an eighth-grade system in dairy cows. (Edmonson et al., 1989) developed a chart for body condition scoring of Holstein dairy cows on a 1 to 5 scale using 0.25 increments. Recently, a new BCS system was developed for Murrah buffalos with a 1-5 scale having 0.5 increments examining eight skeletal checkpoints that adequately reflected the actual fat reserves (Alapati et al., 2010).

In Nepal, livestock is an important part of the national economy and an important sub-sector of agriculture. Agriculture contributes 33% of total GDP and the livestock sub-sector contributes 11 percent to the national GDP, and approximately 27 percent to AGDP (MOALD, 2019). There are in total 5.30 million populations of buffaloes with annual milk production of 1,372,905 liters of which 76,112 buffaloes in Chitwan with total milk production 23,909 liters of annual milk productions (MOALD, 2019). Livestock creates employment opportunities for unemployed and influence on the upliftment of rural economy. Buffalo contributes to milk, meat, manure and draught purposes. Murrah is an important buffalo breed introduced from India for improving indigenous buffalo production and has major cross breed population of buffaloes in Nepal. BCS is an indicator for the energy reserve of an animal which influences the productive and reproductive parameters of the dairy animals. BCS is related to the animal's puberty, sexual maturity, fertility, ovulation, overall health of reproductive tracts and calf, milk production and milk compositions. Body condition score is one of the determining factors for the resumption of the reproductive performances and occurrence of reproductive disorders after calving.

Estimation of the body condition of dairy animals and relating it to productive and reproductive status helps farmers access their overall efficiency of feeding and husbandry practices of farm animals. BCS system helps to acquire optimum management practices for better health, production and reproduction of dairy animals. Poor availability of nutrition during summer season shows higher ovarian inactivity in buffaloes. Poor body condition score leads to the prolongation of the postpartum anestrus and postpartum reproductive disorders (Devkota et al., 2012). Murrah cross buffaloes have better reproductive and productive efficiency than indigenous local buffaloes and has added economic benefits to farmers. Knowing the impact of income and livelihood of buffaloes farming communities and future prospective of making buffaloes as major dairy farming animals, there is also a national policy for cross breeding of high producing murrah buffaloes with indigenous for the genetic improvement of native buffaloes. This study investigates the nutritional status of murrah buffalo in terms of BCS and quantifies relationships between BCS, milk production, milk composition, and reproductive efficiency in murrah crosses buffalo in Chitwan, Nepal.

MATERIALS AND METHODS

Study Area

The present study was conducted at buffalo farming areas of Bharatpur Metropolitan City, Chitwan, Nepal, which lies in the tropical zone around 27 degrees 39 minutes and 14 seconds latitude and 84 degrees, 21 minutes and 6 seconds longitude with the altitude of about 415 m from the sea level. The area has a tropical monsoon climate with high humidity all through the year. The seasonal environmental fluctuation in chitwan, Nepal is typical with cold and semi-dry to dry winter, rapidly increasing hot and dry spring, very hot and rainy monsoon summer and moderate autumn. Annual rainfall and temperatures range between 13- 690 mm and 15.2°C to 27.6°C respectively and relative humidity ranges from 52.85 to 86.87 % (Weather_24, 2021). The Indian Murrah breed or its crosses with indigenous breeds make up the majority of the buffalo population in this area. The main feed supply for buffaloes here is seasonal monsoon fodder during the rainy months and rice straw hay after October/November.

Sampling Procedure and Data Collection

The sampling approach employed was completely randomized design. For documentation a pre-tested semi-structured questionnaire was made. The total 165 Murrah cross buffaloes from 59 household farms were used for the study. Parity (mean $\pm$ S.E.) of buffaloes was 3.65 ± 1.71 (range: 2 to 13). Fifty one buffaloes were of early (0-3 months) lactation stages, forty three were of mid (4-6 months), thirty six were of late (>7months), and thirty five were of dry periods (Yadav et al., 2013). Milk samples from 150 buffaloes were taken from the milking bucket during morning milking and placed in a screw-capped plastic tube, which was then carried to the laboratory in a cool box to determine the fat, SNF,

lactose, and protein levels by Lacto-scan (Ultrasonic Milk Analyzer, Milkotronic Ltd., Bulgaria) in the laboratory of the National Cattle Research Program (NCRP), Rampur, Chitwan.

Determination of body condition score (BCS)

The body condition score (BCS) of each buffalo in the study was determined at calving using a visual technique devised by (Alapati et al., 2010) based on a BCS chart of (1-5) scale with 0.5 increments analyzing eight skeletal checkpoints. A score of 1 indicates emaciated, 2 indicates thin, 3 indicates average, 4 indicates fat and 5 indicates obese condition (Alapati et al., 2010). Based on BCS, the buffaloes were classified into following four groups, namely 2.5-2.99, 3.0-3.49, 3.5-3.99, 4.0-4.99.

Recording of milk yield, reproductive performance parameters and reproductive disorders

The data regarding daily milk yield, peak milk yield, calving to first postpartum estrus interval, number of services per conception, and first service conception rate (%) were obtained from the records of the farm.

Data entry and analysis

The collected information and data were coded, entered and analyzed by using MS-Excel and SPSS 16.0 software. Relationship between BCS with present milk yield, peak milk yield, milk composition, reproductive parameters were assessed using a one-way ANOVA followed by post Hoc Tukey test. The incidence rate of reproductive disorders and mastitis in association with BCS were calculated using simple descriptive statistics and mean were obtained. P value ≤ 0.05 was considered to be significant whereas $0.1 \leq P > 0.05$ considered to have tendency.

RESULTS

Relationship between BCS with present milk yield and peak milk yield

The current daily milk yield for each lactation stage computed in relation to body condition score (BCS) groups of 2.5-2.99, 3.0-3.49, 3.5-3.99, and 4.0-4.99 is shown in Table 1. Buffaloes of BCS group 3.5-3.99 had higher ($P < 0.01$) present milk yields (kg) at early, mid and late lactation, followed by buffaloes of BCS group 4.0-4.49, 3.0-3.49 and 2.5-2.99.

Table 1. Relationship between BCS and present milk yield

BCS	No. of buffalo(n)	Present milk yield (kg/day)		
		Early lactation (n=51)	Mid lactation (n=43)	Late lactation (n=36)
2.5-2.99	52	6.9 $\pm$ 1.04 ^a	5.9 $\pm$ 1.2 ^a	5.6 $\pm$ 0.9 ^a

3.0-3.49	58	8.4±0.6 ^b	7.7±0.9 ^b	7.3±0.5 ^b
3.5-3.99	29	10.3±0.5 ^c	9.5±0.5 ^c	9.4±0.8 ^c
4.0-4.49	26	8.7±0.4 ^b	8.4±0.2 ^{bc}	7.6±0.4 ^b

Note: a, b, c: values with different superscripts within the column differ significantly ($P < 0.05$).

Table 2 shows the relationship between BCS and peak milk yield. Buffaloes of BCS group 3.5-3.99 had higher ($P < 0.01$) peak milk yield followed by buffaloes of BCS group 4.0-4.49, 3.0-3.49 and 2.5-2.99.

Table 2. Relationship between BCS and peak milk yield in test herd

BCS	No. of buffaloes	Peak milk yield(kg/day)	P-value
2.5-2.99	52	7.4 ± 0.8 ^a	<0.0001
3.0-3.49	58	9.2 ± 0.9 ^b	
3.5-3.99	29	11.4 ± 1.1 ^c	
4.0-4.49	26	9.8 ± 0.8 ^b	

Note: a, b, c: values with different superscripts within the column differ significantly ($P < 0.05$).

3.2 Relationship between body condition score (BCS) and milk composition

Milk composition i.e. fat, SNF, lactose, and protein percentages in milk were evaluated independently for three stages of lactation with respect to BCS groups of 2.5-2.99, 3.0-3.49, 3.5-3.99, and 4.0-4.99.

3.2.1 Variation in milk composition with respect to BCS at different stage of lactation

Table 3 shows the milk composition with respect to BCS at different stage of lactation. At all three stages of lactation, the buffaloes of BCS group 4.0-4.49 had the highest ($P < 0.01$) milk fat percentages followed by the buffaloes of BCS groups 3.5-3.99, 3.0-3.49, and 2.5-2.99. Similarly, at all stages of lactation, the buffaloes of BCS 3.5-3.99 had the highest ($P < 0.01$) Solid Not Fat (SNF) percentages followed by the buffaloes of BCS group 4.0-4.49, 3.0-3.49, and 2.5-2.99. Lactose and protein percentages in the milk sample also followed a similar pattern. The buffaloes of BCS 3.5-3.99 had the highest ($P < 0.01$) lactose percentages followed by the buffaloes of BCS group 4.0-4.49, 3.0-3.49, and 2.5-2.99. On overall evaluation, milk fat, SNF, lactose, and protein increased with the advancement of the lactation stage standing on the same body condition score.

Table 3. Relationship between BCS and milk composition

Early lactation					
BCS	No. of buffaloes	Fat%	SNF%	Lactose%	Protein%
2.5-2.99	20	4.80 ^a	7.35 ^a	3.08 ^a	3.13 ^a
3.0-3.49	19	6.72 ^b	8.35 ^b	3.21 ^b	3.40 ^b

3.5-3.99	7	7.83 ^b	9.50 ^c	3.46 ^c	3.88 ^c
4.0-4.49	5	9.19 ^c	8.65 ^b	3.39 ^c	3.69 ^d
Mid lactation					
BCS	No. of buffaloes	Fat%	SNF%	Lactose%	Protein%
2.5-2.99	15	5.88 ^a	7.78 ^a	3.22 ^a	3.21 ^a
3.0-3.49	16	7.11 ^b	8.55 ^b	3.39 ^a	3.47 ^b
3.5-3.99	6	8.10 ^b	9.74 ^c	3.75 ^b	3.97 ^d
4.0-4.49	6	10.20 ^c	8.83 ^b	3.42 ^a	3.72 ^c
Late lactation					
BCS	No. of buffaloes	Fat%	SNF%	Lactose%	Protein%
2.5-2.99	12	6.72 ^a	7.88 ^a	3.27 ^a	3.32 ^a
3.0-3.49	11	7.51 ^b	8.59 ^b	3.45 ^b	3.50 ^b
3.5-3.99	8	8.65 ^c	9.77 ^d	3.82 ^d	4.01 ^c
4.0-4.49	5	10.32 ^d	8.87 ^c	3.61 ^c	3.76 ^d

Note: a, b, c, d: values with different superscripts within the column differ significantly ($P < 0.05$).

3.3. Relationship between body condition score (BCS) and reproductive performance

The reproductive performance of the buffaloes was significantly affected by BCS. Table 4 shows the reproductive performance of buffaloes from various BCS groups. The buffaloes of BCS group 3.5-3.99 had shorter ($P < 0.01$) calving to first postpartum interval (days), fewer ($P < 0.01$) services for conception and higher ($P < 0.01$) first service conception rate as compared to other groups of buffaloes. While summarizing the data, it was shown that reproductive performance improved as the BCS grew up to 3.99, but then began to decline.

Table 4. Reproductive performance of buffaloes of various BCS group

Reproductive Parameter	BCS			
	2.5 - 2.99 (n=52)	3.0 - 3.49 (n=58)	3.5 - 3.99 (n=29)	4.0 - 4.49 (n=26)
Post-partum estrus (days)	82.25 ^b	67.97 ^{ab}	61.14 ^a	62.00 ^a
No. of services per conception	1.5945 ^b	1.3074 ^a	1.2442 ^a	1.3012 ^a
1st service conception rate (%)	50.6779 ^a	74.0498 ^b	79.8724 ^b	74.1338 ^b

Note: a, b, c, d: values with different superscripts within the column differ significantly ($P < 0.05$).

3.3. Relationship between body condition score (BCS) and reproductive disorders

Table 5 showed that out of 200 buffaloes included in this study, the highest incidence of

uterine prolapse was observed in the buffaloes with body condition score (BCS) group 2.5-2.99 with 17.3% which was followed by BCS group 4.0-4.49 with 15.38%, 3.0-3.49 with 10.34%, and 3.5-3.99 with 7.69%. It was showed that highly emaciated and obsessed buffaloes were prone to uterine prolapse. Buffaloes of BCS group 2.5-2.99 were the most susceptible to the retained placenta, with 21.15%, followed by BCS group 4.0-4.49 with 11.53%, BCS group 3.0-3.49 with 6.89%, and BCS group 3.5-3.99 with 3.44%. Occurrence of metritis, on the other hand, followed a slightly different pattern than uterine prolapse and retained placenta. Buffaloes with a BCS score of 2.5-2.99 had the highest prevalence of metritis (25%) followed by BCS groups 3.0-3.49 (13.79%), 4.0-4.9 (7.39%), and 3.5-3.99 (3.44%). Mastitis had a similar trend to metritis in terms of occurrence. Buffaloes with a BCS score of 2.5-2.99 had the highest prevalence of mastitis (26.92%), followed by BCS groups 3.0-3.49 (13.79%), 4.0-4.9 (7.39%), and 3.5-3.99 (6.69%).

Table 5. Association of BCS with various reproductive disorders and mastitis

Disorders	BCS			
	2.5-2.99 (n=52)	3.0-3.49 (n=58)	3.5-3.99 (n=29)	4.0-4.49 (n=26)
Uterine Prolapse	17.3%	10.3 %	7.7%	15.4%
Retained Placenta	21.2%	6.9%	3.4%	11.5%
Metritis	25%	13.8%	3.4%	7.7%
Mastitis	26.9%	8.6%	6.9%	7.7%

DISCUSSION

In this research, the tripod relationship shown between BCS, lactation stages and milk yield is novel approach. The present study revealed that milk production increased with BCS up to a score of 3.99 at all stages of lactation, then began to fall. This finding supported the finding of (Braun et al., 1986), that cows with a BCS 3-3.5 produce more milk than those with a BCS of 2.5 or less. Similarly, (Domecq et al., 1997) observed that cows who gained condition during the dry period provided more milk in the first 120 days of lactation and had a faster rate of growth in milk supply. (Alapati et al., 2010) also showed that lactation yield, 305 day predicted lactation yield, and peak milk yield increased with increase in BCS up to 3.99. In contrast to this study (Ruegg & Milton, 1995) reported that condition score had no effect on either peak or 305-d milk yields. The majority of studies have found a favorable link between BCS and milk output (Roche et al., 2005; Stockdale, 2005), which is similar to the findings of this study. Present research suggested that BCS of (3.50-3.99) is the most appropriate and highly profitable BCS range to maintain in term of peak milk yield. It might be because thin or low BCS animal are more likely to have a higher milk somatic cell count (SCC) and are more prone to mastitis which drastically reduce the milk production (Berry et al., 2007) similarly low

BCS animal or severe negative energy balance may be more susceptible to infection resulting reduced DMI (dry matter intake), poor udder development subsequently reduce in milk production (Bauman & Bruce Currie, 1980; Collard et al., 2000).

Study suggested that buffaloes of BCS group 4.0-4.49 had the highest milk fat percentages 9.19, 10.20, and 10.32 at early, mid and late stages of lactation respectively followed by BCS group 3.5-3.99, 3.0- 3.49, and 2.5-2.99. This is consistent with the findings of (Alapati et al., 2010), who discovered that for every one unit rise in BCS, milk fat percent increased by 1.8 to 2.0 percent. While (Ducháček et al., 2013) observed that milk fat content dropped from 4.89 percent at the start of lactation to 3.27 percent in week 7, then climbed to 4.06 percent in weeks 14 and 16. More the fat reserved in the body more will be the milk fat percentage, it might be because animals with BCS > 3.5 are subjected to significant fat mobilization during the transition period, resulting in higher plasma NEFA to meet the energy demand (Locher et al., 2015). Furthermore, postpartum NEFA discharge typically exceeds the liver's energy requirements and oxidation capacities, resulting in the formation of ketone bodies such as β -hydroxybutyrate and re-esterification to triglycerides (TG) (Locher et al., 2015) might be excreted via milk. In accordance with the present finding (Holter et al., 1990; Roche et al., 2007) also found that under conditioned cow had reduced fat content of milk and it increased with increased in BCS.

Present study revealed that other parameters such as SNF, lactose, and protein were found to be best established at BCS group 3.50-3.99, with declines occurred above and below this level. Study on SNF and lactose by (Alapati et al., 2010) also found buffaloes with BCS group 3.50-3.99 have the highest milk SNF at 6-8 and 16-18 weeks after calving. In contrast to the current findings (Roche et al., 2005) insist that, BCS at calving had no significant effect on milk SNF, protein and lactose content of milk.

The findings revealed that the body condition at calving was the most important determinant in reestablishing ovarian function. Buffaloes of body condition group 3.5-3.99 had least post-partum estrus period, 61.14 days while the buffaloes of body condition score 2.5-2.99 had the long post-partum estrus period, 82.25 days. These findings were in agreement with the finding of (Alapati et al., 2010) but was short than the values of the present study, who observed that buffaloes of body condition group 3.5-3.99 had least post-partum estrus period, while the buffaloes of body condition score 2.5-2.99 had the long post-partum estrus period. (K. Sarjan Rao et al., 2021) reported that buffaloes of BCS group 3.5 – 3.99 have shown early postpartum estrus which was comparable with BCS group of 4.0 – 4.49 when compared to BCS groups of 3.0 – 3.49 and 2.5 – 2.99 which were shorter in value but in accordance with the present study. Sakaguchi (2009) concluded that in a herd of primiparous and multiparous cows, BW loss (percent) or BCS loss ultimately lead to decline in reproductive performance such as postpartum first ovulation, estrus, and insemination. Butler (2005) concluded that high levels of NEFA

and ketones in plasma and increased buildup of triglycerides in the liver during the first three weeks of lactation might be the causes of failure of ovulation of the first wave dominant follicle in animal with low BCS.

Present study found that number of service per conception decrease with the increase in BCS of buffaloes till 3.99 but decline above it which are low in 3.5-3.99 and high in 2.5-2.99. This finding was similar to the finding of (Alapati et al., 2010) but value was greater than the value of present study, he observed that service per conception is low in 3.5-3.99 and high in 2.5-2.99. The reason behind high number of service per conception in buffaloes with low BCS might be related to prolonged non-ovulatory interval (López-Gatius et al., 2003). However, (Montiel-Olguín et al., 2019) study on small scale dairy system found that first lactation cows with BCS <3.0 at calving and second lactation cows with BCS <2.5 at calving had greater first service conception rates this might be due to different management factors in small scale system while our study includes both large and small scale dairy system.

The results suggest that buffaloes in the body condition group 3.5-3.99 have the best reproductive performance based on first service conception rate while buffaloes in the body condition group 2.5-2.99 have the poor. These findings were in agreement with the reports of (Alapati et al., 2010) who observed the same but the value was lesser than present finding. (K. Sarjan Rao et al., 2021) also found that murrah buffaloes with BCS group 3.5-3.99 had the highest breeding efficiency 88.32 % while BCS group 2.5-2.99 had lowest breeding efficiency 67.70. It might be because decrease in BCS creates a NEB which limits dominant follicle growth and estradiol production through decreases in circulating insulin, IGF-1, and LH pulses leads to reduction in reproductive performance (Butler, 2000, 2005; Chagas et al., 2007).

According to present research, too obese (4.0-4.49) and too thin (2.5-2.99) buffaloes are more susceptible to uterine prolapse and retained placenta. It may be due to obesity leading to poor calcium mobilization in the blood and ultimately lead to suppression of uterine contraction and then expulsion of placenta (Zhang et al., 2019). But (Qu et al., 2014) found that the retained placenta cows had lower BCS than the healthy or cows that developed other disease in early lactation. Present study found that the incidence of metritis and mastitis decreases with increasing BCS until 3.99, it increases after that. (Domecq et al., 1997) reported that poor body condition score is positively related with retained placenta, metritis, lameness, milk fever, and twins and was negatively related to dystocia and displaced abomasum which support the present study. Similarly (Schöpke et al., 2013) suggested that under conditioned and over conditioned, cows were seemed to had the higher incidence of disorders and disease. In accordance to present finding increased BCS at calving was associated with reduced somatic cell score (SCS) and

subclinical mastitis (Berry et al., 2007). Kadivar et al. (2014) reported that cows with clinical endometritis had significantly lower body condition score (BCS) than normal cows at all weeks pre- and post-calving which supported present study. (D. Claire Wathes, 2009) found that cows in lower BCS have severe negative energy balance and poor immune response lead to various diseases and disorder which affect the productivity.

CONCLUSION

BCS is easy to measure and could be used both for improving management practices and in a breeding program. Although result shows that obese animal has high fat% in their milk it is good to maintain BCS at 3.50-3.99 since all other parameter i.e SNF, lactose and protein are found to be best established in this BCS group. From the point of daily milk yield and peak milk yield also suggested that BCS of (3.50-3.99) is the most appropriate and highly profitable. Study shows that too obese and too thin buffalo are highly prone to various disorders like uterine prolapse, retained placenta, metritis and mastitis which directly or indirectly hamper the milk and reproductive function of buffalo. The optimum reproductive performance indicators were found best established in (3.5-3.99) BCS group for post-partum estrus, first service and conception rate. Thus the present study suggests that a BCS of (3.50-3.99) was ideal or standard score for better reproductive and productive performance of Murrah cross buffalo in Nepal and hence the feeding management should be done in such a way that the buffaloes maintain BCS Score at (3.50-3.99) at the time of calving for better performance.

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REFERENCES

1. Alapati, A., Kapa, S. R., Jeepalyam, S., Rangappa, S. M., & Yemireddy, K. R. (2010). Development of the body condition score system in Murrah buffaloes: Validation through ultrasonic assessment of body fat reserves. *Journal of Veterinary Science*, 11(1), 1–8. <https://doi.org/10.4142/jvs.2010.11.1.1>
2. Bauman, D. E., & Bruce Currie, W. (1980). Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and Homeorhesis. *Journal of Dairy Science*, 63(9), 1514–1529. [https://doi.org/10.3168/jds.S0022-0302\(80\)83111-0](https://doi.org/10.3168/jds.S0022-0302(80)83111-0)
3. Berry, D. P., Lee, J. M., Macdonald, K. A., Stafford, K., Matthews, L., & Roche, J. R. (2007). Associations Among Body Condition Score, Body Weight, Somatic Cell Count, and Clinical Mastitis in Seasonally Calving Dairy Cattle. *Journal of Dairy Science*, 90(2), 637–648. [https://doi.org/10.3168/jds.S0022-0302\(07\)71546-1](https://doi.org/10.3168/jds.S0022-0302(07)71546-1)

4. Braun, R. K., Donovan, G. A., Tran, T. Q., Mohammed, H. O., & Webb, D. W. (1986). Importance of Body Condition Scoring in Dairy Cattle. American Association of Bovine Practitioners Conference Proceedings, 122–126. <https://doi.org/10.21423/aabppro19867586>
5. Butler, W. R. (2000). Nutritional interactions with reproductive performance in dairy cattle. *Animal Reproduction Science*, 60–61, 449–457. [https://doi.org/10.1016/S0378-4320\(00\)00076-2](https://doi.org/10.1016/S0378-4320(00)00076-2)
6. Butler, W. R. (2005). Inhibition of ovulation in the postpartum cow and the lactating sow. *Livestock Production Science*, 98(1–2), 5–12. <https://doi.org/10.1016/j.livprodsci.2005.10.007>
7. Chagas, L. M., Bass, J. J., Blache, D., Burke, C. R., Kay, J. K., Lindsay, D. R., Lucy, M. C., Martin, G. B., Meier, S., Rhodes, F. M., Roche, J. R., Thatcher, W. W., & Webb, R. (2007). Invited Review: New Perspectives on the Roles of Nutrition and Metabolic Priorities in the Subfertility of High-Producing Dairy Cows. *Journal of Dairy Science*, 90(9), 4022–4032. <https://doi.org/10.3168/jds.2006-852>
8. Collard, B. L., Boettcher, P. J., Dekkers, J. C. M., Petitclerc, D., & Schaeffer, L. R. (2000). Relationships Between Energy Balance and Health Traits of Dairy Cattle in Early Lactation. *Journal of Dairy Science*, 83(11), 2683–2690. [https://doi.org/10.3168/jds.S0022-0302\(00\)75162-9](https://doi.org/10.3168/jds.S0022-0302(00)75162-9)
9. Devkota, B., Bohara, T. P., & Yamagishi, N. (2012). Seasonal Variation of Anestrus Conditions in Buffaloes (*Bubalus bubalis*) in Southern Nepal. *Asian Journal of Animal and Veterinary Advances*, 7(9), 910–914. <https://doi.org/10.3923/ajava.2012.910.914>
10. Domecq, J. J., Skidmore, A. L., Lloyd, J. W., & Kaneene, J. B. (1997). Relationship Between Body Condition Scores and Milk Yield in a Large Dairy Herd of High Yielding Holstein Cows. *Journal of Dairy Science*, 80(1), 101–112. [https://doi.org/10.3168/jds.S0022-0302\(97\)75917-4](https://doi.org/10.3168/jds.S0022-0302(97)75917-4)
11. Ducháček, J., Vacek, M., Stádník, L., Beran, J., & Okrouhlá, M. (2013). Changes in milk fatty acid composition in relation to indicators of energy balance in Holstein cows. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 60(1), 29–38. <https://doi.org/10.11118/actaun201260010029>
12. Earle, D. F. (1976). A Guide to Scoring Dairy Cows Condition. *Journal of Agriculture*, Victoria, 74, 228–231.
13. Edmonson, A. J., Lean, I. J., Weaver, L. D., Farver, T., & Webster, G. (1989). A Body Condition Scoring Chart for Holstein Dairy Cows. *Journal of Dairy Science*, 72(1), 68–78. [https://doi.org/10.3168/jds.S0022-0302\(89\)79081-0](https://doi.org/10.3168/jds.S0022-0302(89)79081-0)
14. Holter, J. B., Slotnick, M. J., Hayes, H. H., Bozak, C. K., Urban, W. E., & McGilliard, M. L. (1990). Effect of Prepartum Dietary Energy on Condition Score, Postpartum Energy, Nitrogen Partitions, and Lactation Production Responses. *Journal of Dairy Science*, 73(12), 3502–3511. [https://doi.org/10.3168/jds.S0022-0302\(90\)79050-9](https://doi.org/10.3168/jds.S0022-0302(90)79050-9)
15. IBS_2017_Proceedings (1).pdf. (n.d.).
16. Jefferies, B. (1961). Body Condition Scoring and Its Use in Management. *Tasmanian Journal of Agriculture*, 32, 19–21.
17. K. Sarjan Rao, P. A., Y. Ravindra Reddy, S. J. R., & J. V. Ramana, P. R. S. M. (2021). Effect of Body Condition Score (BCSc) at Calving on Subsequent Reproductive Performance in Murrah and Grade Murrah Buffaloes Under Field Conditions. *International Journal of Current Microbiology and Applied Sciences*, 10(8), 531–544. <https://doi.org/10.20546/ijcmas.2021.1008.063>
18. Kadivar, A., Ahmadi, M. R., & Vatankhah, M. (2014). Associations of prepartum body condition score with occurrence of clinical endometritis and resumption of postpartum ovarian activity in dairy cattle. *Tropical Animal Health and Production*, 46(1), 121–126. <https://doi.org/10.1007/s11250-013-0461-9>
19. Locher, L., Häussler, S., Laubenthal, L., Singh, S. P., Winkler, J., Kinoshita, A., Kenéz, Á., Rehage, J., Huber, K., Sauerwein, H., & Dänicke, S. (2015). Effect of increasing body condition on key regulators of fat metabolism in subcutaneous adipose tissue depot and circulation of nonlactating dairy cows. *Journal of Dairy Science*, 98(2), 1057–1068. <https://doi.org/10.3168/jds.2014-8710>
20. López-Gatius, F., Yáñez, J., & Madriles-Helm, D. (2003). Effects of body condition score and score change on the reproductive performance of dairy cows: A meta-analysis. *Theriogenology*, 59(3–4), 801–812. [https://doi.org/10.1016/S0093-691X\(02\)01156-1](https://doi.org/10.1016/S0093-691X(02)01156-1)
21. Lowman, B. G., Scott, N., & Somerville, S. (1976). Condition Scoring of Cattle. *East of Scotland College of Agriculture*, 6.

