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EDITORIAL

It is our pleasure to come up with the 37th volume of Nepalese Veterinary Journal (NVJ). The current issue of NVJ includes a range of original research, review articles, short communication, and case studies on several aspects of animal husbandry including health, breeding, nutrition, and management of livestock, pets and wildlife, and public health. Some of the specific areas covered included microbiology; epidemiology, prevention and control of parasitic and infectious animal disease including zoonoses; antimicrobial resistance, COVID-19, ethnoveterinary medicine, and policy review. We believe that the information will be useful to the concerned stakeholders such as veterinarians, animal scientists, public health workers, farmers, and policy makers.

This year has been challenging due to the unprecedented emergence of the novel Coronavirus known as SARS-COV-2 from Wuhan, China in late December 2019. The Coronavirus Disease 2019 (COVID-19), caused by this novel virus, took the pandemic form and affected more than 118.7 million people and killed more than 2.6 million people by 11 March 2021 from more than 200 countries across the world. The economic losses have been tremendous and the full spectrum of it is still to be unfolded. The SARS-CoV-2 emerged from the bat but the intermediate host through which it successfully jumped into the human is still under investigation. This pandemic further underscores the importance of one health approach to tackle diseases that occur at the human-animal-environment interface.

Nepal also remained as one of the affected countries from the COVID-19 pandemic though the mortality rate was lower than expected. By 11 March 2021, more than 275,070 people were affected from COVID-19 out of which 3,012 people died. Five veterinary laboratories were utilized in the COVID-19 diagnosis which was particularly important during the initial days as there were only a few laboratories with diagnostic facilities for COVID-19 in Nepal. This type of one health collaboration needs to be extended in the future and establish a functional one health platform in Nepal. The proposed Center for Disease Control (CDC) and Food and Drug Administration (FDA) of the Government of Nepal need to be developed as multisectoral institutions with fair representation from all one health partners. The operationalization of “One Health Strategy 2019” of the Government of Nepal is the need of the hour to tackle diseases and problems that occur at the human-animal-environment interface that include Antimicrobial Resistance, Zoonotic Influenza, Rabies, Tuberculosis, Brucellosis, and emerging problems like COVID-19. The prioritization of zoonotic diseases to streamline the limited resources is also the need of the hour.

The COVID-19 pandemic equally affected livestock sectors, especially poultry and dairy, due to disruption in the supply chain and reduced consumption due to several media rumours regarding the consumption of animal products. This has highlighted the need for timely government interventions to support farmers in these types of emergencies, the development of cold storage facilities, and effective risk communication strategies. The livestock sector which was badly hit by the indirect effect of COVID-19 is also affected by several endemic and emerging disease problems. A new disease, Lumpy skin disease, which affects cattle and buffaloes, was confirmed for the first time in Nepal in 2020. Thousands of cattle and buffaloes were affected due to which farmers suffered losses due to reduced milk production. Nepal is also at eminent risk of African swine fever, a devastating pig disease after it was confirmed in India in May 2020. High and low pathogenic avian influenza (HPAI and LPAI) continue to affect the poultry industry. Unlike H5N1

outbreaks in earlier years, another strain H5N8 has been dominating in the outbreaks of early 2021. An endemic Newcastle disease (Ranikhet) of poultry also took epidemic form and several outbreaks were reported across the country. The emergence and reemergence of several infectious livestock diseases necessitate the need for the “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 chances of disease spillover events.

Nepal is gradually becoming self-sufficient in finished livestock and fish products such as meat, milk, eggs, and fish. To maintain the current status and fulfill the growing demand for animal protein, there is a need to increase investment in the livestock and fisheries sector from all three tiers of government. Breeding, feeding, and health management of livestock and fish need to be enhanced through effective policies and investments in the public-private partnership model.

The 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 on-line accessible scientific journal indexed in Nepjol. The publication aims to share scientific knowledge and technology among members and stakeholders within the home and abroad. NVJ welcomes original scientific research, review articles, short communication, case studies, and perspectives in the area of veterinary 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 respected authors and reviewers for their hard work and very valuable volunteer 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 correct the technical and grammatical mistakes as far as possible. We respect and welcome constructive suggestions from our readers.

Editorial board

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Seroprevalence of Tuberculosis in Captive Asian Elephants in Nepal

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ABSTRACT

*Tuberculosis (TB) is an infectious zoonotic disease, characterized by the development of tubercles resulting in caseation and calcification in the lungs. In elephants, causative agents *Mycobacterium tuberculosis* and *Mycobacterium bovis* result in chronic weight loss, anorexia, and weakness with occasional dyspnea or coughing. TB has been a major threat to elephants in Nepal. To date, 17 elephants have died in Nepal due to TB alone. Therefore, this study was undertaken to screen the TB seropositive elephants in Nepal. A cross-sectional study was conducted to determine the seroprevalence of tuberculosis (TB) in captive Asian elephants in four protected areas of Nepal from 22 November 2015 to 17 April 2016. The serum samples were examined for tubercle bacillus antibodies by DPP Vet TB Assay. Out of 92 elephants, 10.87% of elephants were found reactive to the bacterium *Mycobacterium tuberculosis* complex. There was a non-significant difference in seroprevalence of TB in captive Asian elephants for the sex, age, and location ($P>0.05$). In a follow-up study, we found that 4 TB seropositive elephants from our study died due to TB and displayed granulomatous tuberculosis lesions with the caseous mass in the lungs on post-mortem examination. Government and non-government stakeholders should jointly formulate effective plans and policies to eradicate tuberculosis from elephants in Nepal.*

Keywords: Captive Asian elephants, DPP Vet TB assay, Tuberculosis (TB), Zoonotic disease

INTRODUCTION

Tuberculosis (TB) is a chronic disease that affects humans and different animal species throughout the world. Elephant TB has been described in domesticated elephants as long as 2000 years ago in Vedic literature (Iyer, 1937). *M. tuberculosis* is the predominant disease-causing agent of TB in elephants while few infections with *M. bovis* and non-tuberculous *Mycobacterium* species are also recorded (Paudel et al., 2014). Because the disease is chronic and insidious, antemortem signs are rarely present and most TB diagnoses are made upon postmortem examination.