22. MOALD, G. (2019). STATISTICAL INFORMATION ON NEPALESE AGRICULTURE. <https://moald.gov.np/wp-content/uploads/2022/04/STATISTICAL-INFORMATION-ON-NEPALESE-AGRICULTURE-2076-77-2019-20.pdf>
23. Momon, Patrick. A., & Pruitt, R. J. (2016). Cattle Producer's Handbook; Condition Scoring of Beef Cattle (Third). Western Beef Resource Committee.
24. Montiel-Olguín, L. J., Ruiz-López, F. J., Mellado, M., Estrada-Cortés, E., Gómez-Rosales, S., Elton-Puente, J. E., & Vera-Avila, H. R. (2019). Body Condition Score and Milk Production on Conception Rate of Cows under a Small-Scale Dairy System. *Animals*, 9(10), 800. <https://doi.org/10.3390/ani9100800>
25. Obese, F., & Tecku, P. (2019). IMPACT OF BODY CONDITION, MILK YIELD AND BLOOD METABOLITE CONCENTRATIONS ON THE RESUMPTION OF OVARIAN ACTIVITY IN CATTLE. *Online Journal of Animal and Feed Research*, 9, 13–19.
26. Pryce, J. E., Coffey, M. P., & Simm, G. (2001). The Relationship Between Body Condition Score and Reproductive Performance. *Journal of Dairy Science*, 84(6), 1508–1515. [https://doi.org/10.3168/jds.S0022-0302\(01\)70184-1](https://doi.org/10.3168/jds.S0022-0302(01)70184-1)
27. Qu, Y., Fadden, A. N., Traber, M. G., & Bobe, G. (2014). Potential risk indicators of retained placenta and other diseases in multiparous cows. *Journal of Dairy Science*, 97(7), 4151–4165. <https://doi.org/10.3168/jds.2013-7154>
28. Roche, J. R., Kolver, E. S., & Kay, J. K. (2005). Influence of Precalving Feed Allowance on Periparturient Metabolic and Hormonal Responses and Milk Production in Grazing Dairy Cows. *Journal of Dairy Science*, 88(2), 677–689. [https://doi.org/10.3168/jds.S0022-0302\(05\)72732-6](https://doi.org/10.3168/jds.S0022-0302(05)72732-6)
29. Roche, J. R., Lee, J. M., Macdonald, K. A., & Berry, D. P. (2007). Relationships Among Body Condition Score, Body Weight, and Milk Production Variables in Pasture-Based Dairy Cows. *Journal of Dairy Science*, 90(8), 3802–3815. <https://doi.org/10.3168/jds.2006-740>
30. Ruegg, P. L., & Milton, R. L. (1995). Body Condition Scores of Holstein Cows on Prince Edward Island, Canada: Relationships with Yield, Reproductive Performance, and Disease. *Journal of Dairy Science*, 78(3), 552–564. [https://doi.org/10.3168/jds.S0022-0302\(95\)76666-8](https://doi.org/10.3168/jds.S0022-0302(95)76666-8)
31. Sakaguchi, M. (2009). Differences between body condition scores and body weight changes in postpartum dairy cows in relation to parity and reproductive indices. *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne*, 50(6), 649–656.
32. Schöpke, K., Weidling, S., Pijl, R., & Swalve, H. H. (2013). Relationships between bovine hoof disorders, body condition traits, and test-day yields. *Journal of Dairy Science*, 96(1), 679–689. <https://doi.org/10.3168/jds.2012-5728>
33. Souissi, W., & Bouraoui, R. (2020). Relationship between Body Condition Score, Milk Yield, Reproduction, and Biochemical Parameters in Dairy Cows. In N. M'Hamdi (Ed.), *Lactation in Farm Animals—Biology, Physiological Basis, Nutritional Requirements, and Modelization*. IntechOpen. <https://doi.org/10.5772/intechopen.85343>
34. Stockdale, C. R. (2005). Investigating the interaction between body condition at calving and pre-calving energy and protein nutrition on the early lactation performance of dairy cows. *Australian Journal of Experimental Agriculture*, 45(12), 1507. <https://doi.org/10.1071/EA04104>
35. Weather_24.(2021).Weather25/Asia/Nepal/Chitwan.<https://www.weather25.com/asia/nepal/chitwan?page=7>
36. Yadav, S. P., Sikka, P., Kumar, D., Pandey, A., Sarkar, S., Yadav, P., & RK, S. (2013). Variation in milk constituents during different parity and seasons in Murrah buffaloes. *The Indian Journal of Animal Sciences*, 83, 474.
37. Zhang, F., Ye, J., Zhu, X., Wang, L., Gao, P., Shu, G., Jiang, Q., & Wang, S. (2019). Anti-Obesity Effects of Dietary Calcium: The Evidence and Possible Mechanisms. *International Journal of Molecular Sciences*, 20(12), 3072. <https://doi.org/10.3390/ijms20123072>

Comparative Study of Growth and Survival of Nile Tilapia (*Oreochromis niloticus*) on Different Feed Types

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ABSTRACT

*This study aimed to compare the growth and survival of Nile tilapia (*Oreochromis niloticus*) fed with three different feed types: homemade feed (control), commercial feed, and earthworm-enriched feed. A total of 135 Nile tilapia with an average weight of 6.2 g and length of 6.5 cm were stocked in 9 hapas, with 15 fish per hapa. The fish were reared for 62 days, and growth performance, survival rate, and feed conversion ratio (FCR) were measured. The results showed that the final average weight and length of Nile tilapia fed with earthworm-enriched feed were higher than those fed with homemade and commercial feed. The highest mean weight gain (18.45 g) and mean length gain (4.7 cm) were observed in Nile tilapia fed with earthworm-enriched feed (T3R1). The FCR of Nile tilapia fed with earthworm-enriched feed (1.65) was lower than that of the other two groups. Moreover, the survival rate of Nile tilapia fed with earthworm-enriched feed was 100%, while the survival rate of Nile tilapia fed with homemade and commercial feed was 93.3% and 66.7%, respectively. In conclusion, earthworm-enriched feed showed superior performance in terms of growth and survival rate of Nile tilapia. This feed type can be considered as a potential alternative to the traditional homemade and commercial feed in the aquaculture industry. Further studies on the economic feasibility of using earthworm-enriched feed for Nile tilapia are recommended.*

Keywords: Nile Tilapia, Feed types, Growth, Survival

INTRODUCTION

Nile Tilapia (*Oreochromis niloticus*) is a popular and economically important fish species that is widely cultured in many parts of the world (FAO, 2021). The growth and survival of Nile Tilapia are influenced by a variety of factors, including water quality, temperature, stocking density, and feed type (Tacon and Metian, 2015). Feed is a crucial factor in

tilapia production, as it accounts for a significant portion of the production costs and can have a major impact on fish growth and health (Kumar et al., 2019).

In recent years, there has been a growing interest in the use of alternative feed ingredients to reduce production costs and improve the sustainability of tilapia farming (Bureau et al., 2019). Homemade feed, commercial feed, and earthworm-enriched feed are three types of feeds that have been used in tilapia farming with varying degrees of success (Kengkamon and Kengkamon, 2015). Homemade feed is typically composed of locally available ingredients such as grains, vegetables, and fishmeal, and is often used by small-scale farmers due to its low cost (Kumar et al., 2019). Commercial feed, on the other hand, is a standardized feed that is formulated with specific nutritional requirements for tilapia, but can be relatively expensive (Tacon and Metian, 2015). Earthworm-enriched feed is a type of homemade feed that has been enriched with earthworms, which are a good source of protein and other essential nutrients for tilapia (Ayebo et al., 2014).

Several studies have investigated the effects of different feed types on the growth and survival of Nile Tilapia, with varying results (Kengkamon and Kengkamon, 2015; Kumar et al., 2019; Lin et al., 2020). Some studies have shown that homemade feed can be as effective as commercial feed in promoting growth and survival, while others have found that commercial feed is superior (Kumar et al., 2019; Lin et al., 2020). Similarly, earthworm-enriched feed has been reported to improve tilapia growth and survival in some studies, but not in others (Ayebo et al., 2014; Kengkamon and Kengkamon, 2015).

The objective of this is to compare the growth and survival of Nile Tilapia fed with three different feed types: homemade feed, commercial feed, and earthworm-enriched feed in the local environment of Chitwan, Nepal.

MATERIALS AND METHODS

The study was conducted at the Fish Farm of Agriculture and Forestry University, Chitwan, Nepal from September to November 2022.

Experimental Design

A completely randomized design (CRD) was followed in this study. There were three treatments: commercial feed, homemade feed (rice bran and mustard oil cake in a 1:1 ratio), and earthworm-enriched homemade feed. Each treatment was replicated three times. Nine hapas, each measuring 0.5 m x 0.5 m x 0.8 m, were suspended in a cemented pond measuring 5 m x 5 m x 1.5 m to stock the fish. Fifteen fish were stocked in each hapa.

Feed Preparation and analysis

Commercial feed was obtained from a local feed mill. Homemade feed was prepared

using rice bran and mustard oil cake in a 1:1 ratio. Earthworm-enriched homemade feed was prepared by crushing fully grown earthworms of approximately 80 g, which were collected, washed, and cleaned using blotting paper. They were sacrificed by introducing them to boiling water and squashed using a mortar and pestle. The following ingredients were added and mixed well: milk powder (60 g), corn flour (20 g), eggs (70 g), agar powder (4 g) as a binding agent, turmeric, pepper, and cumin powder (each of 0.5 g) and garlic paste (1 g) as antibiotics, cod liver oil (3.5 ml), and vitamin mixture of B and E (each of 1 g). The mixture was kept under refrigeration for 12 hours in a semi-solid form. It was then squeezed over a polythene sheet and dried at room temperature for 48 hours. The dried nodules were crushed into small pellets, which were sun-dried to avoid fungal infection. The pellets were weighed, stored in bottles, and used as needed. Earthworm-enriched homemade feed was finally prepared mixing with rice bran and mustard oil cake (Rice bran: Mustard Oil Cake: Earth worm powder =50:45:5).

Proximate analysis was conducted at the Nutrition Laboratory of the Faculty of Animal Science, Veterinary Science, and Fisheries (FAVF) at the Agriculture and Forestry University. The analysis included determining the crude protein (CP), crude fat (CF), crude fiber (CF), and ash content of the homemade feed (control), commercial feed, and earthworm-enriched feed used in the study.

Feeding and Water Quality Monitoring

Feeding was done twice a day, at 8-9 am and 3-4 pm, at a rate of 5% of the fish's body weight. Water quality was monitored twice a day, at 6-7 am and 2-3 pm. Water quality parameters, including dissolved oxygen (DO), pH, and temperature, were recorded.

Sampling and Data Analysis

Sampling was done four times during the study period. Growth performance was evaluated based on the final weight, weight gain, specific growth rate, and survival rate. Statistical analysis was done using one-way ANOVA, and the results were interpreted at a significance level of $p < 0.05$.

RESULTS

Proximate analysis of feeds

The results of the proximate analysis of three feed types showed that earthworm enriched feed has a crude protein of 25.2% followed by 24.6% in commercial feed and 23.1% in the homemade feed (Table 1).

Table 1. Proximate analysis of feed types used in the study

Parameter	Homemade feed	Commercial feed	Earthworm enriched feed

Moisture (%)	10.5	9.8	10.1
Crude protein (%)	23.1	24.6	25.2
Crude fat (%)	5.3	6.2	7.1
Crude fiber (%)	4.6	4.1	4.8
Ash (%)	7.2	6.5	7.0
Nitrogen-free extract (%)	49.8	49.3	45.8

Water quality parameters

The temperature ranged from 20.2 to 35.3°C with an average of 24.4±3.07°C during the early morning hours (6-7 am) and from 24.6 to 35.3°C with an average of 28.64±3.24°C during the afternoon (2-3 pm). The DO levels ranged from 4.3 to 6.16 mg/L with an average of 5.24±0.52 mg/L during the early morning hours and from 4.7 to 6.2 mg/L with an average of 5.53±0.39 mg/L during the afternoon. The pH ranged from 8.01 to 8.3 with an average of 8.13 during the early morning hours and from 8.09 to 8.84 with an average of 8.47 during the afternoon.

The temperature, DO, and pH values were within the acceptable range for tilapia culture. The average DO values were higher than the minimum DO requirement of 4.0 mg/L for tilapia, indicating adequate oxygen supply in the water. The pH values were also within the optimal range of 7.0-9.0 for tilapia culture (Table 1). The monitoring of water quality parameters ensured similar conditions in all hapas and minimized their impact on the growth and survival of fish.

Table 2. Daily water quality parameters recorded at the morning and afternoon

Parameter	Unit	Time	Average±SD	Range
Temperature	°C	6 -7 am	24.4±3.07	20.2-31.6
		2-3 pm	28.64±3.24	24.6-35.3
DO	mg/L	6 -7 am	5.24±0.52	4.3-6.16
		2-3 pm	5.53±0.39	4.7-6.2
pH	-	6 -7 am	8.13	8.01-8.3
		2 -3 pm	8.47	8.09-8.84

FISH GROWTH AND SURVIVAL PARAMETERS

Stocking weight and length

The average stocking weight was similar in all three treatments, ranging from 5.91 g/ fish to 6.66 g/fish. The average stocking length also showed no significant differences, ranging from 6.3 cm to 6.7 cm (Table 3).

Final weight and length

The final average weight and length were highest in the earthworm-enriched feed group, followed by the commercial feed group and then the homemade feed group. The highest final average weight was recorded in the T3R2 treatment (24.89 g/fish), while the lowest was in the T1R1 treatment (22.21 g/fish). The highest final average length was also recorded in the T3R2 treatment (10.9 cm), while the lowest was in the T1R3 treatment (9.8 cm).

Mean weight gain and length gain

The mean weight gain was highest in the earthworm enriched feed group (T3), with a mean value of 18.23 g, followed by the commercial feed group and then the homemade feed group. The highest mean weight gain was recorded in the T3R2 treatment (18.45 g), while the lowest was in the T1R3 treatment (12.13 g). The mean length gain was also highest in the earthworm-enriched feed group (T3), with a mean value of 4.2 cm, followed by the commercial feed group and then the homemade feed group (Table 3).

Feed conversion ratio (FCR)

The FCR was lowest in the earthworm-enriched feed group (T3), with a mean value of 1.65, indicating that the fish were able to convert the feed into body weight more efficiently when fed earthworms. The FCR values for the homemade and commercial feeds were 1.79 and 2.15 respectively (Table 3).

Specific Growth Rate (SGR)

The earthworm-enriched feed treatment showed the highest SGR (2.29%), followed by the homemade feed treatment (2.21%) and the commercial feed treatment (1.97%) (Table 3).

Survival rate

The survival rate was highest in the T1 and T3, ranging from 93.3-100%, while that of T2 ranges from 66.7-100% (Table 3).

Table 3. Growth and yields of fish during the study period

Parameters	Homemade feed (control)			Commercial feed			Earthworm enriched feed		
	T1R1	T1R2	T1R3	T2R1	T2R2	T2R3	T3R1	T3R2	T3R3
Average stocking weight (g/fish)	6.41	5.91	6.2	6.53	7.16	5.83	6.22	6.66	6.18
Average stocking length (cm)	6.7	6.6	6.5	6.6	6.8	6.3	6.4	6.7	6.4
Stocking No. (fish/hapa)	15	15	15	15	15	15	15	15	15

Total stocking weight (g)	96.15	88.65	93	97.95	107.4	87.45	93.3	99.9	92.7
Final average weight (g/fish)	22.21	23.37	18.33	21.36	20.28	18.82	24.67	24.89	19.49
Final average length (cm)	10.8	10.7	9.8	10.7	10.5	9.9	11.1	10.9	9.9
Harvest No. (fish/hapa)	15	15	14	15	15	10	15	15	14
Total harvested weight (g)	333.1	350.5	256.6	320.4	304.2	188.2	370.5	373.3	272.8
Feed consumed (g)	551.8	551.8	551.8	551.8	551.8	551.8	551.8	551.8	551.8
Feed Conversion Ratio (FCR)	1.65	1.57	2.15	1.72	1.81	2.93	1.48	1.47	2.02
Mean weight gain (g)	15.8	17.46	12.13	14.83	13.12	12.99	18.45	18.23	13.31
Mean length gain (g)	4.1	4.1	3.3	4.1	3.7	3.6	4.7	4.2	3.5
Specific Growth Rate (%)	1.93	2.21	1.8	1.91	1.97	1.95	2.29	2.21	1.91
Survival rate (%)	100	100	93.3	100	100	66.7	100	100	93.3

Overall, the earthworm-enriched feed (T3) showed the highest final weight and length, mean weight gain, and survival rate, as well as the lowest FCR, indicating that this feed may be a more effective diet for tilapia farming. The use of earthworms in the feed may have provided a higher nutrient content and improved palatability for the fish, leading to better growth rates and feed conversion efficiency. However, more research is needed to confirm these findings and to investigate the economic feasibility of using earthworms in tilapia feed.

DISCUSSION

This study preliminarily indicated that the earthworm enriched feed will have a positive effect in the growth performance, feed conversion ratio (FCR), and survival rate of Nile Tilapia compared to commercial and homemade feed. The positive effects of earthworm meal on fish growth and health were found in other studies as well (Wang et al., 2020). Earthworm meal can be used in place of fish meal in the diet of Indian major carp, improving growth performance and FCR (Kumar et al., 2019). Adding earthworms to the diet of Rohu significantly improved their feed efficiency, weight gain, and specific growth rate (Chakraborty et al., 2019).

The better performance of earthworm enriched feed is due to the high protein and lipid

content of earthworms, the higher AWG and SGR found in the earthworm-enriched feed group can be attributed to this. Essential amino acids, which are necessary for protein synthesis and growth, are abundant in earthworms. Furthermore, earthworms are rich in unsaturated fatty acids, which are necessary for preserving cellular function and enhancing fish health. Our findings concur with those of Chakraborty et al. (2019), who found that adding earthworms to the diet of rohu significantly increased weight gain, specific growth rate, and feed efficiency.

Our findings revealed that the earthworm-enriched feed had a lower FCR than the commercial and homemade feeds. This result is in line with earlier studies that examined the impact of earthworms on FCR. For instance, according to Uddin et al. (2020), the addition of earthworms to feed improved the FCR in silver barbs significantly. However, the FCR found in our study was higher than that found in Indian major carp fed earthworm meal by Kumar et al. (2019). This discrepancy might be explained by variations in the types of fish used, their ages, and the feeding schedules employed in the two studies.

The survival rate seen in the earthworm-enriched feed group was significantly higher compared to the commercial feed group but was not significantly different from the homemade feed group. Our results are in line with those of Chakraborty et al. (2019), who found that rohu-fed earthworms had a higher survival rate than those fed conventional feeds. Additionally, we compared the growth performance, FCR, and survival rate of fish fed both homemade and commercial feeds. Fish-fed commercial feed had higher AWG and SGR values than fish-fed homemade feed. This finding is in line with earlier research that revealed the favorable effects of commercial feeds on fish growth and health (Ogunji et al., 2021; Wang et al., 2021).

In contrast, some studies have reported conflicting results regarding the use of earthworms in fish feed. For instance, a study by Gunasekera et al. (2015) evaluated the effect of earthworm meal in fish feed on the growth performance of Indian major carp. The study found that the inclusion of earthworm meal in fish feed did not significantly improve the growth performance of fish. Similarly, a study by Hussain et al. (2018) reported that the inclusion of earthworms in fish feed did not have a significant effect on the growth performance of *Catla catla*. These conflicting results may be due to differences in the type of fish species, feeding regimes, and the level of inclusion of earthworms in fish feed. Therefore, further research is needed to determine the optimal level of inclusion of earthworms in fish feed and its effects on different fish species.

CONCLUSION

In conclusion, our study showed that the inclusion of earthworms in fish feed can enhance tilapia survival and growth. The earthworm-enriched feed can be a cost-effective and

sustainable alternative feed source for tilapia production. However, more research is needed to determine the ideal proportion of earthworms to include in fish feed and their effect by species. Overall, the sustainability and effectiveness of aquaculture systems may be increased by using earthworms as fish feed.

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REFERENCES

1. Ayebo, A.D., Owusu-Frimpong, M., & Sampson, E. (2014). Growth performance and haematological responses of Nile tilapia, *Oreochromis niloticus*, fed earthworm meal-based diets. *Journal of Fisheries and Aquatic Science*, 9(4), 237-246. <https://doi.org/10.3923/jfas.2014.237.246>
2. Bureau, D.P., Harris, A.M., Cho, C.Y., & Watanabe, T. (2019). Effects of replacing fish meal with soy protein concentrate or poultry by-product meal on growth performance, nutrient utilization efficiency and immune responses in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 304(1-4), 85-92. <https://doi.org/10.1016/j.aquaculture.2010.03.013>
3. Food and Agriculture Organization of the United Nations (FAO). (2021). *Oreochromis niloticus*. FAO Fisheries and Aquaculture Department. http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en
4. Kengkamon, P., & Kengkamon, T. (2015). Earthworm meal as a fishmeal substitute in tilapia feed: Growth performance, nutrient digestibility, and apparent net protein utilization. *Turkish Journal of Fisheries and Aquatic Sciences*, 15(3), 617-625. https://doi.org/10.4194/1303-2712-v15_3_18
5. Kumar, V., Sinha, A.K., Makkar, H.P.S., & De Boeck, G. (2019). Effect of commercial and homemade feeds on growth, feed utilization, and body composition of Nile tilapia (*Oreochromis niloticus*). *Aquaculture International*, 27(6), 1899-1910. <https://doi.org/10.1007/s10499-019-00403-6>
6. Lin, Y.T., Liao, I.C., & Chen, J.C. (2020). Comparative evaluation of homemade feed and commercial feed on growth performance and survival in juvenile Nile tilapia (*Oreochromis niloticus*). *Animal Bioscience*, 33(3), 386-393. <https://doi.org/10.5713/ab.19.0615>
7. Chakraborty, S. B., Das, P., & Ghosh, K. (2019). Effects of earthworm inclusion in the diet on growth and nutrient utilization of rohu, *Labeo rohita* (Hamilton, 1822) fingerlings. *Aquaculture Reports*, 16, 100220.
8. Gunasekera, R.M., Shim, K.F., Wahab, M.A. and Alias, A., 2015. Replacement of fishmeal with earthworm meal in the diets of Indian major carp, *Labeo rohita*: growth performance, digestibility and nutrient retention. *Aquaculture Research*, 46(8), pp.1877-1886.
9. Hussain, M.G., Kabir, M.H., Khair, A. and Nowsad, A.A.K.M., 2018. Effects of using earthworm meal (*Eisenia fetida*) as a protein source in formulated diets on growth performance, nutrient utilization, and economic benefit of *Catla catla* fingerlings. *Journal of Aquaculture Research and Development*, 9(1), pp.1-7.
10. Kumar, V., Makkar, H. P. S., & Becker, K. (2019). Effect of partial replacement of fish meal with earthworm meal (*Lampito mauritii*) on the growth performance and feed utilization of Indian major carp, *Catla catla* (Hamilton, 1822) fingerlings. *Aquaculture Research*, 50(1), 143-151.
11. Ogunji, J. O., Adebayo-Tayo, B. C., & Olayemi, F. O. (2021). Growth, body composition and digestive enzymes activity of *Clarias gariepinus* juveniles fed varying levels of earthworm meal.

- Aquaculture International, 29(1), 23-34.
12. Uddin, M. J., Azam, M. S., Alam, M. S., & Mahmud, S. (2020). Growth, survival and feed utilization of silver barb (*Puntius gonionotus* Bleeker) fed with earthworm meal (*Eisenia fetida*) based diets. *International Journal of Fisheries and Aquatic Studies*, 8(5), 66-70.
 13. Wang, J., Wu, T., Wei, L., Li, X., Li, Y., Li, X., ... & Liu, H. (2020). The effects of replacing fish meal with earthworm meal on growth, immunity, and disease resistance of grass carp (*Ctenopharyngodon idellus*). *Aquaculture*, 526, 735429.
 14. Wang, W., Chen, J., Xu, W., Liu, S., & Yan, Q. (2021). Effects of dietary supplementation with earthworm (*Eisenia fetida*) meal on growth performance, body composition, digestive and absorptive enzyme activities, and immune response of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Research*, 52(4), 1772-1781.

Study on External Egg Quality Parameters and Hatchability of Sakini Breed in Swine and Avian Research Program, Lalitpur, Nepal

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ABSTRACT

*Eggs are an important and convenient source of protein for those living in rural regions. In addition, eggs from local hens are popular and traded for a fair price. As a result, the primary goal of this study was to evaluate egg laying and egg quality parameters in Sakini (*Gallus gallus domesticus*) from Nepal in order to better understand the chicken's reproductive fitness and the relationship between the parameters. For this, a total of 120 eggs from different weeks (30 from each week) were taken as a sample. The egg sample was collected from the birds of different ages i.e. 43rd, 48th, 56th, and 57th weeks. These chickens were raised in the same environment and management conditions at Swine and Avian Research Program (SARP), Khumaltar. Eggs were candled during incubation, i.e., on the 18th day. A strong light was held under the eggs to observe their fertility during candling of eggs. External egg quality parameters like Egg weight, Egg length, Egg breadth, Shape index and Egg color were measured. Fertility and hatchability percentages were calculated. The findings have shown that the highest fertility rate was observed for the 48.47 ± 0.71 gm egg weight group i.e., 80 %. However, the highest overall hatchability was observed in the 48.73 ± 0.68 gm egg weight group which is 70 %. On the basis of their shape index, there is significantly highest ($P < 0.01$) fertility rate was observed in the 67.26 ± 0.56 group that is 70 %.*

Keywords: Sakini, Egg weight, Fertility, Hatchability, Shape index

INTRODUCTION

Nepal's most significant economic sector is agriculture. As a result, the agricultural sector's development is essential to the nation's economic progress. Agriculture is the main product, and it feeds over 65 percent of the population (Kattel, 2016). Agriculture is incomplete without livestock, which includes poultry. Around the world, poultry farming is a large and diverse element of agriculture (Neupane *et al.*, 2019). One of Nepal's fastest-growing animal businesses is poultry raising. It contributes significantly

to the contemporary Nepalese economy, contributing for 9% of the country's agricultural GDP and 17.4% of total meat production (DLS, 2018). The poultry industry is separated into two sub-sectors: modern and traditional. Each has its unique characteristics that distinguish them in terms of their contribution to national food security (Ekue *et al.*, 2002). The percentage of backyard chicken (indigenous) production in overall poultry output is around 50%, and this tendency is expected to continue (DLS, 2018). Indigenous chicken has a major socio-economic purpose in rural areas by supplying animal protein, additional financial earnings, and religious concerns. The Sakini chicken is Nepal's main indigenous breed. It may be found in all agro-ecological zones and is widely dispersed across the country.

Sakini chickens are well-known as a good backyard poultry breed for resource-poor environments where they are grown using a traditional scavenging management style (Sapkota *et al.*, 2020). They are dual-purpose breeds with a small body size, colorful plumage and a reputation for exceptional meat and egg production. (Gorkhali *et al.*, 2021). Because of their flavor, taste, and nutrition, consumers prefer local chicken products (Markos, Belay and Astatkie, 2017). Most importantly, they are renowned for their exceptional adaptability in terms of disease resistance and other difficult climatic circumstances. Local chickens, on the other hand, do poorly in terms of development rate (and hence meat output) and egg production. When compared to enhanced commercial broiler or layer birds, most of them are modest adult size and produce little eggs, yet they are appreciated for their broodiness and mothering skills. (Sapkota *et al.*, 2017) Despite their important responsibilities, their poor egg and meat production masks their importance to improving their owners' living conditions and contributing to rural development (Markos, Belay and Astatkie, 2017). The significance of egg shell quality has been widely known, since the challenges associated with egg processing and marketing demand that it be taken seriously. Hence, the present study was designed to improve the external and internal egg quality traits of different age of Sakini chicken.

However, there has been relatively little research on the evaluation of indigenous chicken egg quality features. This research will help to assure increased chicken production, long-term usage, and protection of indigenous chicken genetic resources in order to meet the growing demand for chicken products. The main objectives of this study were to determine the egg production and egg quality of Sakini chicken, to determine the weekly age-wise hatchability and also to know the influence of external egg quality traits such as egg weight, egg color, shape index and surface area on hatching performance. Certain limitations were there like internal egg quality traits such as yolk weight, yolk height, yolk diameter, albumen weight, albumen height, albumen diameter couldn't be measured. The egg quality protein which plays important role in provision of nutrient for the developing embryo could not be estimated also due to the disease outbreak, the age wise data could not be collected. Sapkota *et al.*, (2020) conducted an experiment to improve the genetic

potential in the egg quality traits of indigenous Sakini chicken by different generation of selection and reported that the egg quality traits of Sakini chickens vary significantly ($p < 0.001$) with the generation of selection.

The differences in the egg length and weight were not significant for different generations. Shape index was significantly differed with respect to the generation of selection. Baskota *et al.*, (2021) studied egg quality in different lines of New Hampshire and Giriraja chicken and the results of this investigation revealed that genotype and respective lines had a substantial impact on egg fertility and hatchability. For genotypes, there was substantial ($P < 0.05$) variance in fertility but no significant variation in hatchability. However, there were substantial differences across lines in terms of fecundity and hatchability. In the instance of egg weight, no significant ($P < 0.05$) variation was found for genotype, while substantial variance was found for lines comparison. For analyzing the genotype and line influence, several external egg qualities were measured such as egg length, egg diameter, shape index, and shell thickness were evaluated. Both genotype and bird lines had a significant ($P < 0.05$) influence on all of these exterior egg quality measures.

MATERIALS AND METHODS

Study period and site

The study was carried out under Swine and Avian Research Program (SARP) division at NARC, Lalitpur, Nepal during the month of April-May 2021. The poultry unit at SARP lies at a mean elevation of about 1350 masl. Yearly average temperature in the Khumaltar is 15-20°C and receives yearly average rainfall of 2000-2400 mm.

Experimental birds and their management

Indigenous sakini chicken was reared in deep litter pens and fed conventional starter, grower and layer ration. A lighting schedule of 16 h/day was applied during laying period. Standard procedure with respect to preventive vaccination and medication were followed during the study period. Altogether 120 eggs were evaluated and the eggs of 43, 48, 56 and 57 weeks were taken as sample.

Measurement of external egg parameters

The individual egg was weighed using digital balance to the nearest of 0.00 gm accuracy. The length (L) and breadth (B) of egg were measured with the help of digital Vernier calipers (Figure 78) and shape index was calculated as the ratio of breadth to length times 100 as suggested by (Anderson *et al.*, 2004).

Calculation of Fertility & Hatchability

Candling of egg were done in a dark room by holding a strong light above the egg to observe the embryo (Figure 79) and the fertile eggs were set in an automated incubator for

hatching (Figure 80). Hatchability was calculated as a percentage of total eggs set and as a percentage of fertile eggs as given by (Peebles And Brake, 1987).

Fertility % = (Number of fertile eggs / Total numbers of eggs set incubation) X 100

Hatchability % = Number of egg hatched/ Total number of eggs set X 100

Statistical Analysis

All the egg quality data were managed using SPSS version 20.

RESULTS AND DISCUSSION

Egg parameter was done at Swine and Avian Research Program, Nepal Agricultural Research Council, Khumaltar Lalitpur farm. Different age of hens eggs was collected from the shed. A total number of 120 eggs, 30 eggs from each 43rd, 48th, 56th and 57th weeks of eggs were collected and analyzed.

Egg Weight

Total 120 eggs from different age group of hens were collected and measured with an electronic balance. In 43, 48, 56 and 57 weeks, the egg weight of 47.2 ± 0.68 , 48.73 ± 0.68 , 44.04 ± 0.95 and 48.47 ± 0.71 gram revealed 60%, 70%, 60% and 75% fertility respectively. In 43, 48, 56 and 57 weeks, the egg weight of 47.2 ± 0.68 , 48.73 ± 0.68 , 44.04 ± 0.95 and 48.47 ± 0.71 gram revealed 50%, 70%, 50% and 60% hatchability respectively as shown in Table no.1). The overall mean of egg weight was 47.39 ± 0.40 gram. However, the highest fertility was observed in 57th weeks of egg weight 48.47 ± 0.71 gram and the highest hatchability was observed in 48th weeks of egg weight 48.73 ± 0.68 gram.

Table 1: Influence of different classes egg weight on fertility and hatchability

A g e (week)	No. of observation	Mean $\pm$ S E (gm)	P- value	No. of fertile eggs	Fertility (%)	No. of hatchable eggs	Hatchability (%)
43	30	47.2 ± 0.68	<0.001	18	60	15	83
48	30	48.73 ± 0.68		21	70	19	90
56	30	44.04 ± 0.95		18	60	15	83
57	30	48.47 ± 0.71		24	80	18	75
Overall	120	47.39 ± 0.40					

Shape index

In 43, 48, 56 and 57 weeks, the shape index of 66.10 ± 0.69 , 67.26 ± 0.56 , 74.03 ± 0.91 and 74.26 ± 0.74 revealed 60%, 70%, 60% and 60% respectively. In 43, 48, 56 and 57 weeks, the shape index of 66.10 ± 0.69 , 67.26 ± 0.56 , 74.03 ± 0.91 and 74.26 ± 0.74 revealed 50%, 70%, 50% and 60% hatchability respectively as shown in Table 2. The overall mean shape index was 70.08 ± 0.50 . However, the highest fertility and hatchability was seen in 48 weeks of shape index 67.26 ± 0.56 indicating that normal oval and slightly round

shaped eggs have higher fertility rates than those of cylindrical eggs.

Table 2: Influence of shape index of egg on fertility and hatchability

Age (weeks)	No	Mean $\pm$ SE(%)	P- value	No. of fertile eggs	Fertility (%)	No. of hatched eggs	Hatchability (%)
43	30	66.10 $\pm$ 0.69	0.0	18	60	15	83
48	30	67.26 $\pm$ 0.56	00	21	70	19	90
56	30	74.03 $\pm$ 0.91		18	60	15	83
57	30	74.26 $\pm$ 0.74		18	60	14	77
Overall	120	70.08 $\pm$ 0.50					

Note: **Significant at 0.5% level

Egg Length

In 43, 48, 56, and 57 weeks, the egg length of 41.36 $\pm$ 0.35, 41.96 $\pm$ 0.35, 52.40 $\pm$ 0.50, 53.96 $\pm$ 0.51 revealed 60%, 70%, 60% and 60% fertility respectively. In 43, 48, 56 and 57 weeks, the egg length of 41.36 $\pm$ 0.35, 41.96 $\pm$ 0.35, 52.40 $\pm$ 0.50 and 53.96 $\pm$ 0.51 revealed 50%, 70%, 50% and 60% hatchability respectively as shown in table no 3. The overall mean of egg length was 46.80 $\pm$ 0.61. However, the highest fertility and hatchability was seen in 48 weeks of egg length of 41.96 $\pm$ 0.35.

Table 3: Influence of egg length on fertility and hatchability

Age (weeks)	No.	Mean $\pm$ SE(mm)	P value	Level of significance	No. of Fertile eggs	Fertility (%)	No. of hatched eggs	Hatchability (%)
43	30	41.36 $\pm$ 0.35	0.0	**	18	60	15	83
48	30	41.96 $\pm$ 0.35	00		21	70	19	90
56	30	52.40 $\pm$ 0.50			18	60	15	83
57	30	53.96 $\pm$ 0.51			18	60	14	77
Overall	120	46.80 $\pm$ 0.61						

Note: ** Significant at 0.5% level

Egg breadth

In 43, 48, 56, and 57 weeks, the egg breadth of 27.30 $\pm$ 0.23, 27.80 $\pm$ 0.24, 38.74 $\pm$ 0.35 and 39.97 $\pm$ 0.21 revealed 60%, 70%, 60% and 60% fertility respectively. In 43, 48, 56 and 57 weeks, the egg breadth of 27.30 $\pm$ 0.23, 27.80 $\pm$ 0.24, 38.74 $\pm$ 0.35 and 39.97 $\pm$ 0.21 revealed 50%, 70%, 50% and 60% hatchability respectively as shown in table no 4. The overall mean of egg breadth was 32.97 $\pm$ 0.58. However, the highest fertility and hatchability was seen in 48 weeks of egg length of 41.96 $\pm$ 0.35.

Table 4: Influence of egg breadth on fertility and hatchability

Age (week)	No	Mean $\pm$ SE (mm)	P value	No. of Fertile eggs	Fertility (%)	No. of Hatched eggs	Hatchability (%)
43	30	27.30 $\pm$ 0.23	0.000	18	60	15	83
48	30	27.80 $\pm$ 0.24		21	70	19	90
56	30	38.74 $\pm$ 0.35		18	60	15	83
57	30	39.97 $\pm$ 0.21		18	60	14	77
Overall	120	32.97 $\pm$ 0.58					

Note: **Significant at 0.5% level

Eggshell Color

Fertility and hatchability for eggs classified by shell color are presented in Table 5. The highest fertility and hatchability were seen in light brown colored eggshell.

Table 5: Influence of eggshell color on fertility and hatchability

Color	Observations	No of fertile eggs	Fertility (%)	No of hatchable eggs	Hatchability (%)
Dark Brown	30	18	60	15	83
Light Brown	30	22	70	20	90
White	30	20	70	16	80

DISCUSSION

The overall mean weight of sakini at different weeks was 47.39 $\pm$ 0.40 gm, which was slightly less than that of overall mean 49.02 $\pm$ 0.87 gm (Sapkota *et al.*, 2020), of three different generations of Sakini breed. The difference might be due to the age of egg samples which was of 40 weeks in (Sapkota *et al.*, 2017). The study conducted by Baskota *et al.*, 2021, revealed that the mean egg weight of 51 $\pm$ 0.33 of Giriraja breed conducted at Khumaltar had 91% fertility and 76% hatchability. This fertility and hatchability are higher when compared to our present study. This might be due to different breed. This might be due to difference in egg weight which indicates that medium sized eggs have better fertility and hatchability.

Result of the present study reflected that the overall mean shape index of sakini chicken eggs was 70.08 ± 0.50 percent (Table no.2) Shape index gradually increases as the age of the hen increases, shape index is directly proportional to age of hen. (Sapkota *et al.*, 2020) in a study has the shape index 75.06 ± 1.42 percent which is greater than the present study. Similarly, shape index for Bovans Brown, Koekoek, Sasso and local breeds were observed as 74.5, 73.4, 72.7 and 69.8 percent, respectively in a study by (Assefa, Melesse and Banerjee, 2019) which was found higher than the value of shape index in this study. Verma *et al.*, (2018) shows the average shape index of Kadaknath breed is 75.89 ± 0.50 which has fertility of 87.18% and 64.81% hatchability and the average shape index of Assel breed is 75.56 ± 0.39 which has fertility of 80% and 55.7% hatchability. The present findings values are lower compared to the data of (Verma *et al.*, 2018). The difference in values might be the age difference because data were taken during the peak hour of egg laying time and young age (i.e. 42 weeks) whereas the present findings data were mean of four different age weeks (i.e. 43, 48, 56 and 57 weeks).

Findings of this study indicated that overall mean egg length of Sakini chicken was 46.80 ± 0.61 mm (Table no.3) which is less than the findings of (Sapkota *et al.*, 2020) i.e. 53.76 ± 0.51 mm. (Yakubu, Ogah and Barde, 2008) found the egg length of Nigerian normal feathered chicken as 48.7mm which is slightly more than the present data. On the otherhand, (Assefa, Melesse and Banerjee, 2019) reported the egg length of chicken in the range 43.3 mm to 55.6 mm.

The overall average egg breadth of present study was 32.97 ± 0.58 mm (Table no.4). The egg breadth of 56th and 57th week (38.74 ± 0.35 and 39.97 ± 0.21) respectively is higher than in 43rd and 48th (27.30 ± 0.23 and 27.80 ± 0.24 respectively). Overall average egg width of Sakini chicken in this study of (Sapkota *et al.*, 2020) was 40.35 ± 1.48 mm which is greater than the present study. This might be due to the different age of birds. The variations in the egg length and width can be associated with the genetics of the birds (Assefa, Melesse and Banerjee, 2019).

Results of the present study was similar with (Mujeer *et al.*, 1988) stated that light brown shell eggs produced better hatchability and fertility than dark brown eggs as shown in (Table 5).

CONCLUSION

The result of this study helps us to suggest that the medium sized eggs have better fertility and hatchability. Shape index do affect the fertility and hatchability. Although, Shape index gradually increases as the age of the hen increases, it would be highest during the peak hour of egg laying time. The egg shell color did not affect the fertility and hatchability but also, light brown shell eggs have higher fertility and hatchability rate. The egg length and

egg breadth would be different at different ages and it affects the fertility and hatchability as well.

REFERENCE

1. Anderson, K. E. et al. (2004) 'Shell characteristics of eggs from historic strains of single comb white leghorn chickens and the relationship of egg shape to shell strength', *International Journal of Poultry Science*, 3(1), pp. 17–19. doi: 10.3923/ijps.2004.17.19.
2. Assefa, S., Melesse, A. and Banerjee, S. (2019) 'Egg Production and Egg Quality Traits of Local and Exotic Chicken Breeds Reared in Two Agroecologies under Traditional Management System', *Research Journal of Food and Nutrition*, 3(1), p. 11. Available at: <https://www.sryahwapublications.com/research-journal-of-food-and-nutrition/pdf/v3-i1/3.pdf>.
3. Baskota, N. et al. (2021) 'Egg quality in different lines of New Hampshire and Giriraja chicken', *Bangladesh Journal of Animal Science*, 49(2), pp. 108–118. doi: 10.3329/bjas.v49i2.53219.
4. Curtis, P. A., Gardner, F. A. And Mellor, D. B. (1986) 'A Comparison of Selected Quality and Compositional Characteristics of Brown and White Shell Eggs.', *Poultry Science*, 65(3), pp. 501–507. doi: 10.3382/ps.0650501.
5. Ekue, F. N. K. Et Al (2002) 'Survey of the Traditional Poultry Production System in the Bamenda Area, Cameroon', *Institute of Agricultural Research for Development (IRAD) Yaounde, Cameroon, Joint FAO/(June)*, pp. 15–25.
6. Gorkhali, N. et al. (2021) 'Polymorphism of Sakini Chicken Population From Different Locations/Altitudes of Nepal Using Randomly Amplified Polymorphic Dna Markers', *SAARC Journal of Agriculture*, 18(2), pp. 115–124. doi: 10.3329/sja.v18i2.51113.
7. Hays, F. A. And Sumbardo, A. H. (1927) 'Physical Characters of Eggs in Relation to Hatchability.', *Poultry Science*, 6(4), pp. 196–200. doi: 10.3382/ps.0060196.
8. Kattel, P. (2016) 'Socio-Economic Importance of Indigenous Poultry in Nepal', *Poultry, Fisheries & Wildlife Sciences*, 4(1), pp. 9–11. doi: 10.4172/2375-446x.1000153.
9. Markos, S., Belay, B. and Astatkie, T. (2017) 'Evaluation of egg quality traits of three indigenous chicken ecotypes kept under farmers' management conditions', *International Journal of Poultry Science*, 16(5), pp. 180–188. doi: 10.3923/ijps.2017.180.188.
10. Mujeer, K. A. et al. (1988) 'Traits influencing the hatching performance of japanese quail eggs', *British Poultry Science*, 29(1), pp. 101–112. doi: 10.1080/00071668808417031.
11. Neupane, D. et al. (2019) 'Effect of Probiotics on production and egg quality of dual purpose chicken at Kathmundu in Nepal', *Bangladesh Journal of Animal Science*, 48(1), pp. 29–35. doi: 10.3329/bjas.v48i1.44556.
12. Peebles, E. D. And Brake, J. (1987) 'Eggshell Quality and Hatchability in Broiler Breeder Eggs ', *Poultry Science*, 66(4), pp. 596–604. doi: 10.3382/ps.0660596.
13. Sapkota, S. et al. (2017) 'Research Article;Evaluation of egg laying and egg quality parameters of local chicken Sakini', 1(September 2019), pp. 181–188.
14. Sapkota, S. et al. (2020) 'Selective Breeding to Improve Productive and Reproductive Performances and Survivability of Indigenous Sakini Chicken', *Journal of Nepal Agricultural Research Council*, 6(March), pp. 62–69. doi: 10.3126/jnarc.v6i0.28116.
15. Silversides, F. G. and Scott, T. A. (2001) 'Effect of Storage and Layer Age on Quality of Eggs From Two Lines of Hens 1', pp. 1240–1245.
16. Verma, A. K. et al. (2018) 'Comparative Assessment of Fertility and Hatchability of Kadaknath and Aseel Fowls', (7), pp. 1238–1243. doi: 10.13140/RG.2.2.16460.26245.
17. Yakubu, A., Ogah, D. M. and Barde, R. E. (2008) 'Productivity and egg quality characteristics of free range naked neck and normal feathered Nigerian indigenous chickens', *International Journal of Poultry Science*, 7(6), pp. 579–585. doi: 10.3923/ijps.2008.579.585.

Feeding Status of Indigenous Buffalo in Midhills of Gandaki Province, Nepal

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ABSTRACT

More than 50% of the households of Gandaki province have been rearing buffaloes. Whereas in mid hills 1-2 number of buffalo is present in most houses which are reared for sustainable purpose. A cross sectional study was carried out for a year in the mid hills of Gandaki province selecting 20 farms from four locations; Parbat, Nagi ramche, Tarakhola and Lwang ghalel, Kaski. Similarly, for a year a buffalo farm of Directorate of Agricultural research, Gandaki was also studied. The purpose of this study was to determine the feeding system of buffalo in midhills and DoAR farm and compare the milk production. The milk production by the indigenous buffalo of local farms (3.3liters/d) was significantly higher than that of DoAR farms(2.9liters/d) despite of feeding balanced concentrate ration daily. The raulo and khar combination was the main source of roughages whereas maize, barley and millet flour were the common source of concentrate for the indigenous buffalo in mid hills of Gandaki province.

Keywords: Sustainable, Milk Production, Kaski, Raulo, Khar

INTRODUCTION

In Nepal buffalo farming is a main occupation which covers total population of 51,78,612. The contribution of buffalo in meat and milk production is 58.3% and 68.7%, respectively (SINA 2013/14). The buffalo is reared under subsistence farming system rather than for commercial purpose particularly in mid hills part of Nepal. One or maximum two numbers of buffalo are reared and provided feeding that is locally available. The practice of feeding locally available resources doesn't meet the requirement of the buffalo which could be the reason for low milk production. The buffalo should be provided with the Dry matter three percent of their body weight. The one third of the total dry matter should come from concentrate and the remaining two third from the roughages which is the thumb rule of feeding buffaloes. In the country 47% of total livestock nutrition in terms of Dry matter content is fulfilled by crop by-product and residue, and suffers from 31% of feed deficit (Upreti and Shrestha, 2006). The month, duration from June to October is

identified as the peak season of milk production because of the availability of the green grasses which enhances nutrition. In a study done in Chitwan wheat bran and rice bran were identified as the major concentrate feeds and rice straw as major roughages source. The concentrate provided occasionally to the buffaloes in the Chitwan is wheat straw, maize flour, and commercial feed (Shah et al., 2018). The average milk production by Murrah cross in the Chitwan is 2.7 liters.

Lack of knowledge, regular practice of feeding, far from the agro vets and shortage of feeds are some of the reasons for poor feed quality provided to buffaloes. Indigenous buffalo mainly Lime and Parkote are the common breeds of buffalo raised in the mid hills region of Gandaki Province. The villages are far from the road and markets which avoids artificial insemination leading to conservation of pure breeds of buffaloes there. The genetic potentiality of such indigenous buffalo is unknown, the type of feed that they are having and the nutrient content present in them is still not clearly determined which the first step for improving productivity is. The low-quality roughages, agricultural crop-residues and industrial by-products which contain high levels of lingo cellulosic materials, low levels of fermentable carbohydrate and protein are fed to buffalo in mid hills. The productivity improvement program focuses only on breed improvement program rather than the identification of genetic potentiality of the indigenous breed and providing them the nutritious feeds. The purpose of this study was to determine the status of the indigenous buffalo and to determine the year round feeds that are provided to the buffalo. This study was done with a view to help the future programs to provide a valid statistics information on feeds as a basis for the improvement programs.

METHODOLOGY

Year round survey was taken in four different sites (Nagi ramche, Ramja of Parbat, Tarakhola of Baglung and Lwang Ghalel of Kaski) of mid hills. Twenty farmers were selected from each sites and a recorder was kept for each sites. The recorders were kept to determine the daily milk production and types of feed fed to the buffalo. The data were collected in a month interval from each sites and analyzed.

Source of Roughage

Crop by products and fodder trees were major source of roughages for buffalo. Khar (*Imperata cylindrica*) and raulo are the common green forage, maize Stover, rice straw and wheat straw are common crop by-products and khasru (*Quercus semicarpifolia*) and Duhilo (*Ficus neriifolia* var *nemoralis*) are the common fodder trees fed to the buffaloes in mid hills of Gandaki province. They are fed either alone or in combination based on their availability rather than the requirement assessment. The source of roughages fed to the buffalo was collected every month from each house so, they were arranged and entered. The frequency was determined to calculate the most available roughages in the

study sites. On an average 33 kg of roughages and was provided daily to average 3.3 liters' milk producing indigenous buffalo in the mid hills.

Source of Concentrate

Millet bran, maize bran, maize flour, broken rice, local kudo, and barley flour were the identified source of concentrate for buffalo in the study sites. They were fed either alone or in combination based on their availability. Local kudo is a homemade mixture prepared by mixing the waste of kitchen, wheat flour, maize flour, water, and salt, and finally cooking to prepare slurry mixture. The source of concentrate fed to the buffalo was collected every month from each house so, they were arranged and entered. The frequency was determined to calculate the most available concentrate in the study sites. On an average 1.01 kg of concentrate was provided daily to average 3.3 liters' milk producing indigenous buffalo in the mid hills of Gandaki province.