Nepal has more than 200 captive elephants that are in proximity to humans and domestic animals (Pradhan et al., 2011). These captive elephants have been used in patrolling, and tourism; thus, making them more prone to the possible transmission of *M. tuberculosis* from humans as Nepal has a higher load of TB (WHO, 2014). The first case of elephant TB in Nepal was reported in 2002. Since then, 17 elephants have died due to TB alone. During 2006-2007, elephant TB was

the major threats in the elephants in Nepal, when many elephants were diagnosed positive with TB. Routine surveillance and timely treatment were able to curve the number of TB-positive elephants in Nepal. It has been reported that 61 positive TB cases have been successfully treated to date. However, there has been a recent surge in the mortality of elephants due to TB. TB infected captive elephants is not only of concern for a zoonotic disease but also for the sound health of other wildlife species such as wild elephants, rhinoceros, etc. TB has also been reported in the one-horned rhinoceros in Nepal (Thapa et. al., 2015). Therefore, there is an impending need for the proper screening of TB in captive elephants in Nepal.

In this study, the serological method, Chembio DPP Vet[®] TB Assay (Chembio Diagnostic Systems, Inc., Medford, NY), that detects antibodies to ESAT-6/CFP 10 and MPB83 antigens was used for screening the TB in the captive Asian elephants. DPP VetTB[®] assays are licensed by the United States Department of Agriculture and have been used for TB screening in several Asian elephant range countries (Abraham et al., 2008; Ong et al., 2013). Besides that, serological methods are relatively simple, accurate, inexpensive, non-invasive, and non-dependent on the detection of mycobacteria. Thus, the objective of this study was to determine the seroprevalence of Nepalese captive elephants from the different protected areas of Nepal by using DPP Vet[®] TB Assay.

MATERIAL AND METHODS

Study site

A cross-sectional study was carried out in the four protected areas, viz- Chitwan National Park (CNP), Parsa National Park (PNP), Bardia National Park (BNP), and ShuklaPhata National Park (ShNP) of Nepal from November 2015- April 2016. The geographical location of the study area is shown in Figure 1.

Study animal

A total of 92 captive Asian elephants, mainly used for patrolling and tourism purposes were used in this study. The location wise number of elephants were 57, 11, 17, and 7 from CNP, PNP, BNP, and ShNP respectively. The age of the study elephant ranged from 2 to 77 years of age.

Sample collection and serology testing

About 5 ml of blood was collected from the caudal auricular vein of the elephant by using the 19-gauge winged IV infusion set. Thus, collected blood was centrifuged at 3000 rpm for 30 min; serum was separated and aliquoted into cryogenic vials. TB testing was carried out on the same day while the remaining serum was stored at -20 °C for future analysis.

The TB testing was done on the serum sample by using the DPP VetTB[®] assay (Chembio Diagnostic Systems, Inc., Medford, NY). The DPP Vet[®] TB Assay is a single-use immuno-chromatographic rapid test for detection of antibodies to *Mycobacterium tuberculosis* and *Mycobacterium bovis* in serum. The TB testing by using the DPP VetTB[®] assay was carried out by following the manufacturer's instructions. Briefly, 5 µl of serum was loaded in the center of the round sample + buffer well followed by adding buffer in the same well. 5 minutes after loading the sample in well 1, around 4 drops of the buffer were added to the square buffer well 2. Finally,

the test result was read 15 minutes after the addition of the buffer into the buffer well 2. Results were interpreted by observing the test lines in control and test areas

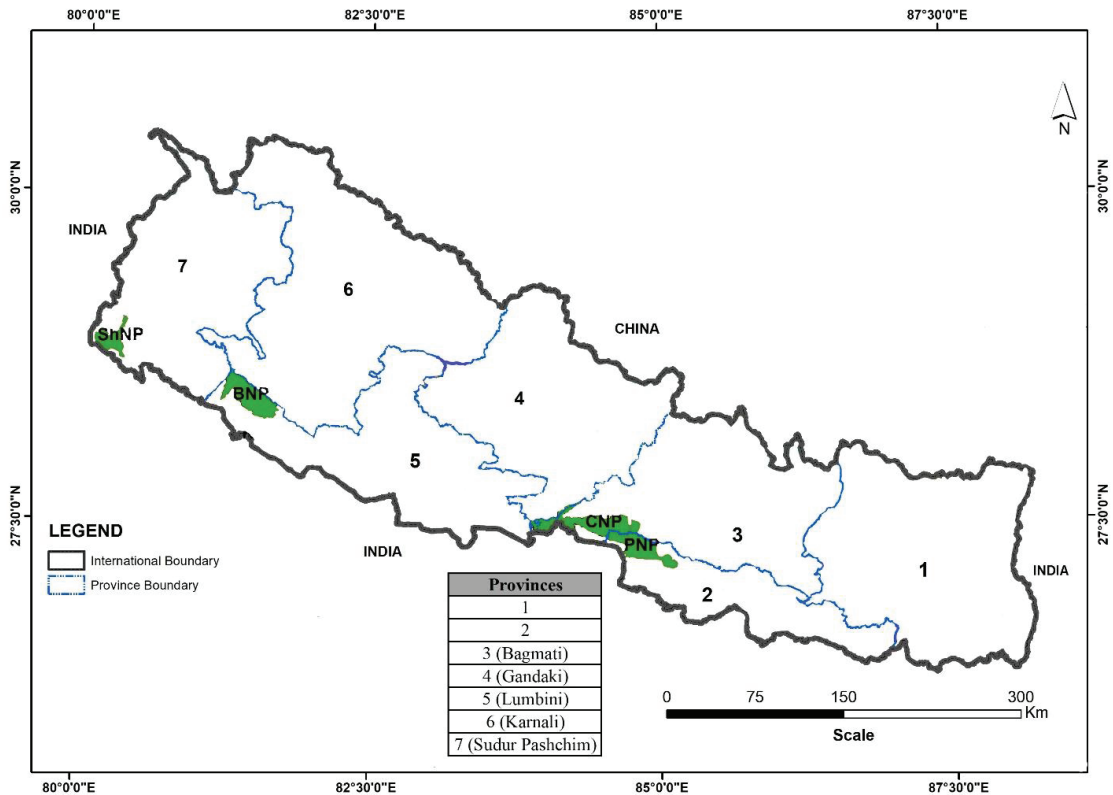


Figure 1. Protected conserved areas that were considered in this study.

Statistical analysis

TB seroprevalence was estimated by calculating the percentage of seropositive out of the total number of tests in the study. The data were analyzed by using MS-EXCEL 2010 and Open Epi version 3. Chi-square test and Fisher Exact Test were used to test the association between variables and results obtained. The P-value of less than 0.05 was considered statistically different.

RESULTS

Out of 92 captive Asian elephants tested during the study period, 10 elephants (10.87%) were seropositive for TB. Thus, the overall seroprevalence of TB was found 10.87% in the captive Asian elephant. The seroprevalence of the TB in the male captive Asian elephants and that of the female captive Asian elephants were 9.38% (3/32) and 11.67% (7/60) respectively. There was no significant difference in the prevalence of TB between male and female captive Asian elephants ($P > 0.05$) (Table 1).