Source of roughages and concentrate in the farm of DOAR, Lumle

The farm consists of 30 indigenous buffaloes out of which only 10 among them were lactating. They were provided daily with 3 kg of concentrate feeds containing 90.4% Final Dry matter and 24.98% crude protein. Whereas in case of roughages they were grazed for 4 hours daily, 5 kg of straws and green grass according to season. During summer season they were provided with 25 kg of grasses like setaria, napier, Khar, Bermuda and local grasses. During winter they were fed with 4 kg of silage and 20 kg of oats grass or fodder leaves. The fodder trees include Katus, Rye khanyu, Nemaro, Dudhilo, and Pakhuri.

RESULTS

The types of roughages and concentrate fed to the indigenous buffalo are given in Table 1 and Table 2, respectively. Table 1 indicates that Raulo and Khar combination was the most available roughages for feeding to the buffaloes in mid hills followed by khar and maize stover combination. Khasru alone was the least available source of roughage for feeding to the buffaloes in study sites. Figure 1 indicates that raulo and khar combination was available for 12 months whereas raulo and dudhilo combination was available only in Baisakh and Mangsir.

Calendar of source of roughages for buffalo

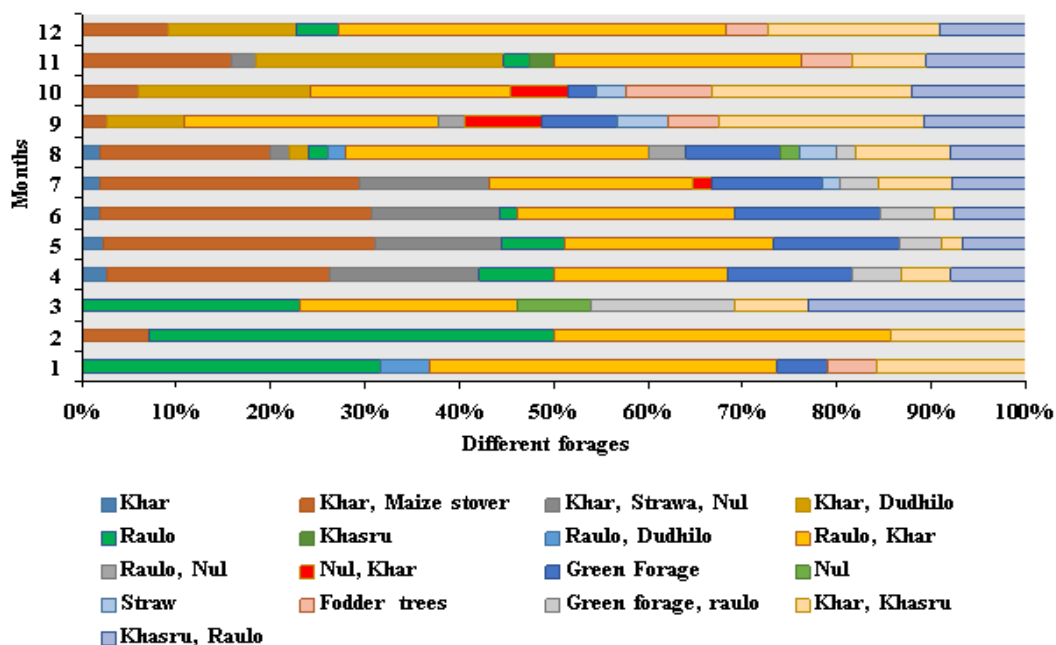


Figure 1. Calendar of source of roughages for buffalo in the study sites

Table 1. Different roughages available for indigenous buffalo in mid hills

S.N.	Roughages	Frequency	Percentage
1	Khar	5	1
2	Khar, Maize stover	72	14.8
3	Khar, rice and wheat straw	28	5.8
4	Khar, Dudhilo	23	4.7
5	Raulo	25	5.1
6	Khasru	1	0.2
7	Raulo, Dudhilo	2	0.4
8	Raulo, Khar	107	22
9	Raulo, Wheat straw	3	0.6
10	Wheat straw, Khar	6	1.2
11	Green Forage	35	7.2
12	Wheat straw	2	0.4
13	Straw	6	1.2
14	Fodder trees	9	1.9
15	Green forage, raulo	12	2.5
16	Khar, Khasru	41	8.4
17	Khasru, Raulo	31	6.4
18	Total	408	100

Table 2. Different concentrate feed available for indigenous buffalo in mid hills

S.N.	Concentrate feeds available	Frequency	Percent
1	Millet, maize bran	76	18.6
2	Millet bran	16	3.9
3	Maize flour	73	17.9
4	Broken rice	6	1.5
5	Maize, broken rice	11	2.7
6	Local kudo	92	22.5
7	Barley	4	1.0
8	Maize, barley, millet flour	130	31.9
9	Total	408	100.0

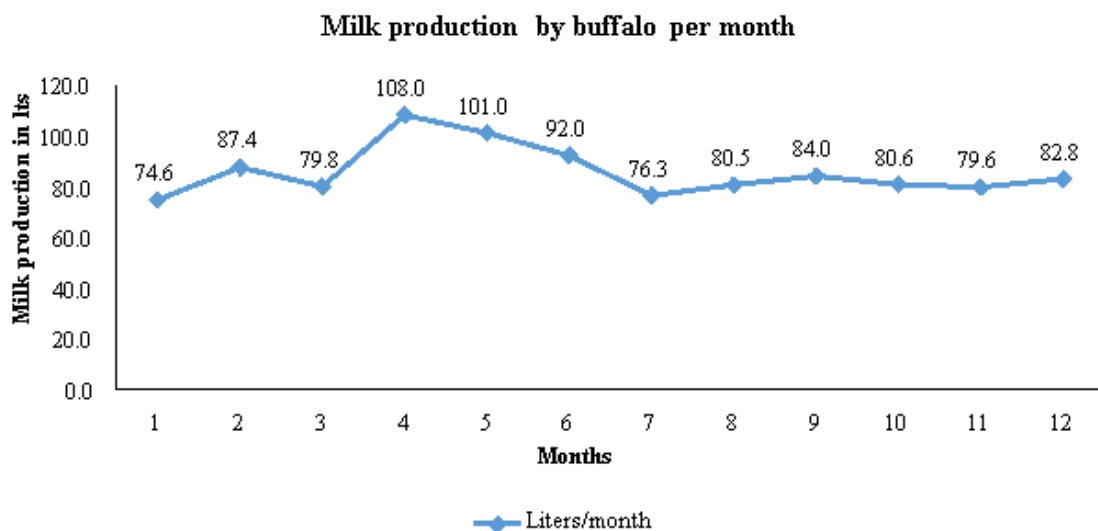


Figure 2. Line graph showing average milk production by buffalo per month

DISCUSSION

Majority of buffalo owners practice open grazing to animals followed by semi stall feeding while very few respondents practiced only grazing on pasture land which is supported by the similar findings of Viswkarma, et al. (2018). Also in Bangladesh, the buffalo are fully dependent on roughages for feed and no concentrate is provided. They are fully dependent on grazing and more than 20% farmers uses cultivated fodders and tree leaves. More than 90% farmers reported that feeds are available in the selected areas (Sarkar et al., 2013).

In the study sites Khar, maize stover and raulo are the common source of roughages rather than the wheat and rice straw contrast to the study done in Bangladesh. Home-

made concentrate, Kudo is also one of the common sources of concentrate in the study sites similar to the study of Bangladesh (Viswkarma, et al., 2018). In Natore district of Bangladesh, buffalo are grazed for 5-7 hours/d, source of roughages are rice straw, locally available green grasses like durba, baghata grass, vadal grass, kaishe grass, khasheri koli etc. and crop byproducts which include sugarcane top, sugarcane pulp and whole sugarcane. The sources of concentrates are rice polish, wheat bran, broken rice, rice gruel and oil cake (til oil cake, mustard oil cake) (Siddiki et al., 2015).

In terai region of Nepal, the lack of green pasture in diet of cattle was compensated by supplying large amount of straw resulting low concentration of CP and TDN than other periods result decrease in milk production. (Hayashi et al., 2005). In Contrast in this study the straw is used in less frequently. Khar (*Imperata cylindrica*) grows well from sea level to 2000 m of altitude, in sub humid or humid grasslands or open woodlands. It grows well at 25°C-35°C temperatures, 250-6250 mm annual rainfall, full sun or light shade on light sandy soils of 4 to 7.5 pH. It is capable of tolerating long drought periods, and burning since its rhizome allows rapid regrowth but cannot withstand flooding and temperatures below 20°C (Ecoport, 2010; FAO, 2010) Though khar contributes much in the feeding of buffalo, it is considered as a poor quality grass whose cp is about 7% DM and generally lower than 12% DM (Feedipedia, 2013). It is also deficient in energy and sodium (Falvey, 1981).

The milk production by indigenous buffaloes was 2.9 liters/day and 3.3 liters/day respectively in the DoAR, Gandaki and local villages of Gandaki Province. This finding is similar to that of Natore district, Bangladesh (3.32 Liters/d) (Gupta et al., 2014) and indigenous Bangladeshi buffalo (2.1 to 2.7 Liters/d) (Amin et al., 2015). In contrast, the average finding is lesser than that produce (7.19 ± 0.18 liters/d) by Azikheli buffaloes of Pakistan (Khan et al., 2014).

CONCLUSION

Lime and Parkote are the most efficient utilizer of the fibers and convert poor quality roughages to high quality milk. Raulo and Khar are the common source of roughages whereas maize, barley and millet flour are the common source of concentrate for the buffaloes in mid hills of Gandaki Province. The balanced concentrate ration has no effect on the milk production potential of indigenous buffalo in the study. Thus, financial burden can be reduced by decreasing amount of concentrate ration and replacing it with more roughage in the feed of buffalo.

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CONFLICT OF INTERESTS

The authors hereby declare there is no any potential conflict of interest(s) including financial or personal with the work submitted for publication.

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REFERENCES

1. Gupta, J. J., Singh, K. M., Bhatt, B. P., and Dey, A. (2014). A diagnostic study on livestock production system in Eastern Region of India.
2. Amin, M. R., Siddiki, M. A., Kabir, A. K. M. A., Faruque, M. O., and Khandaker, Z. H. (2015). Status of buffalo farmers and buffaloes at Subornochar upozila of Noakhali district in Bangladesh. *Progressive Agriculture*, 26(1):71-78.
3. Khan, M., Saleem, M., Gohar, A., Ali, Q., Khan, H., Ahmad, S., and Salim, M. (2014). Assessment of morphometric, productive and reproductive characteristics of Azikheli Buffalo in Swat valley in Northern Pakistan. *Life Science Journal*, 11(11 SPEC. ISSUE 12): 1-8.
4. Siddiki, M. A., Amin, M. R., Kabir, A. K. M. A., Faruque, M. O., and Khandaker, Z. H. (2015). Socio-economic status of buffalo farmers and the performances of buffaloes at Lalpur Upozila of Natore district in Bangladesh. *Bangladesh Journal of Animal Science*, 44(3):157-165.
5. Sarkar, S., Hossain, M. M., and Amin, M. R. (2013). Socio-economic status of buffalo farmers and the management practices of buffaloes in selected areas of Bagerhat District of Bangladesh. *Bangladesh Journal of Animal Science*, 42(2):158-164.
6. Statistical Information on Nepalese Agriculture 2013/2014
7. Ecoport, 2010. Ecoport database.
8. FAO, 2010. Grassland Index. A searchable catalogue of grass and forage legumes. FAO, Rome, Italy
9. Falvey, J. L., 1981. *Imperata cylindrica* for animal production in South-East Asia: a review. *Tropical Grassland*, 15 (1): 52-56
10. Hayashi, Y., Maharjan, K. L., and Kumagai, H. (2005). Feeding traits, nutritional status and milk production of dairy cattle and buffalo in small-scale farms in Terai, Nepal. *Asian-Australasian journal of animal sciences*, 19(2):189-197.
11. Viswakarma, R., Singh, R., Kushram, P., and Kumar, S. (2018). Existing status of buffalo husbandry practices

Study of Prevalence of *Paramphistomum cervi* in domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski

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ABSTRACT

Paramphistomum cervi has been a neglected but very crucial trematode parasite causing huge unseen economic loss in ruminant production globally. It has become an endemic species of fluke predominant in tropics and subtropics of Nepal. This study was aimed to estimate the overall prevalence of *Paramphistomum cervi* eggs in different domestic ruminant species of Pokhara Lekhnath Metropolitan City of Kaski district and to find the interaction of prevalence with other variables. A cross sectional study was performed to study the prevalence of *Paramphistomum cervi* eggs identified by Parasitological unit of Veterinary Hospital and Livestock Service Experts Centre, Kaski from January 2021 to April 2022. Out of 900 observations, 842 observations with complete information were filtered out and included in our study. Statistical analysis was done with EpiInfo (Version 7.2.4.0) and graphical representation was done with MS Excel 2016. The overall prevalence of *Paramphistomum cervi* in different species of domestic ruminants was found to be 16.98% (143/842). The highest prevalence was seen in Cattle that was 18.21% (45/247), followed by goats 16.66% (5/30) and buffaloes 16.46% (93/565). Seasonwise study of prevalence of *Paramphistomum cervi* in all the three ruminant species showed the highest overall infectivity during summer i.e 20.42% (88/431) followed by 13.38% (55/411) in winter and this difference was statistically significant (p -value 0.0065 at 95%CI). The higher prevalence of parasite was observed in adult lactating females of improved breeds. Thus, *Paramphistomum cervi* infection has become an important animal health issue to be addressed for safeguarding animal health and improvement of ruminant production in Pokhara Lekhnath Metropolitan City, Kaski. The biological intermediate host control measures in the pasture and routine screening of the parasite with routine deworming is essential to reduce the fluke burden among ruminants of Pokhara Metropolitan City in the future.

Keywords: *Paramphistomum cervi*, Prevalence, Ruminants, Season

INTRODUCTION

Paramphistomum spp. are gastrointestinal helminths of ruminants belonging to the class trematode and family Paramphistomidae having dorsoventrally flattened and leaf like shape. These are found all over the world tropics and sub-tropics (Hanna,1988). They have indirect type of life cycle like other trematodes involving the freshwater snails (*Bulinus* spp., *Glyptanisis gilberti*, *Indoplanorbis exustus*, *Planorbis planorbis*, *Stagnicola* spp. and *Lymnaea bulimoides*) as intermediate host and ruminants as definitive host. (Chaothary et al.,2015, Hotessa et al.,2020). *Paramphistomum* infects cattle, sheep, goats migrating in the small intestine cause more severe damage and other livestock as well as many wild ruminants. (González et al., 2013).

The common rumen fluke, *Paramphistomum cervi* is considered to be one of the most important species of Paramphistomes. (Khan et al.,2008) The mature stages residing on walls of rumen and reticulum rarely produce clinical disease while immature migrating froms can bury into sub mucosa of duodenum, destroy epithelial cells resulting in severe illness and often death due to anaemia. (Dube et al.,2010). The infection with immature *P. cervi* can result in a gastrointestinal disease in ruminants called paramphistomosis, which can have a deleterious impact on livestock production. The prevalence of this disease has been reported higher in tropical and sub-tropical regions of the world. (Hotessa, et al., 2020). Paramphistomosis belongs to the category of food and water borne trematodiasis and considered one of the most significant parasitic disease of domesticated ruminants (Mursyidah et al.,2017).

Kaski district have more than 44 thousand cattle, 112,000 buffalo and 126,000 goat population which serves as an important economic sources for the farmers (MOALD, Gandaki Province, 2076/7). Paramphistomosis, favored by humid and sub-tropical climate, are causing deleterious impact on economy of the ruminant farmers of Kaski district. Pokhara Lekhnath Metropolitan City is the largest municipality in Kaski district and has largest number of domestic ruminants. Paramphistomosis causes huge economic loss due to various unseen but critical loss such as reduced fertility, delayed growth and development, delayed sexual maturity, poor productivity, cull animals, and poor hide quality (Dube et al., 2010). However, the epidemiology of Paramphistomosis in domestic ruminant of Kaski is poorly understood. This study will provide some basic information about the prevalence of the parasite, interaction with other factors for formulating policies about strategic control of this diseases. The objective of this study to find out the prevalence of *Paramphistomum cervi* eggs in Pokhara Lekhnath Metropolitan Municipality, Kaski and and to study the association between prevalence of *Paramphistomum cervi* eggs and other variables like season, age, breed, location, and type of animal.

MATERIALS AND METHODS

Study site

The study was carried out in largest metropolitan city of Nepal (in terms of area), Pokhara Lekhnath Metropolitan City of Kaski, Nepal. Kaski district lies in the western part of Nepal with co-ordinates 28°12'30"N, 83°59'20"E. The Pokhara Lekhnath Metropolitan city has a humid subtropical climate and receive a high amount of precipitation annually. Faecal samples were brought by the farmers from all the wards of the Metropolitan city for test of helminthes.

Study area of *Paramphistomum cervi* in Kaski district

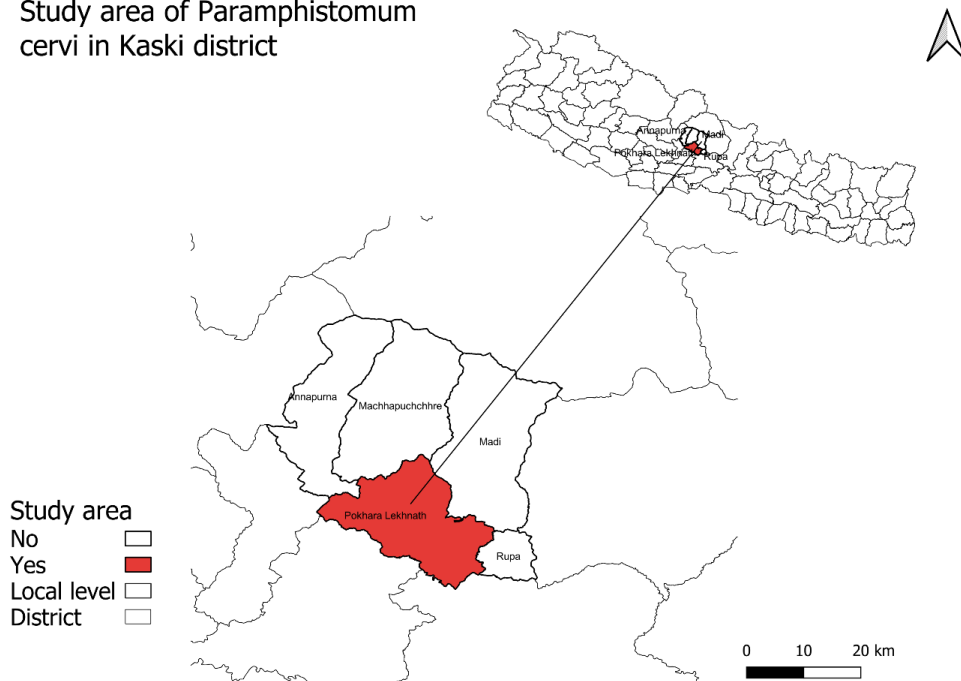


Figure 1. Study site (Pokhara Lekhnath Metropolitan City, Kaski, Nepal)

Study design and sampling

A cross-sectional study was performed to analyze the case data recorded in the case registry book of Parasitological Unit, Veterinary Hospital and Livestock Service Expert Centre, Kaski from January to April 2021 (mid Poush 2077-mid Baishakh 2078). The four months study period was divided into two seasons namely Winter (January-February) and Summer (March-April). There were altogether 900 cases recorded in the register along with 7 variables: date, species of animal, breed, age, location of farm, season of the year and test outcome. All of them were entered in MS Excel-2016 and data cleaning was done manually. The incomplete observations and observations from municipality other than

Pokhara Lekhnath Metropolitan City were removed and final number of cases included in the study was 842. The age group of the animals was categorized as: Young kids, calves and Heifers less than 2 years of age, Adult and lactating doe, cattle and buffalo in early parity -2-5 years, and Adult and lactating doe, cattle and buffalo in late parity- 5-10 years. The hill cattle, lime and parkote breed of buffalo and khari breed of goat are included under local breeds. While the improved breeds include Jersey cross and Holstein Cross cattle, Murrah cross buffalo, Khari × Jamunapari and Khari × Boer breeds of goat.

Procedure of Fecal Test

Samples were brought to the laboratory in sterile polythene bags by the farmers and were examined immediately in the parasitological unit of the hospital. Sedimentation technique was followed to examine the presence of *Paramphistomum* eggs in the faecal samples brought. About five grams of faeces was mixed in 30-50 ml of water and strained through a sieve to remove the coarse material. The mixture was allowed to sediment for half an hour and the supernatant was decanted. Washing was continued until supernatant became clear. Then after, a drop was taken from sediment with a Micropipette on a clean glass slide and was examined under microscope at 10 X for the presence of *Paramphistomum* eggs (Raza, et al., 2012). The identification of eggs of *Paramphistomum cervi* was done according to (Mukhia, et al., 2007) according to whom eggs are 114-176 µm by 73-100 µm in size, Oval in shape, whitish to transparent in colour, distinct operculum, knob-like thickening at the acetabular end of shell and embryonic cells are distinct.

Statistical analysis

All the data entered in MS Excel were first analyzed by pivot table in MS excel 2016. Descriptive analysis was done with EpiInfo (Version 7.2.4.0) and MS Excel 2016 was used to generate graphical presentation of the data. 2 × 2 cross tabulations were performed with EpiInfo and the result were expressed in percentage with P-value and significance was determined when $P < 0.05$

RESULTS AND DISCUSSION

Out of the 842 faecal samples collected from different small and large domestic ruminants of Pokhara Lekhnath Metropolitan City, 16.98% (143/842) were positive for *Paramphistomum cervi* eggs on Fecal test by Sedimentation technique. The result is similar to the overall prevalence of 15.64% *P. cervi* in buffaloes of Kathmandu as reported by Mukia, et al., 2007. Raza et al., 2012 also observed 15% prevalence of *Paramphistomum cervi* in buffaloes of Punjab, Pakistan by similar method. 10.78% *Paramphistomum sp.* in buffalo of Pokharatok VDC in Arghakhanchi district, Nepal was reported by Devi et al., 2012 adopting the method similar to ours. Likewise, (9.69%) of the total faecal samples of different domestic ruminants (cattle, buffalo, goat and sheep) were found to be positive for paramphistome eggs in India according to Maitra, et al., 2014.

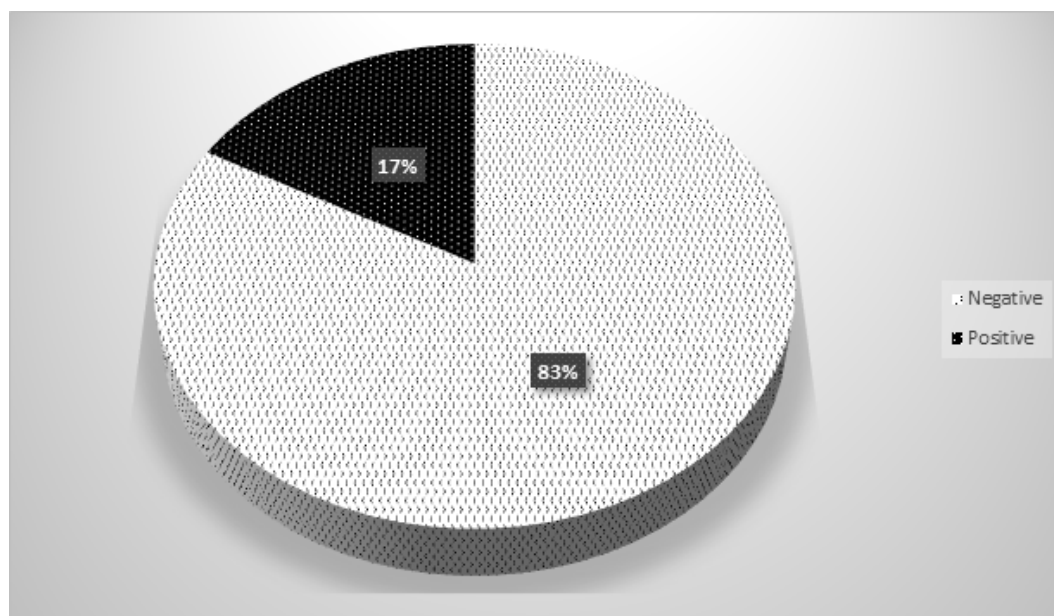


Figure 2: Overall prevalence of *Paramphistomum cervi* in Pokhara Lekhnath Metropolitan City, Kaski

Host-wise Prevalence

The host-wise prevalence of *Paramphistomum cervi* showed that the prevalence of eggs in cattle was found to be the highest i.e Cattle 18.21% (45/247), followed by goats 16.66% (5/30) and buffaloes 16.46% (93/565). As per the study of Khan, et al., 2009, the incidence of paramphistomes was significantly high in buffaloes than cattle that shows dissimilarity to the trend in our study. In our study, the prevalence in cattle is almost 2.5 times lower than prevalence of *Paramphistomum* sp. in cattle (48%) and that in buffalo is slightly higher than 11% as reported by Adhikari, et al., 2003 in cattle and buffaloes of Kathmandu valley through faecal test by sedimentation method. Our result is a bit higher than the result reported by Maitra, et al., 2014 who reported the overall prevalence of paramphistomes in different hosts as, cattle (12.4%) and buffalo (12.3%) followed by goat (4.9%) in uttarakhand, India. Hanna, et al., 1988 reported that of all buffaloes, 92-94% were found to be infected with paramphistomes in India. Contrasting to our study Purja, et al., 2015 reported 4.09% *Paramphistomum* sp. in goats of Puranchour VDC, Pokhara which is much lower than the prevalence found in our study among goats. This is due to the mixed ruminant husbandry. Our study showed slightly higher prevalence in larger domestic ruminants (cattle and buffalo) 16.99% (138/812) than in small domestic ruminants (goats) 16.66% (5/30). But the observed difference among large and small ruminants wasn't statistically significant. (χ^2 value 0.0022, p-value 0.96, OR 1.02, 95%CI (0.38-2.72)).