Among different locations, the seroprevalence of the TB in the captive Asian elephant was found 12.28% (7/57), 5.88% (1/17), 9.09% (1/11), and 14.29% (1/7) in the CNP, BNP, PNP, and ShNP

respectively. No such difference was observed in the seroprevalence of TB among different locations ($P>0.05$). There was no significant difference in the seroprevalence of the TB in the captive Asian elephants among different age groups ($P>0.05$). The seroprevalence of the TB was 3.56%, 20%, 13.33% and 25% for (0-20), (20-40), (40-60) and (60-80) years respectively in the captive Asian elephants.

Table 1. Prevalence of TB among sex, age, and location in captive elephants

	No of elephants	TB negative	TB positive	Seroprevalence %	P-value
SEX					0.7366
Male	32	29	3	9.38	
Female	60	53	7	11.67	
LOCATION					0.8793
CNP	57	50	7	12.28	
BNP	17	16	1	5.88	
PNP	11	10	1	9.09	
ShNP	7	6	1	14.29	
AGE (years)					0.8221
<20	39	38	1	2.56	
20-40	15	12	3	20	
40-60	30	26	4	13.33	
60-80	8	6	2	25	
TOTAL	92	82	10	10.87	

We also did a follow-up study on the TB positive elephants from our study and found that 4 (2 male and 2 female) elephants that were tested positive in our study have already died, while remaining positive (6) are kept inside the national park and are closely monitored. To date, they display normal sound health and are only used for normal work. The dead elephants were emaciated and weak and showed granulomatous tuberculosis lesions with the caseous mass in the lungs on post-mortem examination (Figure 2).



Figure 2. Granulomatous tuberculosis lesion with the caseous mass in the lungs of dead elephants.

DISCUSSION

The overall seroprevalence of TB in captive Asian elephants in this study was observed 10.87%, which was lower than study in the Peninsular Malaysia (20.4%)(Ong et al., 2013) and in India (15%) (Abraham et al., 2008). The lower seroprevalence in this study may be the result of continuous monitoring and surveillance for TB in captive elephants over a period by the Nepal Government, National Park, other NGOs/INGOs. Nepal TB Project was launched in 2006. This project conducted regular screening and treatment for TB in all captive Asian elephants of Nepal. Moreover, Nepal Elephant TB and Management Action Plan (2011-2015) has been instrumental in decreasing the TB infection in Nepalese captive elephants by thorough surveillance and treatment. In fact, Nepal Elephant TB Control and Management Action Plan is the first such program in the Asian elephant range country (Mikota et al., 2015).

In terms of a different location, the seroprevalence of TB was found higher in the captive elephants from the CNP region (12.28%). This finding was in agreement with the previous study done by (Mikota et al., 2015), where they found 15 TB reactive cases from a total of 115 tested captive elephants (13%). CNP, a world heritage site, harbors the highest number of captive elephants in Nepal. Captive elephants in the CNP are used for patrolling, rhino census, and for tourism. Besides,

these captive elephants often intermingle with domestic cattle during grazing in the buffer area. Thus, these elephants are at high risk of getting TB from mahouts, tourists, and domestic animals. This might be one of the reasons for the higher prevalence of TB in captive elephants from CNC. The breeding facility is located adjacent to CNP, where captive elephant cows are bred with wild elephant bulls. So, constant surveillance of TB among the captive elephants is warranted not only to minimize the TB cases in the captive elephants but also to prevent the spread of TB in wild elephants. Although, the prevalence of TB was higher in female captive elephants as compared to males with no significant difference between them. This study also revealed a higher prevalence of TB in the older captive elephants (more than 20 years) than the younger captive elephants (less than 20 years).

In this study, DPP Vet[®] TB Assay was used for determining the seroprevalence of TB in the captive elephants. DPP Vet[®] TB Assay has been used to screen TB in several species of captive cervids and is approved by the USDA Bovine TB Eradication Program (Lyashchenko et al., 2018). Paudel et al. (2018) performed the 3 different serological tests - the Elephant TB STAT-PAK[®], DPP VetTB[®] Assay, and MAPIA along with the culture test on the eight suspected or confirmed TB elephant samples. They reported DPP Vet[®] TB Assay was reactive in all eight cases which were also positive for the culture test. This shows the effectiveness of the DPP VetTB[®] Assay that we used in our study.

Serological tests detect antibodies present in serum using antigen-antibody response. It does not access the clinical state of TB. Therefore, other rigorous diagnostic methods such as culture and molecular identification by using PCR should be carried out along with the serological methods. Considering the recent increase in the mortality of elephants due to TB, routine screening, and monitoring of the elephants are required.

CONCLUSION

The overall seroprevalence of TB in captive Asian elephants in Nepal was found to be 10.87%. In a follow-up study, we also found that 4 positive elephants in our study died due to TB. The remaining positive elephants should be constantly monitored, and further molecular diagnosis approaches need to be considered. Moreover, government and non-government stakeholders should jointly formulate effective plans and policies for the eradication of tuberculosis from elephants in Nepal.

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Gastro-intestinal Parasites of Sheep (*Ovis aries*, Linnaeus, 1758) in Laxmipur VDC, Dang, Nepal

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ABSTRACT

Sheep are being raised by the farmers of the Dang district for an economic purpose, but parasitic diseases remain a significant problem to their health. The study was conducted to determine the prevalence and seasonal occurrence of gastro-intestinal parasites of sheep in the Laxmipur Village Development Committee of Dang district. A total of 110 fecal samples were collected from April to September 2017, preserved in 2.5% potassium dichromate, and microscopically examined using concentration techniques. The study revealed overall 80% prevalence of gastro intestinal parasites including total 15 genera belonging to 5 classes. The gastro-intestinal parasites found in sheep were Eimeria sp., Entamoeba sp., Haemonchus sp., Strongyloides sp., Strongyle sp., Trichostrongylus sp., Oesophagostomum sp., Bunostomum sp., Trichuris sp., Nematodirus sp., Chabertia sp., Ascaris sp., Fasciola sp., Paramphistomum sp. and Moniezia sp., Parasitic infection during rainy season (92.93%) was found significantly higher compared to summer season (67.27) ($\chi^2=9.6023$; $p<0.05$). Most number of sheep (80.68%) were found infected with more than two parasites, among them the combination of Haemonchus sp., Strongyloides sp. and Strongyle sp. were most common. The results suggest that major nematodes belonging to genera Haemonchus, Strongyloides and Strongyle were prevalent in the study areas. The agro-climatic conditions like overstocking of the animals, grazing of young and adult animals together with poorly drained land provide an ideal condition for the transmission of the endoparasites to build up clinical infestation of the host.

Keywords: Concentration, Concurrency, Gastro-intestinal parasites, *Haemonchus sp.*, Seasonal occurrence, Sheep

INTRODUCTION

Ruminants are characterized by the presence of a four - compartment stomach, among these sheep (*Ovis aries*) are globally distributed, widely adapted to different climates, mainly arid and semi-arid areas of the world. Sheep are economically important for meat, manure, milk, hide and skin, wool, horns, bones, medicine etc. Gastro-intestinal parasitic infection is one of the greatest limiting factors in the production of sheep worldwide (Perry and Randolph, 1999; Torres-Acosta and Hoste, 2008; Calvete *et al.*, 2014; Mendoza-de *et al.*, 1998). A total of 8,00,658 sheep population was reported in Nepal in 2016, including 34,091 from

Dang. (SIONA, 2016). The temperature, moisture, rainfall, sharing of grazing area of other ruminants etc. enhance the occurrence of the parasite in Dang. Similarly, the farmers are suffered from the unavailability of veterinarians. Gastrointestinal parasitism is a primary cause of losses in sheep production in dang. Parasitic infections cause a serious threat and limit the productivity of livestock due to the associated morbidity, mortality, and cost of treatment and control measures (Nwosu *et al.*, 2007; Raza *et al.*, 2010; Lashari and Tasawar, 2011). The diseases are usually transmitted by the ingestion of the infective eggs/oocyst or larvae or by its penetration through the skin (Ibrahim *et al.*, 2008). The prevalence of gastrointestinal helminths is related to the agro-climatic conditions like quantity, and quality of pasture, temperature, humidity and grazing behavior of the host (Pal and Qayyum, 1993). The degree of nematode infection varies according to the host immune response to these worms (Greer, 2008). Commonly occurring helminth parasites are *Fasciola* sp., *Haemonchus* sp., *Ostertagia* sp., *Strongylus* sp., *Chabertia* sp., *Trichostrongylus* sp., *Cooperia* sp. (Abouzeid *et al.*, 2010), *Strongyloides* sp., *Trichuris* sp., *Oesophagostomum* sp. (Yaro *et al.*, 2015), *Bunostomum* sp., *Cotylophora* sp., *Dicrocoelium* sp., *Echinococcus* sp., *Moniezia* sp., *Nematodirus* sp., *Paramphistomum* sp. (Raza *et al.*, 2014).

Coccidiosis parasites of small ruminants is a protozoan caused by several species of the genus *Eimeria* located in the different parts of the intestine affects young animals. Coccidian parasites damage the lining of intestine that the animals need to absorb nutrients. Therefore, the most common sign of coccidian infestation is diarrhea (detected by dirty hind ends), and failure to thrive, or weight loss (Villarreal, 2013). Similarly, the most occurring gastrointestinal nematodes infecting sheep are *Haemonchus*, *Strongyloides*, *Strongyle*, *Trichostrongylus* and *Oesophagostomum*. Nematodes adversely affect ruminants, causing hematological, biochemical disturbances, anorexia, weight loss, poor reproductive performance, and even death of sheep (Yaro *et al.*, 2015). The blood - sucking parasite *Haemonchus contortus* which is found in the abomasum of the sheep and goat causes significant blood loss; each worm removes 0.05 ml of blood per day so that sheep with *H. contortus* may lose about 250 ml per day resulting in adecrease in erythrocytes, lymphocytes, hemoglobin, PCV, body weight and wool growth (Urquhart *et al.*, 1987). The present study was undertaken to determine the prevalence and seasonal fluctuation of gastrointestinal parasites in sheep to provide the baseline data for the management of the potential disease.

MATERIALS AND METHODS

Study Area

The study was conducted in Laxmipur Village Development Committee Dang which is located in the Mid-Western Region of Nepal. It is located about 5 km east of the district headquarter (Fig 1. Dang valley is the largest valley in Asia; this district is also known as inner terai. It lies on the geographical coordinates of latitude 27°37' to 28° 39' North and longitude 82°2' to 82°54' East. The maximum and minimum temperatures of this district are around 34°C and 14°C respectively and rainfall averages more than 1300 mm annually.

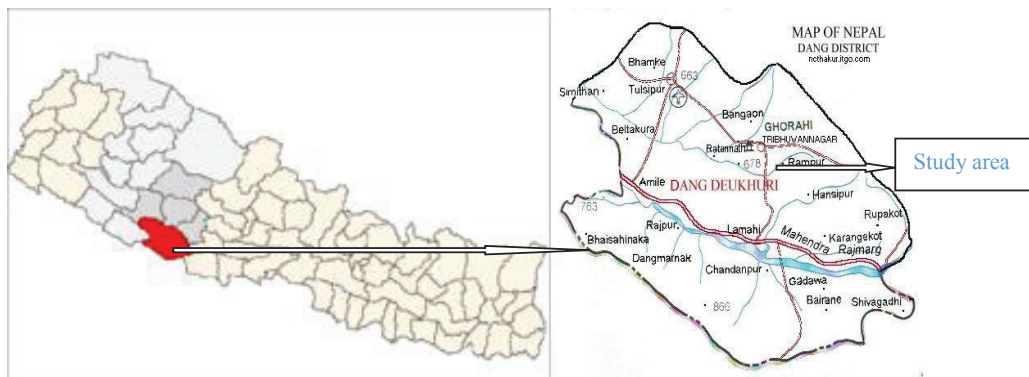


Fig.1 Study area

Collection, preservation, and laboratory examination of sample

A total of 110 faecal samples of sheep were collected purposively from the study area in two seasons (summer and rainy) from April to June and August to September 2017. Fresh fecal samples were collected immediately after voiding, transferred inside the sterile plastic vials, and preserved in 2.5% potassium dichromate. The faecal samples were transported to the Central Department of Zoology TU, Kirtipur Kathmandu and examined by concentration techniques (floatation and sedimentation). Approximately 3 gm of fecal sample was placed in a beaker with 42 ml of water and filtered. The filtrate solution was centrifuged for 5 minutes. The filtrate was saturated with NaCl, and again centrifuged. The top mixture was examined by adding methylene blue and the sediment was stained with an iodine solution to detect eggs, trophozoites and cysts of parasites (Soulsby, 2009).

Identification and data analysis

Identification was determined based on shape and size as described previously (Soulsby, 1982; Indre et al., 2010; Sangma et al., 2012 and Poddar et al., 2017). The data were coded and entered into a Microsoft Excel spreadsheet. Data were used to determine the prevalence of parasites. In addition, the data were tested whether these are equally distributed according to seasons. All data were analyzed in R, version 3.3.1 software packages. The differences in the parasite occurrences on different groups and seasons were compared using Chi-square tests. Data were analyzed using the R Programme (R Core Team 2019). In all cases 95% confidence level (CI) and $p < 0.05$ were considered for statistical significance.