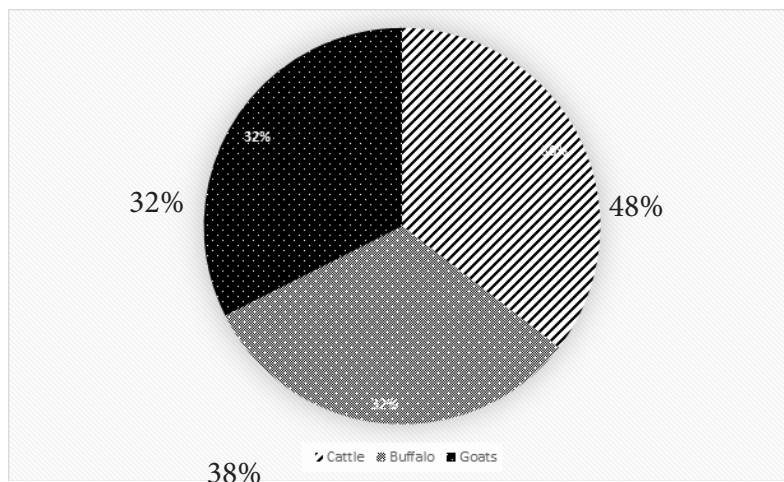


Figure 3: Host-wise prevalence of *Paramphistomum cervi* in different domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski

Month and Season wise prevalence

The monthwise prevalence study showed the highest prevalence during April (20.08%) (46/229) followed by March (17.98%) (41/228). Jan (14.94%) (29/194) and February (14.13%) (27/191). As a whole the prevalence had increasing trend from January to March. The host species wise monthly prevalence showed that cattle showed the highest infectivity in month of January (23.25%) and lowest was during February (14.51%). Buffalo showed the highest infectivity in April (20.68%) and lowest was observed in January (12.16%). Goat showed maximum infection in January (33.33%) and lowest in February (9.09%).

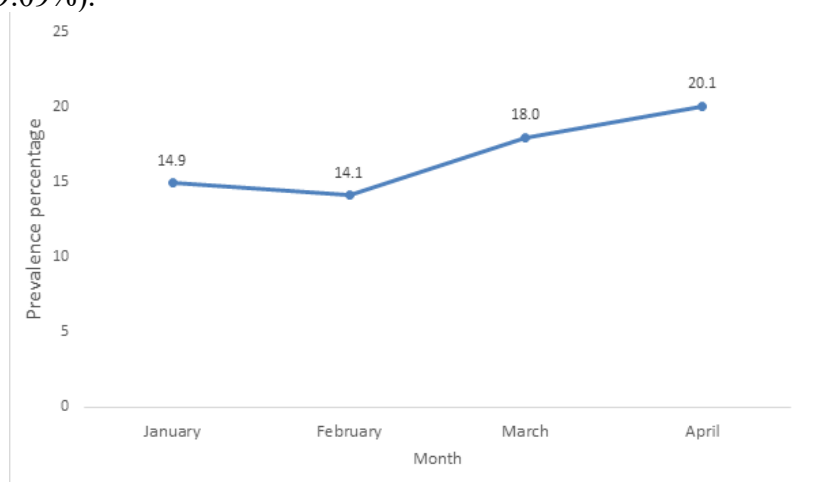


Figure 4: Overall monthly prevalence of *Paramphistomum cervi* in different domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski



Figure 5: Month-wise prevalence of *Paramphistomum cervi* in different domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski

Prevalence of *Paramphistomum cervi* in all the three ruminant species showed the highest infectivity during summer i.e 20.42% (88/431) followed by 13.38% (55/411) in winter. The cross-tabulation of season and overall prevalence showed that the presence of parasite was significantly different in summer and winter (χ^2 value 7.38, p-value 0.00657, OR 0.60, 95%CI (0.42-0.87)). The result is in line with Sardar, et al., 2008 who reported highest prevalence rates of different parasites were observed in the rainy season (July to October). Likewise, Chhabra et al., 1972 reported maximum prevalence of parasite eggs in the faeces of domestic stock in the Punjab during July with lowest prevalence in December and a sudden rise in June. *Paramphistomum* spp infection showed significant ($P < 0.05$) seasonal variation in rainy season in a study by Devi et al, 2012 in Pokharthok VDC of Arghakhachi district Nepal. According to Maitra et al., 2008, the infectivity was the highest during monsoon and post monsoon (12.77%) followed by summer (9.74%) and winter (6.54%). Shah et al., 2015 also reported that 71 (47.01%) samples were found positive in winter and 80 (53.33%) samples were found positive in summer.

The higher overall prevalence during summer or pre-monsoon in our study compared to winter season is due to the increasing humidity triggers the development of larval stages of parasite and starting of premonsoon precipitation in Kaski district from March creates favourable environment for survival of abundant intermediate hosts (snails) of *Paramphistomum*. Thus the ruminants ingest the infective stage (metacercaria) of the parasite through grass from damp and water logged areas or while grazing in such areas. (Akanda, et al., 2014).

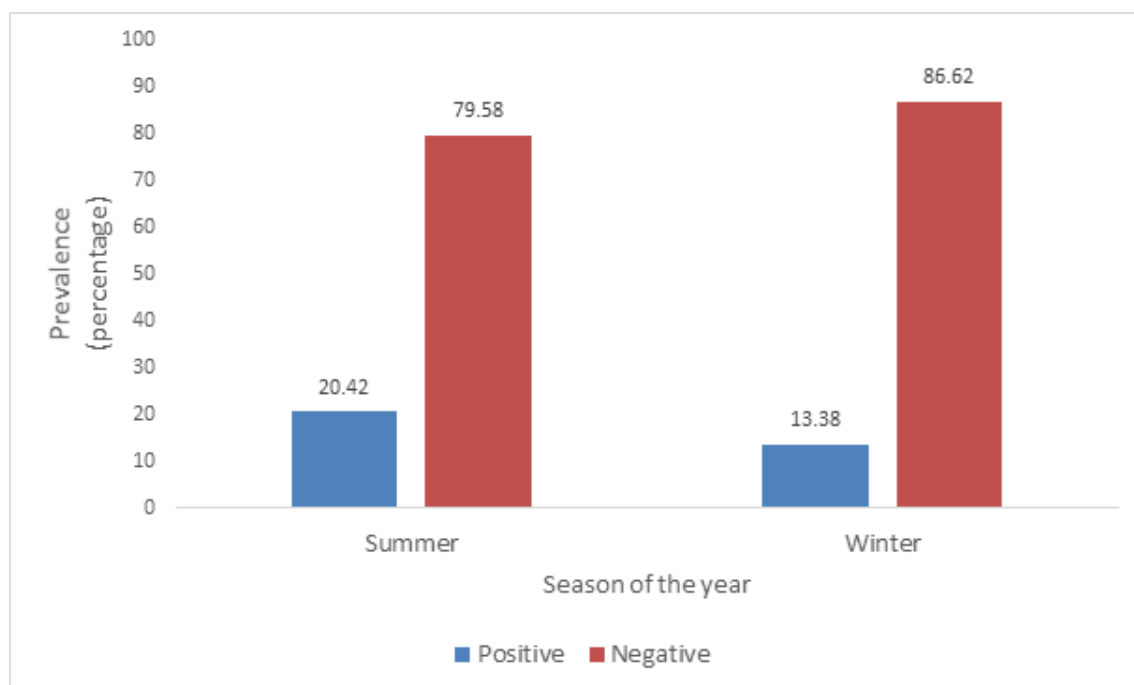


Figure 6: The relationship between season and prevalence of *Paramphistomum cervi* in Pokhara Lekhnath Metropolitan City, Kaski

Age-wise Prevalence

Faecal tests of different age groups showed that the presence of the parasite was highest in greater than 5 years' age group 18.28% (64/350) followed by 17.69% (60/339) in 2-5 years' age group and 12.41% (19/153) in less than 2 years' age group. Further categorizing the age groups of the above age groups as adult and lactating females of greater than 2 years' age group than the young ones and heifers of less than 2 years' age group, higher prevalence 17.99% (124/689) was seen among adult and lactating females and lower prevalence 12.41% (19/153) was seen in young and heifers. But this observed difference wasn't statistically significant (χ^2 value 2.76, p-value 0.09, OR 1.54, 95% CI (0.92-2.59)).

Similar to our study, Akanda et al., 2014 observed that prevalence increased with age in a linear pattern. Sardar et al., 2003 also gave the same line of finding for paramphistomes but the prevalence of *Ascaris*, *Strongylid* and *Strongyloids* were very high in the young animals of age group of < 12 months and gradually lowered in higher age groups. But Raza et al., 2007 reported higher prevalence of gastrointestinal parasites in younger ruminants in Pakistan. Higher prevalence of *Paramphistomum cervi* in larger age groups is supported by the reasons that such ruminants have been kept for longer time in the farm and gone through different kinds of stress like production stress, environmental stress, nutritional deficiency and other various kinds of stressful factors that can weaken their

immune system and predispose to parasitic infection (Akanda et al., 2014).

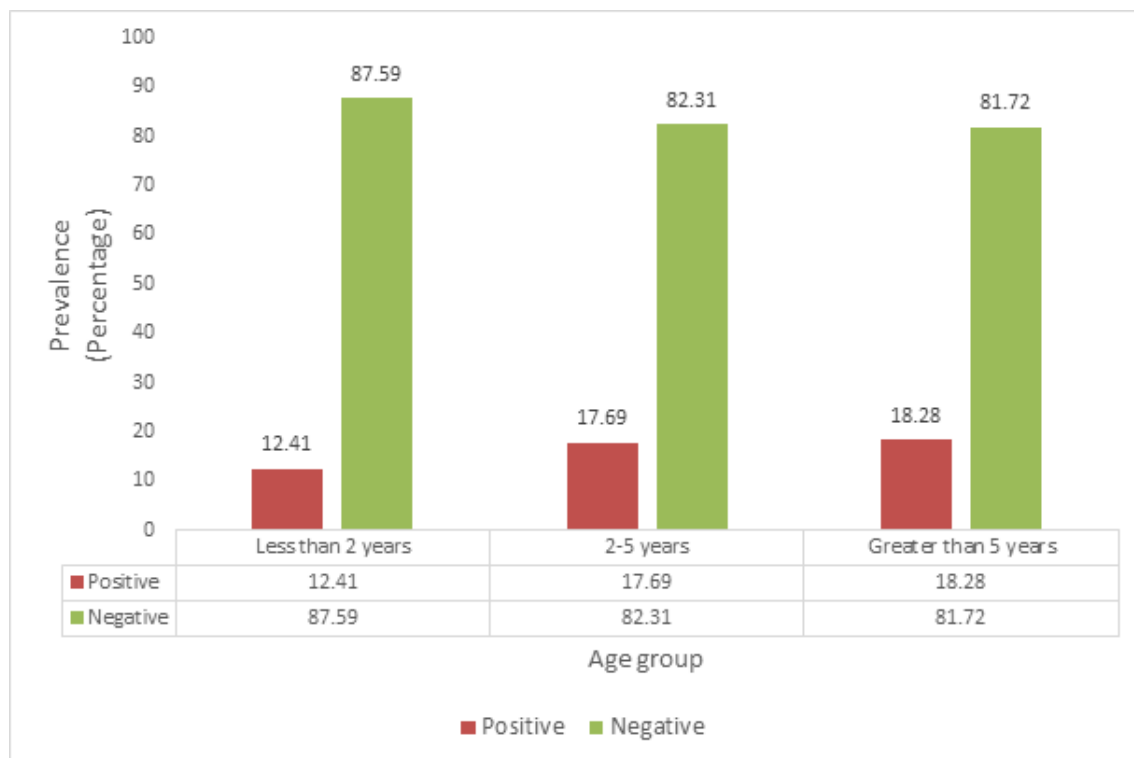


Figure 7: The relationship between the age group and prevalence of *Pramphistomum cervi* in Pokhara Lekhnath Metropolitan City, Kaski

Breed-wise Prevalence

Out of the total observations, 587 were improved breeds of cattle, buffalo and goats while 255 were local breeds. The improved breeds of cattle included 133 Jersey cross and 46 Holstein cross, that of buffalo included 390 Murrah cross and that of goats included 13 Khari*Boer and 5 Jamunapari cross. Likewise, the local breeds included 67 local cattle, 176 local buffaloes, 12 Khari goats. The prevalence was found to be 17.37% (102/587) in improved breeds and 16.07% (41/255) in local breeds. Although higher prevalence was seen in improved breeds compared to the local breeds, the difference was not statistically significant (χ^2 value 0.21, p-value 0.64, OR 1.09, 95% CI (0.73-1.63)).

In a similar study, Bista, et al., 2018 reported higher prevalence of flukes in Cross breed species as, Jersey Cross (39.4%), and Murrah Cross (21.4%) as compared to the prevalence in local buffalo breed (20.0%) and pure Jersey (12.5%) in Western Chitwan by sedimentation method. Ross *et al.* (1959) reported that the *Bos indicus* are more resistant to parasites than *Bos taurus*. Similar findings as the overall prevalence of gastro-intestinal parasites of crossbred cattle (51.11%) was higher than that of native bred (45.28%) was

reported by Sardar et al.,2003. Haque et al., 1986 also reported that the gastro-intestinal parasitic infestation rate was higher in crossbred cattle. The higher prevalence in cross-breed cattle can be justified due to the acclimatization of local breeds to the agro-climatic condition of the particular location of study and are more resistant to disease and parasites while the cross breeds go through some extent of environmental stress and are less resistant to disease and parasites compared to local breeds.

Location wise prevalence

Prevalence of *Paramphistomum cervi* in the wards of Pokhara Lekhnath Metropolitan City inside the city core area was 18.13% (111/612) and the prevalence in the wards outside the city core area was 13.91% (32/198). There was no significant difference between the location and the prevalence of *Paramphistomum cervi* (χ^2 value 2.11, p-value 0.14, OR 1.37,95%CI (0.89-2.09). The difference in agro climatic zone and type of animal husbandry system inside the city area and outside the city area might be accounted for this difference in prevalence inside and outside the city core area of Pokhara Lekhnath Metropolitan City. The farmers outside the city area have minimum access to laboratory faecal test facility at the Veterinary Hospital lying in the city due to which the sample size from outside the city area is quite small. This might have caused lower prevalence of *Paramphistomum* eggs in the wards outside the city core area. On the other hand, the city core area is valley while the wards outside the city core area lie towards hilly topography. The valley has more humid climate than the areas outside the valley.

Table 1 Table showing the relationship between prevalence of *Paramphistomum cervi* and other variables in Pokhara Lekhnath Metropolitan City, Kaski

SN	Variable	X ² value	(P-value)	Odds Ratio(95%CI)
1	Season	7.38	0.006	0.602
2	Breed	0.21	0.64	1.09
3	Age	2.76	0.09	1.54
4	Location	2.11	0.14	1.37

CONCLUSION AND RECOMMENDATIONS

The study shows the overall prevalence status of *Paramphistomum cervi* eggs in domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski district was 16.98%. Present study shows that there is relationship between prevalence and other variables like season, breeds, age, location, etc. but only the season is statistically significant. The prevalence of this parasite is seasonal and more common in summer than winter. The data explored in this study helps to understand the current situation of this parasite and forms the base for the farmers and stakeholders to construct further strategies and programs to control this parasite for management of animal health services. The farmers should be made aware about routine parasite control programs in their farm. The biological control methods for

intermediate hosts (snails) should be brought to practice to ensure safe grazing place and breaking the chain of the parasite's lifecycle.

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REFERENCES

1. Adhikari, B; Shrestha, B; Shrestha, R.M. (2002/03). A study on the prevalence and diversity of *Fasciola* spp in cattle and buffaloes in areas of Kathmandu valley. Central Veterinary Laboratory Annual Technical Report, 1: 59-66.
2. Akanda, M. R., Hasan, M. M. I., Belal, S. A., Roy, A. C., Ahmad, S. U., Das, R., & Masud, A. A. (2014). A survey on prevalence of gastrointestinal parasitic infection in cattle of Sylhet division in Bangladesh. *American Journal of Phytomedicine and Clinical Therapeutics*, 2(7), 850-860.
3. Bista, S., Lamichhane, U., Singh, D. K., & Regmi, S. (2018). Overview of Seasonal Prevalence of Liver Fluke & Rumen Fluke Infestation in Cattle and Buffalo of Western Chitwan, Nepal. *Journal of the Institute of Agriculture and Animal Science*, 35(1), 235-241.
4. Chaoudhary, V., Hasnani, J. J., Khyalia, M. K., Pandey, S., Chauhan, V. D., Pandya, S. S., & Patel, P. V. (2015). Morphological and histological identification of *Paramphistomum cervi* (Trematoda: Paramphistoma) in the rumen of infected sheep. *Veterinary world*, 8(1), 125. *for parasitology*, 18(4), 513-521.
5. Chhabra, R. C., & Gill, B. S. (1975). Incidence of helminthic infections and control of amphistomiasis and fascioliasis in animals in two villages of the Punjab. *J Res Punjab Agric Univ*.
6. Devi, R. 2012. Seasonal prevalence of helminth parasites in Buffaloes of Pokharathok VDC in Arghakhanchi. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal
7. Dube, S., & Aisien, M. S. O. (2010). Descriptive studies on Paramphistomes of small domestic ruminants in Southern Nigeria. *aramphistomosis in cattle*. *Veterinarski arhiv*, 78(3), 243-251.
8. González-Warleta, M., Lladosa, S., Castro-Hermida, J. A., Martínez-Ibeas, A. M., Conesa, D., Munoz, F., ... & Mezo, M. (2013). Bovine paramphistomosis in Galicia (Spain): prevalence, intensity, aetiology and geospatial distribution of the infection. *Veterinary Parasitology*, 191(3-4), 252-263.
9. Hanna, R. E. B., Williamson, D. S., Mattison, R. G., & Nizami, W. A. (1988). Seasonal reproduction in *Paramphistomum epiclitum* and *Gastrothylax crumenifer*, rumen paramphistomes of the Indian water buffalo, and comparison with the biliary paramphistome *Gigantocotyle explanatum*. *International journal*
10. Haque, M. A. (1986). Effects of sex, age, breed, season and system of management in the infectivity of gastro-intestinal parasitism in cattle (Doctoral dissertation, A thesis submitted to the Dept. of Medicine, Faculty of Veterinary Science, BAU, Mymensingh).
11. Hotessa, A. S., & Kanko, D. K. (2020). Review on Paramphistomosis. *Advances in Biological Research*, 14(4), 184-192.

12. Javed Khan, U., Tanveer, A., Maqbool, A., & Masood, S. (2008). Epidemiological studies of paramphistomosis in cattle. *Veterinarski arhiv*, 78(3), 243-251.
13. Khan, U. J. (2008). Epidemiology, Economic Importance and Therapy of Paramphistomosis in Cattle and Buffaloes (Doctoral dissertation, University of Punjab, Lahore).
14. Maitra, A., Yadav, C. L., & Sanjukta, R. K. (2014). Seasonal prevalence of paramphistomosis in domestic ruminants in different agro-climatic zones of Uttarakhand, India. *Asian Pacific Journal of Tropical Disease*, 4, S748-S753.
15. Mukhia, G. (2007). A study on intestinal helminth parasites of buffaloes brought to satungal (Kathmandu) for slaughter purpose (Doctoral dissertation, Central Department of Zoology Institute Of Science and Technology Tribhuvan University Kirtipur, Kathmandu Nepal).
16. Mursyidah, A. K., Khadijah, S., & Rita, N. (2017). Fasciola and paramphistomum infections in small ruminants (sheep and goat) in terengganu. *Malaysian Journal of Veterinary Research*. vol 8no, 2, 8-12.
17. Purja, R. (2015). Gastro-Intestinal Parasites in Goat (*Capra Hircus*) of Puranchour Vdc, Pokhara (Doctoral dissertation, Central Department of Zoology Institute of Science and Technology Tribhuvan University Kirtipur, Kathmandu).
18. Raza, M. A., Bachaya, H. A., Akhtar, M. S., Arshad, H. M., Murtaza, S., Ayaz, M. M., ... & Basit, A. (2012). Point Prevalence of Gastrointestinal Helminthiasis in Buffaloes (*Bubalus Bubalis*) at The Vicinity of Jatoi, Punjab, Pakistan. *Sci. Int.(Lahore)*, 24(4), 465-469.
19. Raza, M. A., Iqbal, Z., Jabbar, A., & Yaseen, M. (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *Journal of Helminthology*, 81(3), 323-328.
20. Ross, J. G., Lee, R. P., & Armour, J. (1959). Haemonchosis in Nigerian Zebu cattle: the influence of genetical factors in resistance. *Veterinary Record*, 71(2), 27-31.
21. Sah, P. K. (2015). Coprological Survey of Gastro-Intestinal Helminth in Buffalo (*Bubalus Bubalis*) of Dhrampur, Dhanusha, Nepal (Doctoral dissertation, Central Department of Zoology Institute of Science and Technology Tribhuvan University Kirtipur, Kathmandu).

Diagnosis and Management of Constipation and Obstipation in Canine: From Current Practices to Future

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ABSTRACT

This study was conducted to understand the present diagnosis and management patterns of constipation and obstipation in dog and accessing about its future holds. A total of 68 dogs both healthy (n=34) and constipated (n=34) brought to a private pet hospital in Kathmandu were included in the study for two-months during Feb-April, 2019 were recorded. The protocol comprised history taking (age, sex, breed and chief complains), physical and clinical examination, hematology/Biochemistry followed by radiography in some cases. Statistical analysis was conducted using MS-Excel 2016 and SPSS v25. We found that starting from the dietary management, rehydration and medical management using laxatives, enemas, antibiotics and supporting liver medications were being followed to manage constipation and obstipation. Anorexia (82.4%) and dehydration were the most common clinical signs. Female dogs (52.9%) were found more affected than male dogs (47.1%). Large and small breed dogs (73.5%) were more prone to constipation than medium sized breeds (26.5%). Older Dogs (>60 months) (34%) were the most affected age groups. Haemato-biochemical blood findings showed significant increase in Total Leucocyte Count (Leukocytosis), Neutrophilia, ALP levels and Blood Urea Nitrogen ($p \leq 0.001$) while there was also an increase in Serum bilirubin and Serum albumin levels ($p \leq 0.05$) in constipated dog. This research showed increased risk of constipation in old and female dogs. Starting from high fiber diets, mild laxatives and enemas, the treatment can be proceeded to fluid therapy, antibiotic therapy and medications managing liver ailments after haemato-biochemical examination. Use of Novel prokinetic drugs practiced in humans like Cisapride and Pruracalopride, can be considered in veterinary practice. In situations of surgical management, most reliable would be the subtotal colectomy to manage idiopathic megacolon causing constipation and obstipation.

Keywords: Constipation, Dog, Management, Novel Drugs

INTRODUCTION

In human medicine, a fecal frequency less than three bowel movements per week is called constipation (Oh et al., 2011; Read, Timms, Barfield, Donnelly, & Bannister, 1986) a constipation induction period, and a fig paste treatment period. We administered fig paste (12 g/kg daily, by gavage. In the dog and cat, it has been more clearly defined as infrequent, difficult or absent defecation with retention of feces within the colon and rectum (Jones, 2000; White, 2002). Obstipation is a form of constipation which is characterized by an inability of animal to evacuate the compacted mass of hard, dry feces where the impaction is observed from rectum to around ileocolic junction, sometimes further beyond that as well (Defarges, 2019). Constipation has a complex etio-pathogenesis resulting from under excretion of toxic gases and gastrointestinal fermentation (Pirkka, Arthur, Erika, & Seppo, 1998). It is also induced by various narcotic analgesics (opioids), anticonvulsants, antihistamines, anticholinergic, calcium channel blocking agents (Elliot, Watts, & Girard, 1983; Enck, 1988; Oh et al., 2011).

Constipation and obstipation are one of common problems in pet dogs in city areas where movement of animals and good fiber diet is compromised. While in developed countries many new technologies and drugs are widely in practice, in developing countries like Nepal, there are limited diagnostic tools and conservative management for handling constipation. Clinical evidence for the use of laxative and dietary management for relieving symptoms of constipation are deficit in both human medicine (Jones, Talley, Nuyts, & Dubois, 2002) although some preparations need to be prescribed by physicians. A meta-analysis was conducted to quantitatively evaluate the published evidence on the efficacy of laxatives in constipation. We found that large, well-controlled, published studies whose data were comparable were lacking. Of 250 articles, 35 met the inclusion criteria but only 11 yielded usable data (N = 375 patients on laxatives, 174 on placebo and veterinary medicine. The way of diagnosing disease using tools like radiographical and hemato-biochemical examination for prompt management varies by cases. Many human labeled products are also used in veterinary medicine to manage secondary organ ailments as veterinary specific drugs are scarce.

In severe cases, constipation often require short time of hospitalization to address and correct metabolic variations. The use of enemas is must for manual clearance of retained feces. Proper use of such techniques is a must in reducing disease severity (Washabau, 2003). The objective of this study is to find various diagnostic and management methods of constipation in current canine practice and novel methods for future practice in Nepal.

METHODOLOGY

The dogs used for this study were those admitted with suspected problems suggesting constipation to the Advanced Pet Hospital and Research Center, Kathmandu. This number included 34 diseased dogs and 34 apparently healthy dogs which were admitted regular veterinary services. The diagnosis protocol sequentially comprised History Taking, Physical and Clinical Examination, followed by Radiography along with Hematology and Biochemistry.

Each cases were registered in Pet Registration Form and Book for date, age, sex, breed, and chief complains of pet owner. The detailed information about complete medical, vaccination, dietary, and environment. History along with clinical signs including recent injuries, surgeries, drug treatment, or any other relevant conditions were noted. Additionally, examination of the distinctive organs and body frameworks was performed along with pulse, heart rate, respiration rate and rectal temperature. Abdominal X-rays in Right/Left Lateral View and Vento-Medial View (VD) to reveal any form of impacted fecal matter in the bowel were done. Blood was sent to Diagnostic Lab for haemato-biochemical abnormalities and to screen for suspected underlying cause of the constipation or any other infections.

Data that were collected on age, sex, breed along with difference in various hematological and Biochemical Parameters of Normal and Dogs with constipation were recorded and to Microsoft Excel 2016[®] spreadsheet, and exported to SPSS v25 for further analysis. First, in accordance to an established normal reference range, Healthy dogs and Dogs with constipation were categorized into lower than normal reference range, within normal reference range and higher than normal reference range and Pearson-Chi-Square Test along with Fisher Exact text was performed. Values of $p \leq 0.05$ were considered as statistically significant.