RESULTS

Overall prevalence of gastro-intestinal parasites in sheep

Overall (80%) samples were found to be positive for parasitic egg, cyst, and larvae. A total of 13 helminths and two protozoan parasites were isolated from the faecal samples and identified. The intestinal protozoan parasites were *Eimeria* sp., and *Entamoeba* sp. The

identified helminths were *Haemonchus* sp., *Strongyloides* sp., *Strongyle* sp., *Trichostrongylus* sp., *Bunostomum* sp., *Oesophagostomum* sp., *Trichuris* sp., *Nematodirus* sp., *Chabertia* sp., *Ascaris* sp., *Moniezia* sp., *Fasciola* sp. and *Paramphistomum* sp. For intestinal protozoan parasites *Eimeria* sp. (15%), showed the highest prevalence than *Entamoeba* sp. Similarly, for intestinal helminths parasites *Haemonchus* sp. showed a higher prevalence than other parasites (Table1).

Table.1 Prevalence of gastro-intestinal parasites in sheep.

S.N.	Class	Parasites Name	Frequency	Prevalence %
1	Sarcodina	<i>Eimeria</i> sp.	4	3.64%
2	Sporozoa	<i>Entamoeba</i> sp.	3	2.73%
3	Nematoda	<i>Haemonchus</i> sp.	37	33.64%
4		<i>Bunostomum</i> sp.	11	10%
5		<i>Oesophagostomum</i> sp.	15	13.64%
6		<i>Strongyle</i> sp.	28	25.45%
7		<i>Strongyloides</i> sp.	34	30.91%
8		<i>Trichostrongylus</i> sp.	16	14.55%
9		<i>Nematodirus</i> sp.	6	5.45%
10		<i>Trichuris</i> sp.	9	8.18%
11		<i>Ascaris</i> sp.	2	1.82%
12		<i>Chabertia</i> sp.	5	4.54%
13	Trematoda	<i>Fasciola</i> sp.	12	10.91%
14		<i>Paramphistomum</i> sp.	8	7.27%
15	Cestoda	<i>Moniezia</i> sp.	7	6.36%

Season-wise prevalence and concurrency of the parasite in sheep

The study period was categorized as rainy and summer seasons. The relationship between the two seasons indicated that among 110 faecal samples (55 from rainy and 55 from summer) occurrence of parasitic infection in sheep showed higher infection of gastrointestinal parasites in the rainy season (N=51) than in the summer season (N=37) (Fig

2). The occurrence of gastro-intestinal parasites in sheep at two seasons was varied ($\chi^2=9.6023$; $p<0.05$). Overall *Haemonchus* sp. was found the highest prevalence in both the season; followed by *Strongyle* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Trichuris* sp., *Fasciola* sp., *Bunostomum* sp., *Paramphistomum* sp., *Chabertia* sp., *Moniezia* sp., *Eimeria* sp., and *Entamoeba* sp. Similarly, *Nematodirus* sp. and *Ascaris* sp. were not found in summer seasons but recorded only in the rainy season (Table 2).

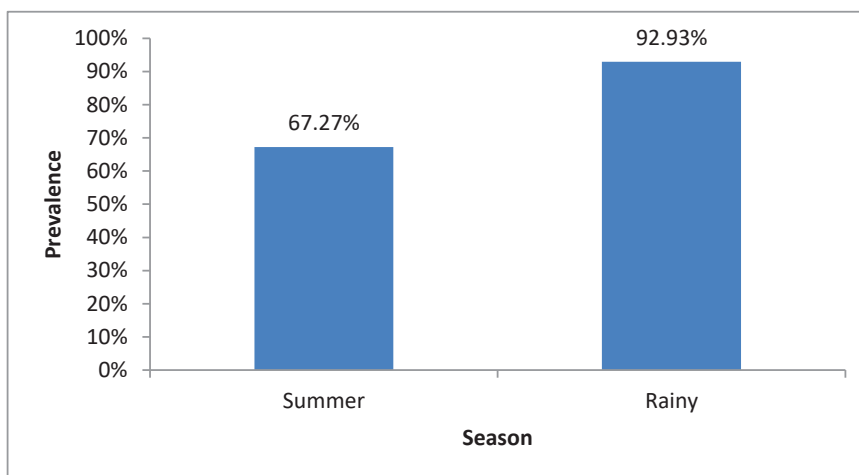


Figure 2. Season-wise prevalence of gastro-intestinal parasite in sheep

Table 2. Overall season-wise prevalence of gastro-intestinal parasite in sheep

S.N	Parasite Name	Summer season (April)		Rainy season (August)		χ^2	p-value
		No. of positive	Prevalence (%)	No. of positive	Prevalence (%)		
	<i>Eimeria</i> sp.	2	3.63	2	3.63	0	1
	<i>Entamoeba</i> sp.	1	1.82	2	3.63	0	1
	<i>Haemonchus</i> sp.	18	32.73	19	34.55	0	1
	<i>Bunostomum</i> sp.	5	9.09	6	10.91	1.121	1
	<i>Oesophagostomum</i> sp	6	10.91	9	16.36	0.309	0.578
	<i>Strongyle</i> sp.	11	20	17	30.91	1.198	0.274
	<i>Strongyloides</i> sp.	12	21.82	22	20	3.448	0.06
	<i>Trichostrongylus</i>	7	12.73	9	16.36	0.073	0.787
	<i>Nematodirus</i> sp.	0	0	6	10.91	4.407	0.036
	<i>Trichuris</i> sp	2	3.63	7	12.73	1.936	0.164

	<i>Ascaris</i> sp.	0	0	2	3.63	0.509	0.476
	<i>Chabertia</i> sp.	2	3.63	3	5.45	0	1
	<i>Fasciola</i> sp.	5	9.09	7	12.73	0.093	0.76
	<i>Paramphistomum</i> sp.	3	5.45	5	9.09	0.135	0.713
	<i>Moniezia</i> sp.	3	5.45	4	7.27	1.127	1

Polyparasitism was observed among the examined fecal samples of the sheep. The result indicated that the maximum number of sheep were suffered from mixed parasite infection, either protozoan or helminth parasites showing prevalence of 71 samples for mixed infection and 17 samples were detected to have a single infection (Fig 3). Among the mixed infection the combination of *Haemonchus* sp., *Strongyloides* sp. and *Strongyle* sp. were most common in maximum samples followed by *Trichostrongylus* sp., *Oesophagostomum* sp., *Trichuris* sp., *Fasciola* sp., *Bunostomum* sp., *Nematodirus* sp., *Paramphistomum* sp., *Chabertia* sp., *Moniezia* sp., *Eimeria* sp., *Entamoeba* sp. and *Ascaris* sp. also common parasites in single infection during both seasons. Multiple infections was not observed in the summer season. However, the distribution of single and multiple parasites was not varied among the examined sheep $p>0.05$) (Fig 3).