Management and Treatment of constipated Dogs

As dehydration is a usual sign seen in constipation, it is one of the important aspect to be considered while managing patient. Fluid therapy can be administered via different route (IV, Subcutaneous, Orally or using feeding tube) to organize the electrolyte balance, in which potassium being of the essential to be corrected. The method referred to the recent publication by Davis et al. (2013) was used for correcting hydration level. High fiber containing diets like Pumpkin Pulps, Carrots, peas or green beans, bran flakes, cooked oats were advised to use after grinding and steaming it altogether if possible. Commercially available Dog diets with high fiber contents with the quantity prescribed by company were also suggested. Along with this, fiber also reduces bile salt concentration which implicit reduction in gut contractibility activity. Along with this, proper hydration and regular exercise were advised to animal owner.(Gonzalez-Martinez, Ortiz-Olvera, & Mendez-Navarro, 2014).Laxatives are the agents that promotes defecation by increasing the frequency of defecation or by enhancing consistency and fecal volume. Osmotic

Laxatives (Lactulose solution, Duphalac®, Polyethylene Glycol, PEGLEC®), Rectal Laxatives; Enemas Surfactant or Stool softener (Solution of Glycerin and Sodium Chloride, EZIVAC®) laxatives are commonly used.

After checking for the increased infection counts via TLC and Neutrophils counts, different type of oral (Clavum®625 mg, Amoxicillin+Clavulanic Acid, Tab.Megapen® 250mg) and systemic antibiotics (Keftragard®1500/1000mg; Ceftriaxone+Sublactam, Megapen® 1000mg; Ampicillin + Cloaxicillin) were used to reduce underlying primary as well as secondary bacterial infections. Along with this, for managing liver ailments Ursodiol;Ursodeoxycholic acid(Livopan ®, Ursocol ®Agalstone ®,) which slows the progression of hepatotoxicity and diminishes intestinal retention of cholesterol along with suppressing hepatic synthesis and secretion of cholesterol. Also, L-ornithine-L-aspartate (Tab. Hepa-Meraz, ® Inj. Hepa-Meraz ®) is most commonly used orally and by needle (by IV) for a liver illness that reduces brain function called hepatic encephalopathy.

RESULTS AND DISCUSSIONS

The major clinical signs observed were Anorexia (82.4%), Problems in fecal passage (85.2%), Problem in urinary passage (41.2%), Dehydration with various severity (94.1%) and vomiting (52.8%). After manual palpation, Abdominal X-ray in ventro-dorsal view and lateral view, performed in 19 animals showed radiopaque stool mass in colon, large intestine along with radiopaque areas of gases formation inside various bowel areas. Female dogs (52.9%) were shown more affected with constipation than male dogs (47.1%). Older Dogs (>60 months) were found more affected among constipated dogs than other age groups. The occurrence of constipation was found high in large and small mediums (73.5%) than medium breeds (26.5%).

Haemato-biochemical blood findings showed highly significant increase in Total Leucocyte Count (Leukocytosis), Neutrophilia, ALP levels and increased Blood Urea Nitrogen ($p < 0.001$) while increase Serum Bilirubin and Serum albumin was found significant ($p < 0.05$) in constipated dog. Other parameters were not significantly different among healthy and constipated dog. Therapeutic Management started with hydration using DNS or RL after considering glucose level and severity of liver from blood report. Laxatives with high fiber diet were advised in all the cases, while enema was given to 13 cases where the fecal mass was obstructed more towards the colon and rectum areas. The patients with liver problems were given Ursodiol and L-ornithine-L-aspartate in recommended dose. All the cases were managed by therapeutic and dietary management. Surgical management was not practiced in any of the case.

Table 1 Haemato-biochemical profile in Normal and Constipated Dog (Mean $\pm$ S.E.)

Parameters(units)	Healthy Dog	Dog with Constipation	Normal Reference Range
Total Leucocyte Count(units/ μ L)	13369.12 $\pm$ 2950.655	21285.29 $\pm$ 6579.082	5000-16000
Neutrophils (%)	70.12 $\pm$ 9.874	86.5 $\pm$ 6.106	58-85
Hemoglobin(gm/dl)	13.1 $\pm$ 1.5152	14.576 $\pm$ 2.5041	12 to 19
PCV (%)	40.41 $\pm$ 4.593	44.68 $\pm$ 6.79	35 to 57
Glucose (Random) (mg/dl)	101.74 $\pm$ 20.367	95.06 $\pm$ 32.354	70-125
Blood Urea Nitrogen(mg/dl)	21.4118 $\pm$ 6.51859	49.0588 $\pm$ 29.39691	7 to 27
Serum Creatinine(mg/dl)	1.2971 $\pm$ 0.39118	1.6029 $\pm$ 0.73008	0.5-1.8
Serum Bilirubin Total(mg/dl)	0.2529 $\pm$ 0.1022	0.2265 $\pm$ 0.06183	0.1-0.3
Alkaline Phosphatase(U/L)	106.29 $\pm$ 27.967	155.44 $\pm$ 60.916	10-150
Alanine Aminotransferase(SGPT)(U/L)	41.18 $\pm$ 3.247	45.35 $\pm$ 13.865	5 to 60
Serum Protein(Total)(g/dl)	6.626 $\pm$ 0.4231	7.456 $\pm$ 2.4294	6.0-7.5
Serum Albumin(g/dl)	3.344 $\pm$ 0.3457	4.165 $\pm$ 0.5092	2.7-4.4

Table 2 Haemato-biochemical profile in Normal and Constipated Dog in various ranges

Parameters	Type of Dog	No. of Dogs (%)			p-value
		Lower than Normal Reference Range	Normal Reference Range	Higher than Normal Reference Range	
TLC	Healthy Dog	-	29(85.3)	5(14.7)	0.000**
	Dog with Constipation	-	9(26.5)	25(73.5)	
Neutrophils	Healthy Dog	3(8.8)	28(82.4)	3(8.8)	0.001**
	Dog with Constipation	-	18(52.9)	16(47.1)	

Hb	Healthy Dog	8(23.5)	26(76.5)	-	0.287
	Dog with Constipation	4(11.8)	29(85.3)	1(2.9)	
PCV	Healthy Dog	5(14.7)	29(85.3)	-	0.468
	Dog with Constipation	3(8.8)	30(88.2)	1(2.9)	
R.Glucose	Healthy Dog	5(14.7)	25(73.5)	4(11.8)	0.205
	Dog with Constipation	8(23.5)	18(52.9)	8(23.5)	
BUN	Healthy Dog	-	25(73.5)	9(26.5)	0.000**
	Dog with Constipation	-	2(5.9)	32(94.1)	
S.Creatinine	Healthy Dog	-	31(91.2)	3(8.8)	0.171
	Dog with Constipation	-	27(79.4)	7(20.6)	
S.Bilirubin	Healthy Dog	-	26(76.5)	8(23.5)	0.003*
	Dog with Constipation	-	34(100)	-	
ALP	Healthy Dog	-	31(91.2)	3(8.8)	0.000**
	Dog with Constipation	-	14(41.2)	20(58.8)	
ALT	Healthy Dog	-	31(91.2)	3(8.8)	0.452
	Dog with Constipation	-	29(85.3)	5(14.7)	
S.Protein_	Healthy Dog	1(2.9)	33(97.1)	-	0.076
	Dog with Constipation	-	30(88.2)	4(11.8)	
S.Albumin	Healthy Dog	-	34(100)	-	0.020*
	Dog with Constipation	-	29(85.3)	5(14.7)	

** p < 0.001 (highly significant)

* p < 0.05 (significant)

Dehydration and Anorexia was common problem in humans as well, supported by Brosshard, Dreber & Schnegg (2004). Radiographic findings were similar to reported by (Lee & Leowijk, 1982; Rakha et al., 2015). Hydration using DNS or RL after considering glucose level and severity of liver were supported by Allen & Holm (2008) and Davis et

al., (2013) chief complaint, physical exam findings, and indicated additional testing will determine the need for fluid therapy. Fluid selection is dictated by the patient's needs, including volume, rate, fluid composition required, and location the fluid is needed (e.g., interstitial versus intravascular. Laxatives with high fiber diet advised in managing constipation were supported by Kanauchi et al. (2010). Female were found to be more affected with constipation which was similar to (Rakha et al., 2015). Although the pet owners usually prefer male dog, so constipated female were not significantly higher. Age group greater than 60 months (or 5 years) developed constipation more frequently, also (Rakha et al., 2015) showed similar findings for digestive problems, primarily constipation. It is also supported by Higgins & Johanson (2004) and Brosshard, Dreber & Schnegg (2004), where researches mentioned constipation is seen mostly in female and old humans. Also (Knowles et al., 2001) mentioned about increased risk of constipation after childbirths. This might owe due to sedentary and lazy lifestyle in old age as well as in female dog.

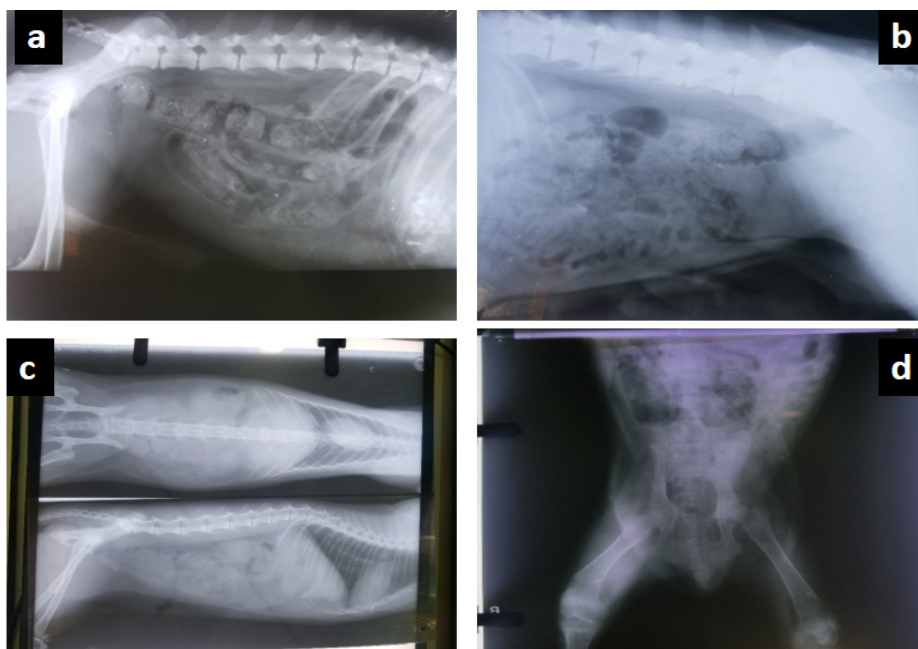


Figure 1 Radiographic Images of various constipated dogs.

a) Right lateral radiographic views (RL view) showing radiopaque stool mass in the colon of 5 years old German Shepherd with severe constipation. (b) Left lateral radiographic views (LL view) with radiopaque areas with gases formation inside the colon and large intestine of 2 years old Japanese Spitz dog. (c) RL view and VD view showing radiopaque stool mass in maximum bowel length of 6 years old Dachshund Dog. (d) VD view showing areas of gases and stool in 9 months old Lhasa Apso dog.

The increase in Blood Urea Nitrogen is significantly higher which is seen other gastrointestinal diseases (Prause & Grauer, 1998); Dehydration is also found to increase BUN level (Polzin, 2013) CKD results from loss of functional nephrons; however, the specific disease process responsible for this loss usually cannot be determined due to development of chronic changes (eg, fibrosis. Significant Leukocytosis was also found in (management of elderly patients' constipation), which might be due to increase in bacterial infection due to impaired immune system, also cases were presented to hospital only after more than 2 days of symptoms in maximum instances. This might be due to inflammation and secondary bacterial infections. There was significant rise in AST levels along with serum bilirubin suggesting liver disorder or elevated liver enzymes of idiopathic origin. Use of polyethylene glycol(PEGLEC) was supported by (Portalatin & Winstead, 2012) in managing constipation. Some of the therapeutics used in human medicines that can be considered novel in veterinary practice are:

Pruracalopride Pruracalopride, a first in class dihydro-benzofuran-carboxamide, is used in symptomatic treatment of chronic constipation, in the case where laxatives can't provide intended relief (Camilleri & Deiteren, 2010; Washabau, 2003) It has high affinity with 5-HT₄ receptor, without showing any effect on other 5-HT receptor (Gonzalez-Martinez et al., 2014; Schiller, 2004; Tack, Van Outryve, Beyens, Kerstens, & Vandeplasseche, 2009) osmotic laxatives, stimulant laxatives, prokinetic agents, biofeedback training, and surgery. These often are tried sequentially and episodically and have little evidence of long-term efficacy. Patients often report inadequate relief of symptoms. There is room for improvement, therefore, in the therapy of chronic constipation. Future advances largely will be based on insights into the enteric nervous system (ENS. It stimulates defecation via giant contractions (GMC's) in dogs, showing a dose dependent stimulation (0.2-1.25mg/kg).

Cisapride

Cisapride, a gastro prokinetic drug, increases motility of upper gastrointestinal tract. It directly acts serotonin 5-HT₄ receptor agonist. It increases lower esophageal peristalsis along with sphincter pressure which aids in accelerating gastric emptying. Cisapride has been reported to be useful in managing constipation in dogs and megacolon in cats (Plumb, 2015). For Cisapride, dose rate of 0.1–0.5 mg/kg PO 2–3 times per day given 30 minutes before meals. Its prokinetic effect is similar to metoclopramide and domperidone, but is a weak antiemetic drug (Washabau, 2003)

Although there was no necessity of performing surgical management in this research, In some severe cases of constipation, primarily idiopathic megacolon, the therapeutic management using laxatives, enemas, fiber diet (dietary management), have not been able to permanently relieve clinical signs and sufferings in animals as reported by Hasler & Washabau (1997) and White (2002). Surgical management of the condition of megacolon are described by various authors; including partial colectomy (Webb, 1985) and Subtotal

Colectomy (Gregory, Guilford, Berry, Pederson, & Olsen, 1990) physical condition, body weight, blood chemistry panel, fasting and postprandial serum bile acids, serum cobalamin concentration, serum folate concentration, fecal weight, fecal water content, fecal fat content, fecal osmolality and electrolyte concentration, quantitative anaerobic fecal bacterial culture, partial thromboplastin time, prothrombin time, breath hydrogen concentration, urinary calcium, phosphorus and electrolyte concentrations, and abdominal radiographic examination with air contrast studies (Sweet, Hardie, & Stone. 1994) 22 cats with megacolon underwent colectomy by end-to-end anastomosis of the distal colon to either the proximal colon (preserving the ileocolic junction in 14 cats.

CONCLUSION AND RECOMMENDATIONS

As this research showed increased risk of constipation in old and female dogs, owner must pay more attention and care in diet and exercise while rearing such pet. Dehydration should be managed at the initial phase to manage the patients' physical condition. Radiographical and haemato-biochemical examination to search for prompt diagnosis which differs from case to case in management of constipation should be practiced. Starting from high fiber diets, mild laxatives and enemas, the treatment can be proceeded to fluid therapy, antibiotic therapy and medications managing liver ailments after haemato-biochemical examination.

Since, clinical evidence for the efficacy of laxative therapies and their use in long-term is not available in veterinary and even human medicine. Further studies can be performed to know the efficacy of Cisapride and Prucalopride usage in management of constipation in dog at various dose rate. Along with this, surgical management, most reliably Subtotal colectomy, can be used to manage idiopathic megacolon causing constipation and obstipation.

REFERENCES

1. Allen, S. E., & Holm, J. L. (2008). Lactate: Physiology and clinical utility. *Journal of Veterinary Emergency and Critical Care*, 18(2), 123–132. <https://doi.org/10.1111/j.1476-4431.2008.00286.x>
2. Bosshard, W., Dreher, R., Schnegg, J. F., & Büla, C. J. (2004). The treatment of chronic constipation in elderly people. *Drugs & aging*, 21(14), 911-930.
3. Davis, H., Jensen, T., Johnson, A., Knowles, P., Meyer, R., Rucinsky, R., & Shafford, H. (2013). 2013 AAHA/AAFP Fluid Therapy Guidelines for Dogs and Cats*. *Journal of the American Animal Hospital Association*, 49(3), 149–159. <https://doi.org/10.5326/JAAHA-MS-5868>
4. Defarges, A. (2019). Constipation and Obstipation in Small Animals.
5. Elliot, D. L., Watts, W. J., & Girard, D. E. (1983). Constipation. Mechanisms and management of a common clinical problem. *Postgraduate Medicine*, 74(2), 143–149. <https://doi.org/10.1080/00325481.1983.11698384>
6. Enck, R. E. (1988). Constipation: etiologies and management. *Am J Hosp Care*, 5(5), 17–19.
7. Gonzalez-Martinez, M. A., Ortiz-Olvera, N. X., & Mendez-Navarro, J. (2014). Novel pharmacological therapies for management of chronic constipation. *Journal of Clinical Gastroenterology*, 48(1), 21–28. <https://doi.org/10.1097/01.mcg.0000436440.05887.02>

8. GREGORY, C. R., GUILFORD, W. G., BERRY, C. R., PEDERSON, N. C., & OLSEN, J. (1990). Enteric Function in Cats after Subtotal Colectomy for Treatment of Megacolon. *Veterinary Surgery*, 19(3), 216–220. <https://doi.org/10.1111/j.1532-950X.1990.tb01173.x>
9. Hasler, A. H., & Washabau, R. J. (1997). Cisapride stimulates contraction of idiopathic megacolon smooth muscle in cats. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine*, 11(6), 313–318. <https://doi.org/10.1111/j.1939-1676.1997.tb00472.x>
10. Higgins, P. D., & Johanson, J. F. (2004). Epidemiology of constipation in North America: a systematic review. *The American journal of gastroenterology*, 99(4), 750.
11. Jones, B. D. (2000). Constipation, tenesmus, dyschezia, and fecal incontinence. *Textbook of veterinary internal medicine*, 129-135.
12. Jones, M. P., Talley, N. J., Nuyts, G., & Dubois, D. (2002). Lack of objective evidence of efficacy of laxatives in chronic constipation. *Digestive Diseases and Sciences*, 47(10), 2222–2230. <https://doi.org/10.1023/A:1020131126397>
13. Kanauchi, O., Mitsuyama, K., Komiyama, Y., Yagi, M., Andoh, A., & Sata, M. (2010). Preventive effects of enzyme-treated rice fiber in a restraint stress-induced irritable bowel syndrome model. *International journal of molecular medicine*, 25(4), 547-555.
14. Knowles, C. H., Scott, S. M., & Lunniss, P. J. (2001). Slow transit constipation. A disorder of pelvic autonomic nerves? *Digestive Diseases and Sciences*, 46(2), 389–401. <https://doi.org/10.1023/A:1005665218647>
15. KUDISCH, M., & PAVLETIC, M. M. (1993). Subtotal Colectomy With Surgical Stapling Instruments Via a Trans-cecal Approach for Treatment of Acquired Megacolon in Cats. *Veterinary Surgery*, 22(6), 457–463. <https://doi.org/10.1111/j.1532-950X.1993.tb00422.x>
16. Lee, R., & Leowijik, C. (1982). Normal parameters in abdominal radiology of the dog and cat. *Journal of Small Animal Practice*, 23(5), 251–269. <https://doi.org/10.1111/j.1748-5827.1982.tb01664.x>
17. Oh, H.-G., Lee, H.-Y., Seo, M.-Y., Kang, Y.-R., Kim, J.-H., Park, J.-W., ... Chae, S.-W. (2011). Effects of Ficus carica paste on constipation induced by a high-protein feed and movement restriction in beagles. *Laboratory Animal Research*, 27(4), 275. <https://doi.org/10.5625/lar.2011.27.4.275>
18. Pirkka, V. K., Arthur, C. O., Erika, I., & Seppo, J. S. (1998). The ability of probiotic bacteria to bind to human intestinal mucus. *Fems Microbiology Letters*, 167(2), 185–189. Retrieved from <http://dx.doi.org/10.1111/j.1574-6968.1998.tb13226.x>
19. Plumb, D. C. (2015). *Plumb's Veterinary Drug Handbook*.
20. Polzin, D. J. (2013). Evidence-based step-wise approach to managing chronic kidney disease in dogs and cats. *Journal of Veterinary Emergency and Critical Care*. <https://doi.org/10.1111/vec.12034>
21. Portalatin, M., & Winstead, N. (2012). Medical management of constipation. *Clinics in Colon and Rectal Surgery*, 25(1), 12–19. <https://doi.org/10.1055/s-0032-1301754>
22. Prause, L. C., & Grauer, G. F. (1998). Association of Gastrointestinal Hemorrhage with Increased Blood Urea Nitrogen and BUN/Creatinine Ratio in Dogs: A Literature Review and Retrospective Study. *Veterinary Clinical Pathology*, 27(4), 107–111. <https://doi.org/10.1111/j.1939-165X.1998.tb01028.x>
23. Rakha, G. M. H., Abdl-Haleem, M. M., Farghali, H. A. M., & Abdel-Saeed, H. (2015). Prevalence of common canine digestive problems compared with other health problems in teaching veterinary hospital, Faculty of Veterinary Medicine, Cairo University, Egypt. *Veterinary World*, 8(3), 403–411. <https://doi.org/10.14202/vetworld.2015.403-411>
24. Read, N. W., Timms, J. M., Barfield, L. J., Donnelly, T. C., & Bannister, J. J. (1986). Impairment of defecation in young women with severe constipation. *Gastroenterology*, 90(1), 53–60. [https://doi.org/10.1016/0016-5085\(86\)90074-0](https://doi.org/10.1016/0016-5085(86)90074-0)
25. Schiller, L. R. (2004). New and emerging treatment options for chronic constipation. *Rev Gastroenterol Disord*, 4(2), S43–S51.

26. Sweet, D. C., Hardie, E. M., & Stone, E. A. (1994). Preservation versus excision of the ileocolic junction during colectomy for megacolon: A study of 22 cats. *Journal of Small Animal Practice*, 35(7), 358–363. <https://doi.org/10.1111/j.1748-5827.1994.tb01718.x>
27. Tack, J., Van Outryve, M., Beyens, G., Kerstens, R., & Vandeplasseche, L. (2009). Prucalopride (Resolor) in the treatment of severe chronic constipation in patients dissatisfied with laxatives. *Gut*, 58(3), 357–365. <https://doi.org/10.1136/gut.2008.162404>
28. Washabau, R. J. (2003). Gastrointestinal motility disorders and gastrointestinal prokinetic therapy. *The Veterinary Clinics of North America. Small Animal Practice*, 33(5), 1007–28, vi. [https://doi.org/10.1016/S0195-5616\(03\)00076-7](https://doi.org/10.1016/S0195-5616(03)00076-7)
29. WEBB, S. M. (1985). Surgical management of acquired megacolon in the cat. *Journal of Small Animal Practice*, 26(7), 399-405.
30. White, R. N. (2002). Surgical management of constipation. *Journal of Feline Medicine and Surgery*, 4(3), 129-138.

Global Pattern of Epizootic Ulcerative Syndrome Outbreaks in Freshwater Farmed and Wild Fish from 2005 to 2012

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ABSTRACT

*Epizootic ulcerative syndrome (EUS) is an infectious disease of freshwater farmed and wild fish caused by a fungus, *Aphanomyces invadans*. This disease is an important economic disease causing huge socio-economic losses to fish farmers across the world. While this is an important fish disease, its global epidemiology is poorly understood. The objective of this study is understand the global spatiotemporal epidemiology of EUS outbreaks from 2005 to 2022. The data were retrieved from open access database of the World Organization for Animal Health (WOAH). Results showed that there were a total of 277 outbreaks of EUS reported to WOAH from 2005 to 2022 from 15 countries and territories with 76% outbreaks reported from African countries. Democratic Republic of Congo reported the highest number of EUS outbreaks. Higher number of outbreaks were reported during 2014 to 2017. The observed epidemiological pattern might be biased due to the underreporting of the disease. Nevertheless, it offers important insights to understand the latest global pattern of EUS. We suggest to increase country level awareness on EUS so that outbreaks are reported in a timely manner.*

Keywords: *Aphanomyces invadans, Epidemiology, Infectious fish disease, Spatiotemporal pattern*

INTRODUCTION

Epizootic ulcerative syndrome (EUS), also known as red spot disease, mycotic granulomatosis and ulcerative mycosis, is an infectious disease of fish caused by a fungal pathogen, *Aphanomyces invadans* (WOAH, 2023). This disease is prevalent among both domestic and wild fresh water species and estuarine fish and is characterized by necrotic ulcerative lesions leading to granules (WOAH, 2023). This is a disease of international concern and is reportable to World Organization for Animal Health (WOAH) because of its widespread distribution among fish species covering more than 16 orders, 54 families and 160 species (Herbert et al., 2019).

The EUS can cause mortality up to 100% and is therefore considered an important disease of economic importance (Iberahim et al., 2018). Small and mixed fish farms are particularly prone to the infection of this disease. A study has shown that EUS has caused massive economic losses to the fish farmers from the Asia-Pacific region which estimates up to 20,000 to 100 million economic losses (cumulative losses USD 114 million) from different countries that include Australia, Bangladesh, India, Pakistan, Nepal, Thailand, Indonesia and Sri Lanka (Chong, 2022). Several factors are responsible for the spread of EUS. Studies has shown that higher stocking density, older ponds with high bottom disposition and shade, stress conditions, cultures fish connected to rice fields and flooding contributes in the spread of EUS (Kar and Aurobindo, 2021, Khan and Lilley, 1999 and Kumar et al., 2017).

This pathogen was first isolated in Japan in 1971 and has been detected in various countries from North America, Australia and African countries (Iberahim et al., 2018). However, there are limited studies describing the global epidemiology of EUS. The objective of this study is to describe the global spatiotemporal epidemiology of Epizootic Ulcerative Syndrome (EUS) among freshwater farmed and wild fish species based on the reported incidences to the World Animal Health Information System (WAHIS) of the WOA. H.

MATERIALS AND METHODS

This is a descriptive epidemiological study of Epizootic Ulcerative Syndrome (EUS) globally from 2005 to 2022.

Data collection

Open source data of EUS outbreaks reported to the World Animal Health Information System (WAHIS) were collected from the World Organization for Animal Health (WOAH) website from the year 2005 to 2022. Data reported from all the countries across the world were included in this study.

Data Analysis

The data were collected in Microsoft Excel 2016 and made graphs and pivot tables using the same Excel version.

Mapping

The maps to show the spatial pattern of EUS were prepared using the open access software QGIS version 3.28.0.

RESULTS AND DISCUSSION

Temporal pattern of EUS outbreaks

There were a total of 277 outbreaks of EUS reported to the WOA from 2005 to 2022. The highest number of outbreaks were reported in 2016 (63 outbreaks) followed by 44 outbreaks in 2015 and 27 outbreaks in 2008 (Figure 1). The pattern shows that in the earlier years from 2005 to 2007, there were only limited number of outbreaks reported while it increased to 27 outbreaks in 2008 (Figure 1). The outbreaks again decreased until 2014 and remained below a total of 10 outbreaks (Figure 1). Thereafter, the number of outbreaks rapidly increased and reached to 63 outbreaks in 2016 after which it began to decrease (Figure 1).

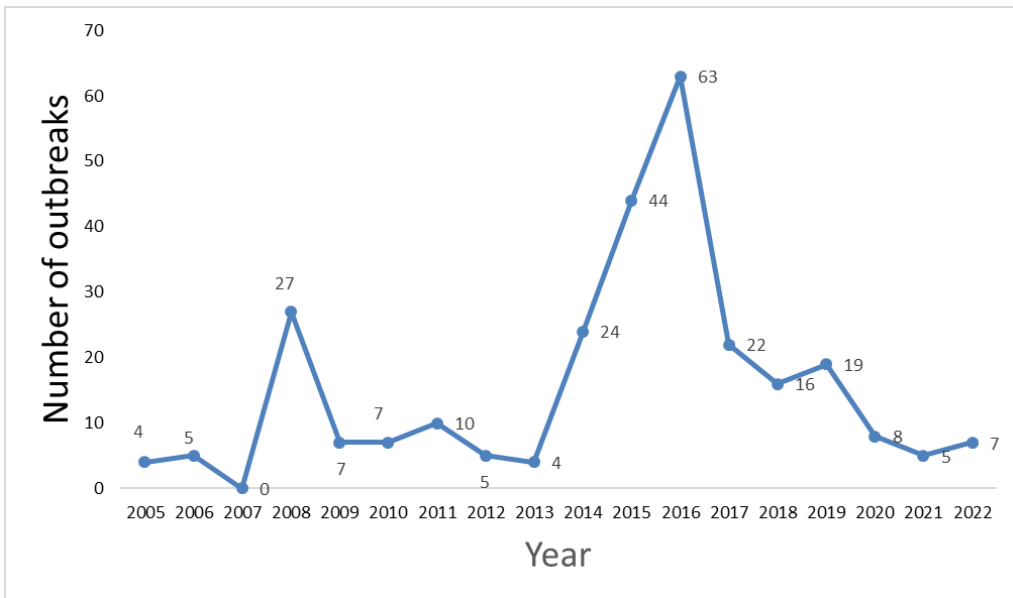


Figure 1. Temporal pattern of EUS from 2005- 2022

Spatial pattern of EUS outbreaks

The spatial pattern showed that outbreaks of EUS were reported from 15 countries from 5 continents, Australia, Americas, Asia, Oceania and Europe (Figure 2). The countries and territories that reported outbreaks of EUS to the WOA during 2005- 2022 include Cameroon, Canada, Chinese Taipei, Thailand, United Kingdom, Bangladesh, Zimbabwe, Malawi, Japan, Botswana, India, Namibia, South Africa, Australia and Democratic Republic of Congo (Figure 2). The highest number of outbreaks were reported from Democratic Republic of Congo (159 outbreaks) followed by 58 outbreaks from Australia and 27 outbreaks from South Africa (Figure 2). The highest number of outbreaks were reported from Africa (76%, 210/277 outbreaks) followed by Oceania (16%, 44/277 outbreaks) and Asia (8%, 21/277 outbreaks) (Figure 3).

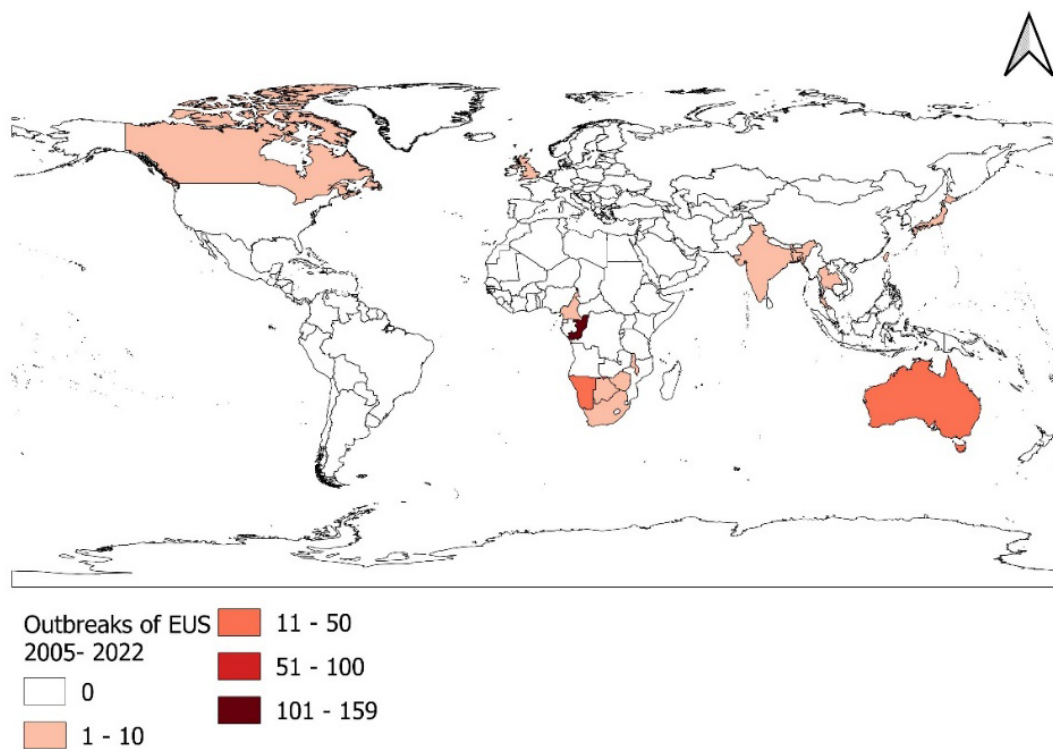


Figure 2. Spatial distribution of EUS outbreaks from 2005- 2022

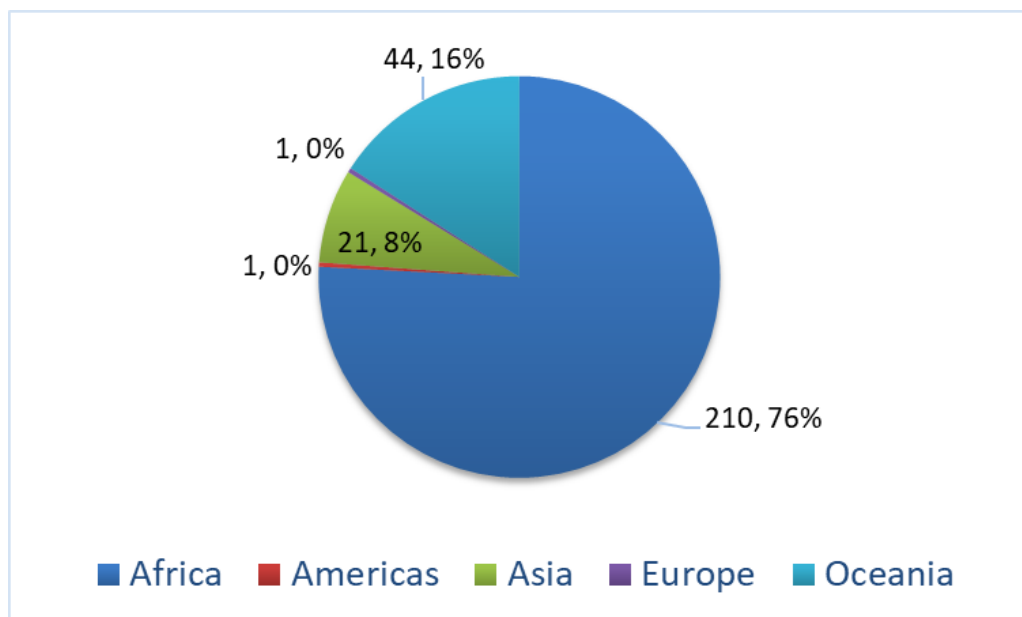


Figure 3. Continent-wise distribution of EUS outbreaks from 2005- 2022

Spatiotemporal pattern of EUS outbreaks

The spatiotemporal pattern shows that Congo, Australia, South Africa and Namibia have reported EUS outbreaks frequently with annual variations in the number of outbreaks in different countries (Figure 4). Outbreaks were reported in 1 or only few years in most of the countries.

For example, among the South Asian countries, India reported outbreaks in 2017 and 2018 while Bangladesh reported only in year 2022 (Figure 4). WOA database shows that Nepal has not reported EUS to the WOA during 2005 to 2022. However, literature review clearly indicated EUS is widely prevalent in Nepal. EUS was first reported in February 1989 (Shrestha, 1994) and has been reported from terai, mid-hills, Kathmandu and Pokhara valley (Dahal, 2002 and Dahal, 2003), eastern districts of Morang and Sunsari (Thapa and Pal, 2022) and Chitwan (Baidya and Prasad, 2013). Further, EUS was observed in fish farmed in reservoirs of Nepal that include Trishuli, Begnas and Mirmi (Shrestha et al., 2019).

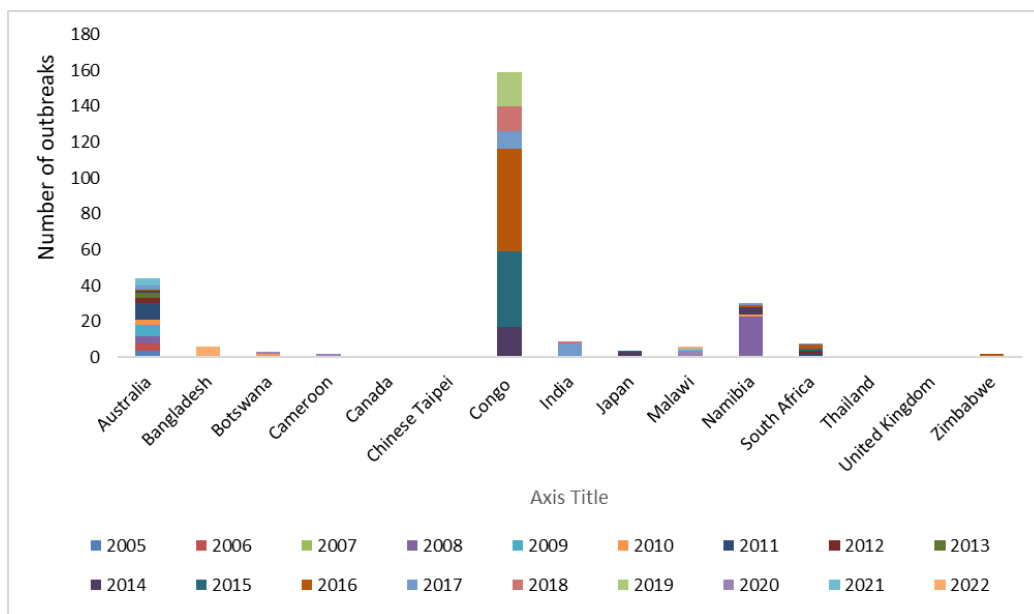


Figure 4. Spatiotemporal distribution of EUS outbreaks from 2005- 2022

Species affected by EUS outbreaks

Outbreaks were reported from freshwater farmed and wild fish species. The species in which EUS outbreaks were reported during the study period include African sharptooth, Catfish, Banded tilapia, *Clarias ngamensis*, Dashtail barb, Largemouth bass, Mochokidae, Redbreast tilapia, Sand whiting, Silver catfish, Straight fin barb and Striped mullet, Yellowfin bream and several unidentified species of farmed and wild fish species.

CONCLUSION

This study analysed the publicly available data of WOAHA to understand the pattern of EUS outbreaks from 2005 to 2022. It was found that during this study period, 277 outbreaks of EUS were reported from 15 countries and territories. Temporal pattern shows that majority of the outbreaks occurred during 2014 to 2017 while spatially, majority of the outbreaks were reported from Africa, particularly Congo and South Africa. Australia also reported significant number of EUS outbreaks. Though the number of countries reporting the disease are only 15, its spread in 5 regions indicates that the disease is globally widespread. While the epidemiological pattern might be biased due to underreporting of disease incidences, nevertheless, it provides important insights to understand the global spatiotemporal pattern of EUS from 2005 to 2022. Country level awareness need to be increased to increase the reporting of the disease outbreaks which will pave a way forward to design country specific control programs.

ACKNOWLEDGMENT

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REFERENCES

1. Dahal, S.P., Shrestha, M.K., Pradhan, S.K. and Jha, D.K., 2008. Occurrence of epizootic ulcerative syndrome in pond fish of Kapilvastu district of Nepal. In Proceedings of the Sixth Symposium on Diseases in Asian Aquaculture (pp. 169-178).
2. Shrestha, G.B. (1994). Status of epizootic ulcerative syndrome (EUS) and its effects on aquaculture in Nepal. In: R.J. Roberts, B. Campbell and I.H. MacRae (eds.) Proceedings of the ODA Regional Seminar on Epizootic Ulcerative Syndrome, 25-27 January 1994. pp. 49-57. Aquatic Animal Health Research Institute, Bangkok.
3. Thapa, G.B. and Pal, J., 2022. Histopathology of the fish infected with the epizootic ulcerative syndrome in Eastern Nepal. *Nepalese Journal of Zoology*, 6(1), pp.20-29.
4. Baidya, S. and Prasad, A., 2013. Prevalence of epizootic ulcerative syndrome (EUS) in carps. *Nepalese Journal of Zoology*, 1(1), pp.41-47.
5. Shrestha, S.P., Bajracharya, P. and Rayamajhi, A., 2019. Study on status of fish diseases in Nepal. *Nepalese Veterinary Journal*, 36, pp.30-37.
6. Herbert, B., Jones, J.B., Mohan, C.V. and Perera, R.P., 2019. Impacts of epizootic ulcerative syndrome on subsistence fisheries and wildlife. *Rev. Sci. Tech. Off. Int. Epiz*, 38(2), pp.459-475.
7. Iberahim, N.A., Trusch, F. and Van West, P., 2018. *Aphanomyces invadans*, the causal agent of epizootic ulcerative syndrome, is a global threat to wild and farmed fish. *Fungal Biology Reviews*, 32(3), pp.118-130.
8. Chong, R.S.M., 2022. Epizootic ulcerative syndrome. In *Aquaculture Pathophysiology* (pp. 621-627). Academic Press.
9. Kar, D. and Aurobindo, R., 2021. Epizootic Ulcerative Syndrome (EUS) Fish Disease Chronology, Status and Major Outbreaks in the World. *Transylvanian Review of Systematical and Ecological Research*, 23(2), pp.29-38.

10. Khan, M.H. and Lilley, J.H., 1999. Risk factors and socio-economic impacts associated with epizootic ulcerative syndrome (EUS) in Bangladesh. PREPARATION OF THIS DOCUMENT, p.27.
11. kumar Pagrut, N., Ganguly, S., Jaiswal, V. and Singh, C., 2017. An overview on epizootic ulcerative syndrome of fishes in India: A comprehensive report. J Entomol Zool Stud, 5, pp.1941-1943.

Oleander Poisoning in Cattle in Devghat, Nepal- A Case Report

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ABSTRACT

Oleander poisoning, though widely seen in the Mediterranean region, is rarely seen in Nepal. The poisonous effect of the fodder is due to the accidental ingestion of the different parts of 'oleandrin' which contains cardiac glycosides called cardenolides causing mortality in animals. In our study, 8 lactating cows were affected after oleander leaves were accidentally mixed and chopped with Napier. After the ingestion of the mixed fodder, out of 8 cows, 5 died instantly and the remaining 3 were showing severe clinical signs such as severe depression, anorexia, ruminal atony, diarrhea, serous nasal discharge, tachycardia, and irregular heartbeat. The diagnosis of the poisoning was made by finding the chopped leaves of oleander in the feed and ruminal content. The visual observation of the oleander leaves along with gross lesions specifically in the heart causing coagulative necrosis- as it acts as cardiac glycosides- we came to diagnose the oleandrin poisoning. As there was not the availability of digoxin-specific antibodies for the therapy, symptomatic and supportive treatment was done, and the good prognosis was seen on morbid animals within a week.

Keywords: HPLC, Glycosides, NSAID_s

INTRODUCTION

Oleander is the decorative shrub grown in gardens for aesthetic purposes primarily based in the Mediterranean and Asian regions of the tropical and subtropical regions (Mahin et al., 1984, as cited in (Oryan et al., 1996). The oleander leaves, flowers and seeds and all parts, if ingested are poisonous to human, animals and certain insects (Langford & Boor, 1996). The toxic compound present in the shrub having cardiac glycosides activity called cardenolides is detected by HPLC (Us et al., 2012). Karawya et al. (1969) performed an experiment to conclude that seeds and roots contain the highest percentage of total cardenolides, followed by fruits and leaves. Cardenolides act their effect by halting the

Na⁺-K⁺-ATPase pump in cardiomyocytes. As a result, the level of intracellular Na level is increased, which in turn aggravates the level of calcium hindering the positive inotropic effect. The resting membrane is also increased, causing increased excitability and slow atrioventricular conduction due to altered vagal tone (Ceci et al., 2020).

In the present study, the authors describe the occurrence of a cattle herd intoxication in Devghat, Tanahun due to the accidental incorporation of oleander leaves in the Napier fodder. The diagnosis was done by clinical signs, post-mortem and the presence of oleander leaves in the ruminal content and residual fodder in the manger.

CASE HISTORY

A case was reported in the cattle farm located at Devghat, Tanahu, Nepal during the month of September 2022. The farmer was unaware of the accidental incorporation of the oleandrin neither was aware about the poisonous effects oleandrin in animals. In the morning 10 am, the feed was ingested by cattle. The farm owner called a local veterinary technician. The technician administered NSAIDs, but the condition did not get better. Later in the morning, the conditions aggravated with diarrhea, severe depression, and prolonged sternal decubitus. The technician later consulted with the Department of Veterinary Surgery and Pharmacology of the Agriculture and Forestry University. The team from the university went there and examined the remaining 3 animals before starting a therapy.

CASE FINDINGS

After the ingestion of feed, the animal began to show symptoms within 6 hours, that include depression, anorexia, lack of rumination, and pedaling. The case was so acute that, out of 8 animals, 5 animals were found to be dead by the night time.

There were the most common symptoms among the remaining animals and all showed varying degrees of depression and weakness. Out of 3 animals, one was showing nasal discharge and was severely dehydrated with dry mucus membranes and shrunken eyes. All of them were anorectic and had varying degrees of abdominal colic. Yellowish diarrhea was observed with no blood taints. Severe respiratory signs like labored breathing (dyspnea) with respiratory rales (crackle sound) and coughing were observed. The heartbeat was abnormal with tachycardia. During the feed observation, crushed Nerium oleander leaves and flowers were found.

Necropsy was performed on all dead animals and all of them showed similar lesions like congestion on visceral organs like lungs, liver, and kidneys. Myocarditis with both external and internal hemorrhage (endocardium) was seen on the heart. Lungs were severely

congested with pneumonic changes. The liver was severely congested and hemorrhagic. Diffuse hemorrhage was observed on the gastrointestinal system with unidentified reddish nodules. There was also mild hydrothorax, hydropericardium, and ascites. We suspected the case might be poisoning. The clinical signs of the poisoning are similar with the study of Galey et al. (1996) and with the visual traces of oleander in leaves and ruminal content we came to diagnose the poisoning.



(A)



(B)

Figure 1

(A) Oleander around the vicinity of the farm; (B) oleander leaves in chopped fodder.



Figure 2: Animal in sternal recumbency pooled in diarrhea with head extended.



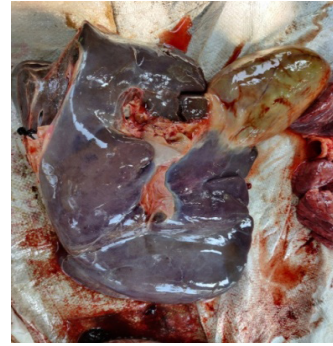
Figure 3: Post mortem of animal showing oleander traces in ruminal content.



(A)



(B)



(C)



(D)



(E)

Figure 4: (A) Diffused hemorrhagic myocardium; (B) Hemorrhagic endocardium (C) Congested liver; (D) Congested and hemorrhagic lungs; (E) Unidentified black nodules in intestine

Management

Rajapaksa (2009), in his review, described the treatment of toxicity with supportive care, symptomatic treatment, gastric decontamination, managing cardiac arrhythmia and digoxin-specific antibodies. The treatment in our case was done duly as aforementioned. The digoxin-specific antibody was not used in our therapy due to the inaccessibility of the antibody. After confirming the oleander poisoning, we started to treat the remaining 3 animals and remaining 3 cattle recovered within one week. One pregnant cattle gave birth a healthy calf one month earlier of its term.

Table 1. Drug Regimen for the Therapy of Oleander Poisoning at Devghat.

Medication and Therapy	Dosage	Route	Total Days of Administration
NS + DNS	25 ml/kg, once daily	IV	3
Magnesium Sulfate	250 gm/animal, twice Daily	PO	3
Rumenatas Boli	3 boli/animal, twice daily	PO	5
Atropine Sulfate	0.5 mg/kg, twice daily	IV	2
Furosemide	0.5mg/kg, once daily	IV	2

Note. IV stands for Intra Venous, PO for Per Oral.

DISCUSSION

In our study, we found the most striking gross lesions in the heart, lungs, liver, intestine and rumen. The clinical signs and gross lesions were in line with the experimental study of poisoning in monkeys (Schwartz et al., 1974). The good prognosis of the morbid animals showed that time management and therapy of the animal are crucial. As the blood, serum, heart, liver and milk samples of oleander-poisoned animals show traces of oleandrin (Gosetti et al., 2019; Ceci et al., 2020). So, we advised farmers not to have milk of suspected oleander poisoning.

CONCLUSION

Though poisoning from oleander is not a common toxicological problem in Nepal, their plantation around the vicinity of the farm for aesthetic value can be menacing. There have been many cases of poisoning, both in animals and humans, around the globe, and it is a clinical emergency. The main clinical features caused by the ingestion of oleander are nausea, vomiting, abdominal pain, diarrhea, dysrhythmias, and hyperkalemia. Management is done with symptomatic and supportive therapy. If therapy is given time, there is a good chance of recovery as seen in our case study.

ACKNOWLEDGEMENT

We would like to thank Devghat Local Governance for their support during treatment and Veterinary Teaching Hospital for their help during post mortem.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Ceci, L., Girolami, F., Capucchio, M. T., Colombino, E., Nebbia, C., Gosetti, F., Marengo, E., Iarussi, F., & Carelli, G. (2020). Outbreak of Oleander (*Nerium oleander*) Poisoning in Dairy Cattle, Clinical and Food Safety Implications. *Toxins*, 12(8), 471. <https://doi.org/10.3390/toxins12080471>
2. Gale, F. D., Holstege, D. M., Plumlee, K. H., Tor, E., Johnson, B., Anderson, M. L., Blanchard, P. C., & Brown, F. (1996). Diagnosis of oleander poisoning in livestock. *Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, 8(3), 358–364. <https://doi.org/10.1177/104063879600800314>
3. Gosetti, F., Nebbia, C., Ceci, L., Carelli, G., & Marengo, E. (2019). UHPLC-MS/MS determination of oleandrin in blood and tissues of dairy cattle poisoned by oleander (*Nerium oleander*). *Analytical Methods*, 11(43), 5562–5567. doi:10.1039/c9ay01800j
4. Karawya, M., Balbaa, S., & Khayyal, S. (1973). ESTIMATION OF CARDENOLIDES IN *NERIUM OLEANDER*. *Planta Medica*, 23(01), 70–73. doi:10.1055/s-0028-1099414
5. Langford, S. D., & Boor, P. J. (1996). Oleander toxicity: an examination of human and animal toxic exposures. *Toxicology*, 109(1), 1–13. doi:10.1016/0300-483x(95)03296-r
6. Schwartz, W. L., Bay, W. W., Dollahite, J. W., Storts, R. W., & Russell, L. H. (1974). Toxicity of *Nerium oleander* in the Monkey (*Cebus apella*). *Veterinary Pathology*, 11(3), 259–277. doi:10.1177/030098587401100307
7. Us, P., Gowda, Y., Vg, N., & Bm, M. (2012). Detection of Residues of Cardenolides of *Nerium oleander* by High-Performance Thin-Layer Chromatography in Autopsy Samples. *International Journal of Medical Toxicology and Forensic Medicine*, 2(4), 135–142.

Pregnancy Toxaemia in Goat: a Case Report

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ABSTRACT

A four years old Khari goat was brought to Madi Veterinary Hospital with the history of parturition 3 days ago with triplet's kid, in-appetence and weakness. Vitals signs were within normal physiological range. Laboratory diagnosis revealed low glucose level (27 mg/dl) and was diagnosed as pregnancy toxemia on the basis of clinical and laboratory findings. Calcium-borogluconate, corticosteroid, B-complex and jiggery were given as glucose replacement, glucose precursor, metabolic supporter and source of glucose respectively. Goat became responsive with after two days of treatment.