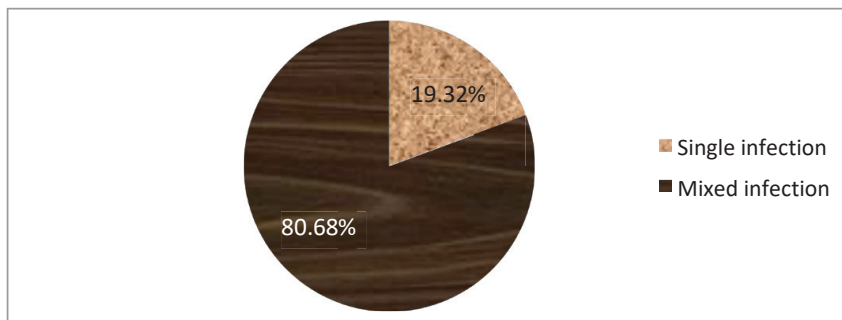


Figure 3. Polyparasitism of gastro-intestinal parasites

DISCUSSION

Plenty of researches has been done on sheep in a global context, but hardly a few in the national context. The study sets to investigate the presence of gastro-intestinal parasites in sheep in Dang. The prevalence of gastro-intestinal parasites is considerably influenced by climatic and geographical factors. Generally, the warm and humid conditions, continuous high rainfall, a decrease of grazing area, contaminated pasture, nutritional factors, and traditional rearing system provide a good condition for many gastro-intestinal parasites to flourish in the study area. In rural areas such as Dang, veterinary services to support farmers technically was not enough and people don't frequently report the need for treatment of that farm animals. In addition, the current climatic fluctuation in seasons such as rainy and summer might also increase the more parasitic prevalence. The climatic conditions such as temperature, moisture, rainfall, humidity, etc. in the grazing area,

faecal pellet, and grasses enhance the high prevalence of gastro-intestinal parasites in the study area. Apart from the above, these factors also increase the rapid life cycle of parasites from eggs to larvae in warm wet conditions (Mekonnen, 2007). Sheep are grazing in different grazing areas according to the season, while moving sheep from one pasture land to another they might be infected by parasites. Generally, the mode of infection is high in moveable livestock than in domesticated animals (Svensson *et.al.* 2000 and Rook *et. al.*, 2004). Furthermore, a higher prevalence of the nematodes in the present area might be due to the grazing of both young, and adult in poorly drained land, malnutrition, weak immunity and sharing of the same land for grazing by cattle, sheep, and goats (Asif *et al.*, 2008). Nematode infection seems higher in the studied sheep might be due to overcrowding of sheep and cattle, inadequate nutrition, the susceptibility of entry of parasites, the longevity of larval stage in the soil for a long period of time and finally, intermixing of pasture land and exposure of eggs and embryo. Similarly, the age of host dietary level, physiology, exposure of parasites, etc. increase the eggs output of nematodes (Miller *et al.*, 2012).

A total of 110 faecal samples of sheep was collected from Dang and examined by sedimentation and concentration methods. The majority of samples (80%) were found to be positive for both protozoan and helminth parasites. This prevalence rate of sheep was almost similar results of Getachew *et al.* (2016) from Ethiopia, Africa, Mavrot *et al.* (2015) from Europe and Koinari *et al.* (2013) from Papua New Guinea, Australia who reported 86.6%, 86% and 72% respectively with minor differences. Similarly, the present result is also in harmony with the range of 72% to 80% in different South Asian countries (Yadav *et al.*, 2006; Asif *et al.*, 2008 and Sangma *et al.*, 2012). Previous studies among sheep from different area of Nepal has shown the positivity ranging from 78% to 87% Khakural *et al.*, 2005 and Acharya, 2017) though prevalence rates were seen to vary according to locality. The prevalence of parasites in these sheep suggests that parasitic infections are among the main health problems in small ruminants globally (Mbuh *et al.*, 2008; Kantzoura *et al.*, 2012). The prevalence of parasites in sheep observed in the present study is lower than the study carried out by (Ibrahim *et al.*, 2014; Pedreira *et al.*, 2006 and Bansal *et al.*, 2015). The higher prevalence observed in different parts of countries could be due to overstocking, poor nutrition, poor management practice of the animals (lack of sanitation) and frequent exposure to the communal grazing lands that have been contaminated. Whereas, in comparison to other studies conducted in different countries present study recorded higher prevalence in sheep which range from 7% to 66% (Sultan *et al.*, 2016; Kantzoura *et al.*, 2012; Poddar *et al.*, 2017; Mohammedameen, 2016; Vijayalakshmi, 2015 and Lemma and Aber 2013). Lower prevalence in those countries could be due to good management practice, existence of unfavorable climatic or environmental factors that could support prolonged survival and development of the infective larval stage of parasites. The high infection rates of GIT helminths in sheep in the study area could be due to the presence of favorable environmental conditions for the existence and development of the gastro-intestinal parasites, foraging behavior of sheep (Tefera *et al.*, 2009; Bansal *et al.*, 2015).

A total of 13 helminths and two protozoan parasites were isolated from the faecal samples and identified from the study area. Among them *Eimeria* sp. and *Entamoeba* sp. from the protozoan and *Haemonchus* sp., *Strongyloides* sp., *Strongyle* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Trichuris* sp., *Nematodirus* sp., *Chabertia* sp., *Ascaris* sp., *Moniezia*

sp., *Fasciola* sp., *Bunostomum* sp. and *Paramphistomum* sp. were identified as helminths parasites. In the current study, *Haemonchus* sp. (33.64%) showed the highest prevalence in helminths parasites followed by others which is almost similar to the work of Almalaik *et al.* (2008), Tariq *et al.* (2008), Tefera *et al.* (2009) and Khajuria *et al.* (2013) who also reported *Haemonchus* sp. as predominant parasite by 53.4%, 59.6%, 67.5% and 61.18% from Tulus Area, Sudan, Kashmir valley, India, Bedelle, South- Western Ethiopia and Jammu, India respectively although the prevalence rate is higher than present finding. The higher prevalence of *Haemonchus* sp. could be due to poor management practices, grazing, traditional rearing system, resistance of parasites, nutritional factors, etc. The prevalence rate of *Ascaris* sp. (1.82%) in the present study is less than the report of Ibukun and Oludunsin (2015) who reported 9.4% from Nigeria.

Among the protozoan parasites the prevalence rate of *Eimeria* sp. is almost similar with the report of Yadav *et al.* (2006) who reported 6.73%, from R.S. Pura, Bishnah and Samba tehsils of Jammu district, India, less than the report of Bhat *et al.* (2012), Koinari *et al.* (2013), Ibrahim *et al.* (2014) and Sultan *et al.* (2016) who reported 9.8%, 17.3%, 11.7% and 16.52% from Kashmir valley of India, Papua New Guinea, Jimma town, Western Ethiopia and Nile Delta, Egypt respectively and less than the report of Gizachew *et al.* (2014) who reported 30.9% from Bako town, Western Ethiopia. From these results, it was seen that cooler and temperate climate is suitable for the development of *Eimeria* sp. than warm and tropical climates. Only a few studies have been done regarding protozoan parasites. Furthermore, *Entamoeba* sp. (2.73%) is another protozoan parasite identified in study area.

Regarding the concurrency of gastro intestinal parasites both singles and mixed infections were recorded. Out of positive samples, (80.68%) samples for mixed infection and (19.32%) samples were detected to have single infection. Among the mixed infection the combination of *Haemonchus* sp., *Strongyloides* sp. and *Strongyle* sp. were most common in maximum samples followed by other parasites. This finding is in harmony with reports of previous studies of Raza *et al.* (2014) and Getachew *et al.* (2016) conducted in Cholistan desert of Pakistan and Areka, Ethiopia who also detected mixed infection in their studies. In present study, mixed infection was detected in 80.68% samples which is higher than the finding of previous studies conducted by Kenea *et al.* (2015) and Getachew *et al.* (2016) who reported 17.7% and 26% from, Kaffa and Bench Maji zones, Southwest Ethiopia and Areka, Ethiopia respectively and almost similar with the study conducted in Patiala and its adjoining areas (Kaur and Kaur, 2008) who reported mixed infection in 85.71% samples. The variance in mixed infection in different studies could be due to a lack of sheep de-worming, less use of anti-parasitic drugs, dietary deficiency, poor management system, lack of knowledge for sanitation and climatic and geographical variation.

Gastro-intestinal parasitic infection in a sheep in the rainy season was found 92.93%, whereas in summer season, 67.27% with a significance difference ($\chi^2=9.6023$; $p<0.05$). The highest prevalence of parasites in rainy season is in consent to the research carried by Bansal *et al.* (2015) and Varadharajan and Vijayalakshmi (2015) in Mhow, Indore and Coastal areas of Tamil Nadu. On the contrary few workers have also observed and reported a high incidence of these parasitic infections in summer (Tariq *et al.*, 2008), autumn (Khalafalla *et al.*, 2011) and spring (Mir *et al.*, 2013) season which is not in agreement with the present

study. During rainfall the number of infective larval stage of nematodes is increased, that's why the animal becomes more susceptible to infection whereas, the infection is generally less during the dry season (Kantzoura *et al.*, 2012). The development and distribution of gastro-intestinal parasitic helminths will show the maximum prevalence during the rainy season, and lower incidence during summer (Dappawar *et al.*, 2018). The high level of gastro-intestinal helminth parasite infections in sheep is because of lack of management and parasite treatment program, mainly on traditional farms. In traditional sheep rearing enclosure sanitation is not possible for accurate attention, so the transmission of different gastro-intestinal parasites occurs.

CONCLUSION AND RECOMMENDATIONS

The present study demonstrates that the sheep in Dang were affected by various gastro-intestinal parasites. Good husbandry practices, veterinary health program and appropriate prevention, treatment with broad spectrum anthelmintic and control strategy actions should be adopted by both stockowners and government to minimize the gastro-intestinal parasites of sheep.

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Evaluation of Ethno-veterinary approaches to Control Ticks in Nepal

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ABSTRACT

Tick borne diseases such as Babesiosis and Theileriosis are common in Nepal. Farmers are using several plants as traditional ethnoveterinary medicines to control ticks in their animals. However, their efficacy have never been assessed. A study was conducted to evaluate the efficacy of plant extracts used in ethnoveterinary medicine by Nepalese farmers to control ticks. 100 rural farmers, 50 each from Tanahun and Nawalparasi districts, were interviewed through semi-structured questionnaires to document the ethnoveterinary plants used to control ticks. Among the 26 plants documented, Neem (*Azadirachta indica*) was used by 42 out of 100 respondents, Titepati (*Artemisia* spp.) by 34 respondents, Sisno (*Utrica dioica*) by 10 respondents and Bozo (*Acorus calamus*) by 6 respondents. The choice of plants used was determined by their local availability. Besides, mapping of livestock tick was conducted by collecting ticks from 21 districts. A total of five genera of ticks were identified viz. *Boophilus microplus*, *B. annulatus*, *Hyalomma marginatum issaci*, *Rhipicephalus haemaphysaloides*, *R. Sanguineus*, *Haemaphysalis bispinosa* and *Ixodes ovatus*. *Boophilus microplus* was found in all study districts and all the livestock species indicating the risk of enzootic stability of Babesiosis in local cattle. In a field trial conducted with 70 cattle at Nawalparasi district, Cypermethrin 0.02% (Clinar®) was found to be most effective in reducing the number of ticks (97.17% reduction) followed by 20% Neem extract (91.46% reduction) and 20% Bozo extract (89.88% reduction in ticks) ($p < 0.05$). All the plant extracts exhibited more than 50% reduction in ticks at 3 days after treatment (DAT). Neem and Bozo extracts were found superior in tick control among the traditionally used plant extracts. Based on their local availability, Neem in Terai and Bozo in both Terai and Hills might be effectly used for the control of livestock ticks in Nepal in an eco-friendly manner.

Keywords: Eco-friendly, Plant extracts, Tick control

INTRODUCTION

Ticks, the haematophagus parasites, are the most common external parasite of economic importance in the small holder farming system of Nepal. High mortalities are reported in cattle due to ticks and tick borne diseases in Africa and across the tropical areas (Muchenje *et al.*, 2008). Ticks transmit a wide variety of pathogenic agents than other groups of arthropods (Oliver 1989), Babesiosis, Theileriosis, Trypanosomiasis and Ehrlichiosis are some of the examples of tick-borne livestock diseases which cause morbidity and mortality in animals. Besides, ticks cause tick worry,

blood loss and damage to skin, hides, and teats leading to formation of wounds predisposing animals to secondary bacterial infections. (Peter *et al.*, 2005).