Keywords: Corticosteroids, Glucose replacement, Khari

INTRODUCTION

Pregnancy toxemia (PT) is also known as twin lamb disease, a metabolic disorder of pregnant small ruminants caused by an abnormal metabolism of carbohydrates and fats, which occurs at the final stage or few days after pregnancy (Brozos, C et.al, 2011). The occurrence of this disorder may be either due to poor nutrition or excessive demand from multiple rapidly growing fetuses but sometimes both contributed together (Lima et al., 2012). Nearly 80% of fetal growth occurs during the last six weeks of gestation (Bergman E.N., 1993, Navarre and Pugh, 2002). The normal blood glucose and ketone bodies range in does is 40-60 mg/dl and 1-4 mg/dl respectively. There is significant decrease in the blood glucose level and increase in the level of ketone bodies in PT in goat. During the final month of gestation, the energy requirement of a pregnant doe carrying twins or triplets is 180% or 240% greater, respectively, than that of a doe with a single fetus and obese ewes and does are at greater risk for developing PT (Navarre and Pugh, 2002). Obese ewes or does carrying multiple fetuses are at higher risk to develop the disease because of the limited space for adequate intake of feed (Ermilio EM, Smith MC. 2011). It is characterized by hypoglycemia and hyperketonemia. Rapid fetal development at the late gestation periods causes rapid mobilization of the fat stores to assure adequate energy. The liver also increases gluconeogenesis to facilitate glucose availability to the

fetus. However, in a negative energy balance, this increased mobilization may overwhelm the capacity of liver resulting in hepatic lipidosis. At the same time, ketone bodies are being produced and accumulated, which eventually leads to excessive ketone bodies in blood circulation, thus increasing the susceptibility to pregnancy toxemia (Menzies, 2011). In severe outbreaks, morbidity can reach up to 20% with a mortality rate of 80% of affected animals (Ismail et al., 2008). Early clinical signs of PT in ewes and does include in-appetence, listlessness, aimless walking, muscle twitching, fine muscle tremor, opisthotonos, and grinding of teeth. As the condition progresses, blindness, ataxia and finally recumbence, coma and death will occur in animals that are not diagnosed and treated with the condition. This study outlines the clinical approach in management of PT in a doe. This clinical case management highlighted that institution of correct treatment at the right time was able to give better prognosis and success.

CASE HISTORY

History of patient

A four-year-old Khari cross female goat weighing 30 kg is brought in with the main complaints of weakness and lack of appetite. She had the history of parturition 3 days ago having three kids. The state of the deworming and vaccinations were out of date.

Physical examination, clinical and laboratory findings

The doe was unable to stand properly on its legs with the body condition score (BCS) 2 out of 5 according to Ghosh (2019). Physical examination shows normal vitals with decreasing ruminal motility, lethargic, weak, off feed. Animal had pale mucous membrane and increased capillary refill time. Blood glucose level was found 27 mg/dl. Based on the history, physical examination, clinical examination, and laboratory findings, pregnancy toxemia was determined in this instance (Ketosis).

TREATMENT

20 ml of calcium-borogluconate injection (THICAL, Vetoquinol containing 20% glucose along with calcium, magnesium) was given S/C once daily for two days as glucose replacement. 40mg of triamcinolone as corticosteroids (TRICORT 40, 1 ml ampule, CADILA pharmaceuticals) was given IM once daily for two days for the initiation of gluconeogenesis. 10 ml of vitamin B-complex injection (Conciplex, Concept Pharmaceuticals) was given IM once daily for two days to support metabolic process of the goat. 100gm of jiggery was provided orally in drinking water for 3 days as the chief source of glucose and energy. The main objective of treatments in pregnancy toxemia is to increase the formation of glucose for utilization at tissue level, and increase the usage of ketone bodies to prevent acidosis and electrolytes disorder. However, treatments with drugs are only effective in an early disease diagnosis.

PROGNOSIS

The doe was bright and alert and responsive with increase in appetite after 2 days of treatment. Recovery rate was higher with better performance after the treatment.

RESULTS AND DISCUSSION

Jones and Navarre (2014) state that PT is typically detected in malnourished animals, however in the current case, the affected goat was underweight and carrying multiple fetuses, which placed her at higher risk due to her limited rumen area for a sufficient intake of feed and low blood glucose level. Tinkler (2014) asserts that clinical indicators including anorexia, depression, and detachment from the herd are the first step in the diagnosis of PT in a pregnant doe, which is the primary concern in the current case. As the disease progressed the animal developed neurological signs such as depression, tremors, star-grazing, ataxia, circling, teeth grinding, and blindness as a result of hypoglycemia-induced encephalopathy. (Andrews 1997). In the final stage without proper diagnosis and treatment, affected animal will be in recumbency and coma where the prognosis at this stage is very poor with low survival rate (Tinkler 2014). Biochemically, significant reduction in the level of glucose and calcium, and an increased level of Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Blood Urea Nitrogen (BUN), creatinine, β -hydroxyl Butyric Acid (BHBA), and Non-Esterified Fatty Acid (NEFA) in the pregnancy toxemic goats had been observed by Vasava, P. R. et.al., 2016 in Gujarat, India. In this case blood glucose was found level were found to be decreased. Laboratory diagnosis of serum BHBA and urine ketone level can be performed to further diagnose pregnancy toxemia (Ramin et.al., 2007).

According to Ermilio and Smith (2011), based on their observations from a study conducted on sheep and goats, the primary therapeutic strategy for pregnant toxemia is to restore glucose level with re-establish metabolism and dehydration condition. In this instance, calcium borogluconate, 20 ml S/C, was administered to the doe to make up for the low glucose level. In addition to borogluconate, oral glucose replacement with 100gm of jiggery was given once daily as source of glucose for 3 days. Corticosteroids and/or ACTH have been used as treatments to enhance gluconeogenesis and raise blood glucose levels (LW, H. 1958). Dexamethasone, a glucocorticoid, has been used to successfully treat hyperglycemia in field situations. (Yadhav et al., 2018). In the present case, the doe was parturited three days prior, therefore we utilized the corticosteroid triamcinolone to boost gluconeogenesis and subsequently the blood glucose level. There are a few crucial things to keep in mind when preventing and treating pregnancy toxemia. For example, during the latter few months of gestation, high-energy supplement feed like 0.5–1 kg of cereal grain like corn, oats, or barley should be provided (Edmondson et.al., 2012). Prior to the two months of parturition, the does with BCS less than 2 must be identified

and fed with high energy concentrates and essential minerals that contain calcium and magnesium. Animals require a high performance deworming product as well as potable water. Additionally, vitamin B12 may be administered as an appetite stimulant. It accelerates the goat's body metabolic rate, increases the absorbable glucose concentration in the body.

CONCLUSION

Glucose is the animal's major energy source, and the demand for glucose in the body increases several times during pregnancy. PT results from alternating the glucose hemostatic mechanisms in response to the increased nutritional demand of the developing fetus and pregnant mother. Early diagnosis and treatment are crucial to save the life of the dam and fetus. Treatment mostly includes the administration of the rich energy glucose source to the affected animals. It is suggested to take a sample of late gestation doe to test for serum BHB or glucose concentration to determine the extent of the risk in the rest of the flock.

REFERENCES

1. Abba Y, Abdullah FFA, Chung ELT, Sadiq MA, Mohammed K, Osman AY, et al. Biochemical and pathological findings of pregnancy toxaemia in Saanen doe: A case report. *Journal of Advance Veterinary and Animal Research*. 2015; 2(2):236-239.
2. Andrews A (1997). Pregnancy toxemia in the ewe. *In Practice*, 19, 306-312.
3. Bergman EN. Disorders of carbohydrate and fat metabolism. In: Swenson MJ, Reece WO, eds. *Dukes physiology of domestic animals*. 11th ed. Ithaca, NY: Cornell University Press, 1993, 492-516.
4. Brozos, C., Mavrogianni, V. S., & Fthenakis, G. C. (2011). Treatment and control of peri-parturient metabolic diseases: pregnancy toxemia, hypocalcemia, hypomagnesemia. *Veterinary Clinics: Food Animal Practice*, 27(1), 105-113.
5. Edmondson MA, Roberts JF, Baird AN, Bychawski S, and Pugh DG. (2012). Theriogenology of Sheep and Goats. In: Pugh D.G., Baird A.N., eds. *Sheep and Goat Medicine*, 2nd Edition. Maryland Heights, MO: Elsevier-Saunders, 150-231
6. Ermilio E.M. and Smith M.C. (2011). Treatment of Emergency Conditions in Sheep and Goats. *Veterinary Clinical Food Animal*, 27, 33-45
7. Ermilio EM, Smith MC. Treatment of emergency conditions in sheep and goats. *Veterinary Clinics of North America: Food Animal Practice*. 2011; 27:105-106.
8. Ghosh, C. P., Datta, S., Mandal, D., Das, A. K., Roy, D. C., Roy, A., & Tudu, N. K. (2019). Body condition scoring in goat: Impact and significance. *Journal of Entomology and Zoology Studies*, 7(2), 554- 560.
9. Ismail ZAB, Al-Majali AM, Amireh F, and Al-Rawashdeh OF (2008). A metabolic profile in goat does in late pregnancy with and without subclinical pregnancy toxemia. *Veterinary Clinical Pathology* 39 (4), 434-437.
10. Jones M and Navarre C, 2014. Fluid therapy in small ruminants and camelids. *Vet Clinic Food Anim*, 30: 441-453
11. Kahn CM, Line S. *The Merck Veterinary Manual* 9th Edition. USA: Merial. 2005; 14.
12. Lima MS, Pascoal RA, Stilwell GT and Hjerpe CA, 2012. Clinical findings, blood chemistry values

- and epidemiologic data from dairy goats with pregnancy toxemia. *Bov Practitioner*, 46: 102-110
13. LW, H. (1958). Studies on the treatment of ovine pregnancy toxemia with corticosteroids and ACTH. *The Cornell Veterinarian*, 48(3), 348-357.
14. Menzies PI (2011). Pregnancy Toxemia in Ewes: Hepatic Lipidosis: Merck Veterinary Manual. Merial: USA.
15. Navarrei CB, Pugh DG. Diseases of the gastrointestinal system. In: Pugh DG (ed.): *Sheep and Goat Medicine*. WB Saunders Company Ltd, London, UK. 2002, 69-105.
16. Ramin AG, Asri-Rezaie S, and Macali SA (2007). Evaluation on Serum Glucose, BHB, Urea and Cortisol in Pregnant Ewes. *Medycyna Wet.*, 63 (6), 674-677.
17. Tinkler SH. Common Nutritional and Metabolic Diseases of Small Ruminants: http://c.ymcdn.com/sites/www.invma.org/resource/resmgr/2014_speaker_proceedings/dr._tinkler_metabolic_diseases.pdf on 28th April 2015.
18. Vasava, P. R., Jani, R. G., Goswami, H. V., Rathwa, S. D., & Tandel, F. B. (2016). Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. *Veterinary world*, 9(8), 869–874. <https://doi.org/10.14202/vetworld.2016.869-874>.
19. Yadav, Sampurna Nand & Kalita, D & Phukan, Arabinda & Das, B & Dutta, T & Mahato, G & Tamuly, Shantanu & Barman, Ditul & Bharali, Kadambari. (2018). A comparative therapeutic study on subclinical ketosis of goat. *Journal of Entomology and Zoology Studies*. 6. 673-676.

Abdominal Myolipoma in Dog- A Case Report

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ABSTRACT

A 6-year-old female dog was referred to Veterinary Teaching Hospital of Agriculture and Forestry University, Rampur, Chitwan, Nepal. The complaint was an abdominal mass around the umbilicus centrally and cranial to the sternum without any clinical manifestation. Histopathologically, mature adipocytes of different sizes were seen and were interposed within spindle shaped smooth muscle fibre. The muscle fibres were seen separated due to the proliferative infiltration of these adipocytes in between. Abdominal myolipoma was diagnosed based on the clinical manifestation, gross and histopathological lesions. This could have been misdiagnosed with mammary tumour but the absence of proliferative myoepithelial cells ruled out the possibility.

Keywords: *H and E staining, Histopathology, Mesentery, Myolipoma*

INTRODUCTION

Lipomas are commonly called as tumor of subcutaneous as neoplasm of fat cells are encapsulated by fibrous tissues. Most of the cases present are benign and frequent problem of senescing dogs, can be found anywhere in the body, but more often in the axillae, legs, abdomen, and subcutaneous areas of the chest (Rao & Krishna, 2011). It is a rare case and is described as soft tissue tumor, admixture with maturing adipocytes and smooth muscle cells. The differential diagnosis includes: liposarcoma, angioliipoma, leiomyosarcoma (Takahashi et al., 2004). Moore et al. (2006) found that cases of lipoma suggest a partially or fully capsulated mass with a uniform admixture of fat cells and smooth muscle. Similarly, Kim et al. (2013) reported similar findings of myolipoma in adult dogs based on their previous studies in the mesentery.

METHODOLOGY

The excision of the abdominal mass was done under general anesthesia. After the excision, the mass was preserved in 10% buffered formalin. Later on, the gross morphology of the mass was assessed using electric balance, vernier caliper and measuring cylinder. Immediately, histopathology was carried out and stained with H & E stain

CASE DESCRIPTION

A 6-year-old female dog, named Lucy, was referred to Veterinary Teaching Hospital of Agriculture and Forestry University, Rampur, Chitwan, Nepal in October 2021 with a 6-month history of painless muscular mass around umbilicus caudal to sternum distal to the mammary glands.

Vital parameters revealed normal parameters. Temperature, heart rate and respiration rate were 37°C, 85 beats/min and 45 breaths/min respectively. Hematological parameters provided no diagnostic evidence of clinical manifestations but abdominal palpation revealed a soft mass around the umbilicus. Aspiration of the mass revealed absence of pus or any sort of sepsis. Later, the mass was surgically resected under general intravenous anesthesia and taken lab for further investigation. The dog showed good post-operative recovery.

Gross morphology was assessed and histopathology of the mass was done. Gross morphology revealed a creamy white soft mass with sides measuring 58.37 mm × 45.03 mm × 30 mm, weighing 47.09g and volume of 50 ml.

Histopathology revealed numerous mature adipocytes and were interposed with clearly defined spindle shaped smooth muscles. Another major finding was the gap between the muscle fibers which was due to interposition of fat cells inside the fibers seeming as loose separation of muscle bundles. Blood vessels were seen congested. The location, gross morphology and histopathology of the mass confirmed the case of myolipoma of abdominal muscle.

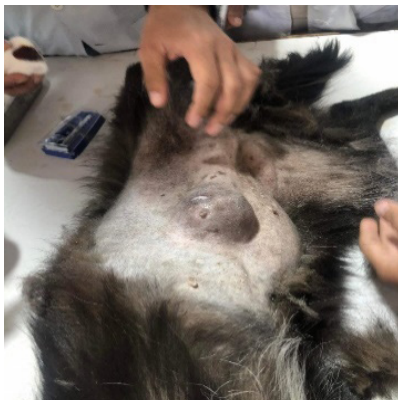


Figure 1: Gross appearance of abdominal mass before excision. The red arrow defines the area.



Figure 2: The mass was surgically exposed under general anesthesia.

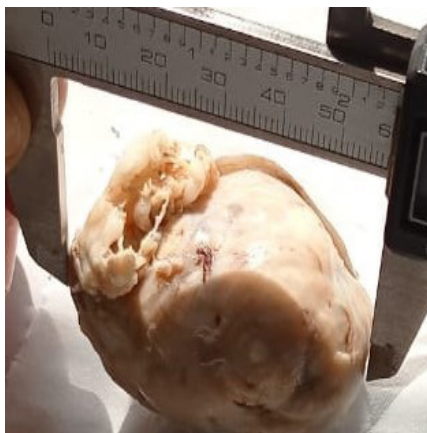


Figure 3: Gross section of resected tumor mass. The tumor's cut surface shows capsulation, hard, creamy white round mass.

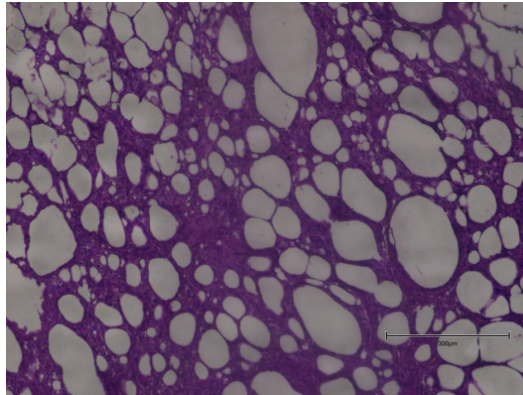


Figure 4: Microscopic Finding. The tumor shows admixture of smooth muscle fiber and adipocytes with congested vessels under H&E stain. Red arrows show distinguished adipocytes.

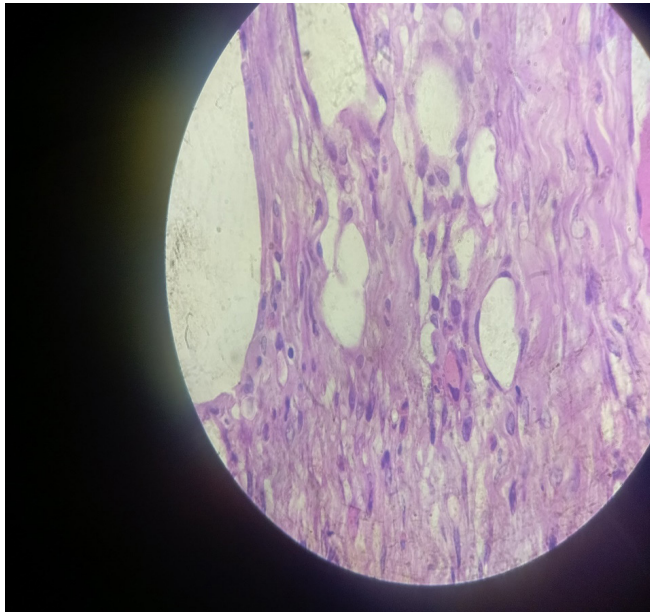


Figure 5: Microscopic Findings. The tumor shows prominent smooth muscle fibers (nucleus stained) and interspersed adipocytes under H&E stain. The red arrow shows well defined adipocyte between muscle fibers. The blue arrow shows gap in muscle fibers due to infiltration of small adipocytes.

CONCLUSION

This was a rare case of abdominal myolipoma in the dog diagnosed at Teaching Hospital, Rampur. The tumor is more frequent seen in female dogs and more predisposed with

old age. Correct diagnostic and imaging technique with histopathology is needed to accurately assess and resect the tumor. This surgery goes good with proper resection and follows good post-operative recovery. There was no evidence that the tumor recrudesces after 5 months of constant follow-up.

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REFERENCES

1. Kim, H. S., Kim, S., Kim, K., Choi, K. U., & Kim, J. Y. (2013). Myolipoma of Mesentery: A Case Report. *Case Reports in Pathology*, 2013, 1–4. <https://doi.org/10.1155/2013/823823>
2. Moore, M. O., Richardson, M. L., Rubin, B. P., & Baird, G. S. (2006). Abdominal cavity myolipoma presenting as an enlarging incisional hernia. *Radiology Case Reports*, 1(1), 7–12. <https://doi.org/10.2484/rcr.v1i1.5>
3. Rao, C. M., & Krishna, N. V. V. H. (2011). Surgical Management of Lipoma in a Dog Reproduced with permission of the copyright owner . Further reproduction prohibited without permission . 4(1).
4. Takahashi, Y., Imamura, T., Irie, H., Tanaka, F., Fukushima, J. I., Fukusato, T., Harasawa, A., & Shiga, J. (2004). Myolipoma of the retroperitoneum. *Pathology International*, 54(6), 460–463. <https://doi.org/10.1111/j.1440-1827.2004.01646.x>

Dhakeri-Bange Syndrome in Goats: A Clinical-laboratory Investigation

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ABSTRACT

An outbreak of syndrome of unknown etiology associated with the feeding of moldy dry forage and green fodder among goats in Dhakeri village of Banke District on October-November 2008. Goats suddenly became ill with symptoms of knuckling of the fetlocks of the pelvic limbs, with no apparent ataxia or flexor weakness. Weight bearing was possible while the digits were extended, but with knuckling, weight was supported on the dorsal surface of the foot. The more severely affected goats were paraplegic and recumbent. Anorexia, apathy, diarrhea and ruminal stasis, flaccid posterior paralysis, hindlegs stretched forward both side of abdomen, paralysis of all four legs, head bented to sides, aimlessly head and rear shaking. On clinical examination based on history these goats were provisionally diagnosed as Dhakeri-Bange as being called locally and Endemic Mycotic polyneuropathy syndrome as seen first time in Nepal due to moldy forage/fodder poisoning in natural pasture were treated with Antidegnala liquor. On mycological and microbiological examination of tissue samples from post-mortem of dead goat and forage/fodder samples from pasture and goats feeding stalls on respective medium revealed the growth of fungal pathogens like Aspergillus and Penicillium spp with E. coli. These results provide circumstantial evidence that feeding of moldy forages and green fodder leaves infected by Penicillium and Aspergillus spp may cause outbreaks of a systemic Mycosis in these goats.

Keywords: *Moldy forage, Green fodder, Penicillium, Aspergillus Spp, Fungus, Mycoticpolyneuropathy, Banke, Antidegnala liquor.*

INTRODUCTION

During the autumn season (October-November) of 2008, around 300 goats raised by farmers of different villages in Banke districts of Nepal started showing paralytic symptoms like Kumri and were treated with the preparation of Diethylcarbamazin but disease did not subside. The goats included in this study had knuckling of the fetlocks of the pelvic limbs, with no apparent ataxia or flexor weakness. Weight bearing was possible while the digits were extended, but with knuckling, weight was supported on the dorsal surface of the foot. The more severely affected goats were paraplegic and recumbent. The

syndrome was consistent with sciatic or peroneal nerve disease. The paraplegic goats also have had a component of femoral nerve involvement. A field and clinical laboratory investigation was initiated to find out the cause of disease and to provide the appropriate curative and preventive measure.

FINDINGS

Clinical findings



Clinical examination of goats showed that animals were exhibiting the symptoms of anorexia, ataxia, diarrhea, dullness, dysmetria, generalized weakness and were found to be in the various stage of polyneuropathic condition. Besides, knuckling of the fetlocks of the pelvic limbs with no apparent ataxia or flexor weakness were found. Weight bearing was possible while the digits were extended but with knuckling weight was supported on the dorsal surface of the foot similar to reported by Dr. Maurice E. White 2008, and ruminal stasis, Schneider DJ, et. al. (1985). R. W. Medd, et. al. (2008), L. W. Whitlow and W. M. Hagler, Jr. (2008).

Post-Mortem findings

Postmortem examination of all 16 goats showed mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum; and congestion and hemorrhages in the abomasum. Liver was shrunken with pale to yellowish discoloration

with bile filled, distended bladder pin point hemorrhage in kidney, and small intestine with excessive mucus. These lesions were similar to lesions experimentally induced by Schneider DJ, et.al. (1985); Dhama et.al. (2007), R. W. Medd, et.al. (2008); Hussein S. Hussein, and Jeffrey M. Brasel (2001).

Microbial/mycobial culture examination of tissue samples

On mycological and microbiological examination of tissue samples from post-mortem of dead goat and forage and fodder from natural pasture on respective medium revealed the growth of fungal pathogens that include *Aspergillus* and *Penicillium* spp with *E. coli* similar to the findings of Karki et. al. (2008); C. Wendell Horne (2008); Sabreen, M. S. and Zaky, Z. M. (2001). All nasal and rectal swabs from sick and dead animals tested for PPR with pen-side test turned out to be negative.

Treatment of clinically affected goats and remaining herds

All goats that were showing clinical symptoms were treated with Antidegnala liquor (s.r) 2 ml s/c followed by 1 ml daily for next four days. Similarly, rest of animals in herds were provided with same drugs at the dose rate of 1 ml orally for four days. Those goats received the treatment as earliest time recovered promptly. The delayed treated goats too recovered but took longer duration. Similar longer recovery time was reported in earlier findings of Karki et.al. (2008).

RESULTS AND DISCUSSION

As during warm humid climate of tropics and subtropics favors growth of mold and fungus in feed grains and fodder especially after heavy monsoon rain feeding of exclusively such grain to livestock and poultry seems to cause the detrimental effect in the health these animals. As in this investigation clinical signs of anorexia, apathy, diarrhea and ruminal stasis and Clinical pathological findings included mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum and congestion and hemorrhages in the abomasum. Liver with shrunken appearance pale to yellowish discoloration with bile filled distended bladder pin point hemorrhage in kidney, small intestine with excessive mucus. On mycological and microbiological examination of tissue samples from post-mortem of dead goat on respective medium revealed the growth of fungal pathogens like *Aspergillus* and *Penicillium* spp with *E. coli*. These results provide circumstantial evidence that feeding of moldy maize grain and green fodder leaves infected by *Penicillium* and *Aspergillus* spp and timely use of Antidegnala liquor has controlled the further mortality in sick goats and when remaining animals in herd there was no further appearance of syndrome indicative of the above polyneuropathic syndrome was caused by a systemic Mycosis in these goats need to be looked into.

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REFERENCES

1. Schneider DJ, Marasas WF, Collett MG, van der Westhuizen GC (1985): An experimental mycotoxicosis in sheep and goats caused by *Drechslera campanulata*, a fungal pathogen of green oats. Onderstepoort J Vet Res. 1985 Jun; 52(2): 93-100. [www.ncbi.nlm.nih.gov/pubmed/ 4047622](http://www.ncbi.nlm.nih.gov/pubmed/4047622) -: Retrived on 13 october 2008.
2. R. W. Medd, G. M. Murray and D. I. Pickering: Review of the epidemiology and economic importance of *Pyrenophora semeniperda*. Australasian Plant Pathology 32(4) 539 – 550. [www.publish.csiro.au/act=view_file&file_id= AP03059.pdf](http://www.publish.csiro.au/act=view_file&file_id=AP03059.pdf): Retrieved on 13 october 2008.
3. DhamaK, Chauhan R S, MahendranMahesh, SinghKP, TelangAG, SinghalLokesh, Tomar Simmi (2007): Aflatoxins-hazard to livestock and poultry production: A review Journal of Immunology & Immunopathology 9 (1& 2). Indianjournals.com/ Retrieved on 13 october 2008.
4. Outbreaks called “moldy corn toxicosis,” “poultry hemorrhagic syndrome, ... Adult cattle, sheep, and goats are relatively resistant to the acute form of the ...[www.merckvetmanual.com/mvm/ index.jsp?cfile=htm/bc/212202.htm](http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/212202.htm):-Retrived on 13 october 2008.
5. C. Wendell Horne, Mycotoxins in Feed and Food Producing Associate Department Head and Extension Program Leader for Plant Pathology