Ticks are commonly controlled using conventional acaricides however, it has certain shortcomings. They are expensive and not readily available to rural farmers. They are toxic to livestock and humans, their residues remain in animal tissues and are harmful to the environment. Another important threat is that, ticks are developing resistance against a range of conventional acaricides available in the market. Due to these shortcomings of the conventional acaricides, resource-limited farmers are reverting to alternative methods and remedies that fall within ethnoveterinary practices. One of such method is the exploitation of eco-friendly, economical, safe and effective indigenous plants. These plants are the miraculous laboratories of nature that provide various kinds of molecules. Plant extracts provide valuable alternatives to and complements conventional veterinary medicine and is increasingly evident in the western world where herbal medicine and other alternative approaches are becoming the mainstream practices. Ethnoveterinary practices are of specific value in developing countries where allopathic medicines are often beyond the reach of livestock producers. In Nepal, farmers are using several traditional plants that include Neem (*Azadirachta indica*), Titepati (*Artemisia* spp.), Sisno (*Utrica dioica*), Bozo (*Acorus calamus*) and several other plants to control ticks, the choice of plants largely driven by local availability. However, their efficacy have never been assessed. This study was conducted to understand the common plants farmers are using to control the ticks and to measure their efficacy against commonly found livestock ticks in Nepal.

MATERIALS AND METHODS

The research was conducted from 15th July 2011 to 15th October 2011. *In-vitro* trials were conducted at Parasitology laboratory at Animal Health Research Division (AHRD), Nepal Agriculture Research Council (NARC). In-vivo trial was conducted at Gaidakot Municipality, Nawalpur District. For questionnaire survey an open-ended questionnaire was prepared. Survey on the existing practices on ectoparasite control and use of plants for tick control was conducted in Tanahun (Hilly district) and Nawalparasi (Terai district). Altogether 100 farmers were interviewed focusing on the ethnoveterinary remedies for tick control.

Mapping of livestock tick

Ticks were collected from the naturally infested animals of 21 districts of Nepal regardless of age, sex and ecological region. The districts from where ticks were collected include Jumla, Okhaldhunga, Kaski, Lalitpur, Kathmandu, Baglung, Myagdi, Bhaktapur, Arghakhachi, Morang, Sunsari, Jhapa, Siraha, Saptari, Dhanusha, Nawalparasi, Rupendehi, Chitwan, Bara, Parsa and Udyapur (Figure 1). Ticks were hand-picked from different parts of the body viz. ear, dewlap, tail base, foreleg, hindleg, udder, inguinal region, perennial region etc. The ticks were immediately preserved in vials containing 5% glycerine in 70% alcohol. Ticks containing vials were labeled and transported to AHRD, NARC for further identification. The preserved ticks were examined for their morphological characteristics under stereo-microscope and identified according to the figures and keys described by acarology division (Kaiser, 1987 and Morel, 1989).

Preparation of plant extracts

Four plants namely; *Azadirachta indica* (Neem), *Acorus calamus* (Bozo), *Artemisia vulgaris* (Titepati) and *Utrica dioica* (Sisno) were used in the trial. Leaves of Neem, rhizomes of Bozo collected from Chitwan District and the leaves and soft aerial parts of Sisno and Titepati collected from Kavre district were taken to AHRD, NARC, Khumaltar, Lalitpur. Aqueous extracts having concentrations of 20%, 10% and 5% were prepared and stored in a refrigerator at 4° C until used in the experiment.

In-vivo trial

In-vivo trial was conducted at Gaidakot Municipality, Nawalpur District. Altogether 70 cattle, naturally heavily infested with ticks were selected. The animals were of different breeds, colour, age, sex, parity and body conditions. One square foot area at three distinct regions in the body was marked at dewlap, tail-base and udder respectively. The total number of ticks present on the selected areas were counted. 12 different solutions containing aqueous extract of Neem, Sisno, Bozo and Titepati having 5%, 10% and 20% were used in the test. Distilled water and Cypermethrin 0.02% (standard) were used as negative and positive control respectively. Altogether 14 treatments with 5 replications each were made. Test solution was applied on the demarcated area with paint brushes after counting the number of ticks on the first day. The number of ticks remaining on the site was counted on 2nd, 3rd and 4th day of application of the extracts. The percentage reduction in number of ticks was then analysed using MSTAT-C, computer software program.

RESULTS

Documentation of plants used in ethno-veterinary practices to control ticks

Altogether 26 plants and plant parts were documented (Table 1). We found that Neem (*Azadirachta indica*) was the most used plant for tick control. Ten out of fifty respondents in Hilly region and 32 out of 50 respondents in Terai region used Neem for tick control. Titepati (*Artemisia* spp.) was used by 34 farmers (26 out of 50 farmers in hilly region and 8 out of 50 farmers in terai). This indicates that these plants are common to both the highland areas and low land areas.

Table 1. List of plants used to control ticks

Common name	Scientific name	Number of respondents using plants as a method of tick control		
		Tanahun	Nawalparasi	Total
Neem	<i>Azadirachta indica</i>	10	32	42
Titepati	<i>Artemisia japonica</i>	26	8	34
Banmara	<i>Ageratina adenophora</i>	21	6	27
Sisno	<i>Utrica dioica</i>	8	2	10
Lemon extract	<i>Citrus limon</i>	7	3	10
Gandhe	<i>Ageratum hausronianum</i>	5	5	10
Salt	Sodium chloride	6	3	9
Tulsi	<i>Ocimum tenuiflorum</i>	3	6	9

Tobacco	<i>Nicotiana tabaccum</i>	-	7	7
Bojo	<i>Acorus calamus</i>	3	3	6
Haldi/ Besar	<i>Curcuma longa</i>	2	3	5
Ghiukumari	<i>Aloe vera</i>	2	3	5
Asuro	<i>Adhatoda vasica</i>	4	-	4
Timur	<i>Zanthoxylum armatum</i>	2	2	4
Bakaino	<i>Melia azadirach</i>	-	4	4
Mustard cake	<i>Barassica campestris</i>	1	2	3
Tejpatta	<i>Cinnamomum tamala</i>	2	-	2
Amala	<i>Emblica officinalis</i>	2	-	2
Guava leaves	<i>Psidium spp.</i>	1	1	2
Garlic	<i>Allium sativum</i>	1	1	2
Simali	<i>Vitex negundo</i>	1	-	1
Jangli tulsi	<i>Croton bonplandianum</i>	1	-	1
Cow butter oil	-	1	-	1
Chilaune bark	<i>Schima wallichii</i>	1	-	1
Bhojpatra	<i>Betula utilis</i>	1	-	1
Zeera	<i>Cuminum cyminum</i>	-	1	1
Peach leaves	<i>Prunus Persica</i>	-	1	1

Tick mapping

From 21 study districts, the total numbers of ticks examined were 1268, of which 1001 were identified as female, 216 as male and 57 as nymphs. A total of five genera of ticks were identified from livestock in 21 districts (Table 2).

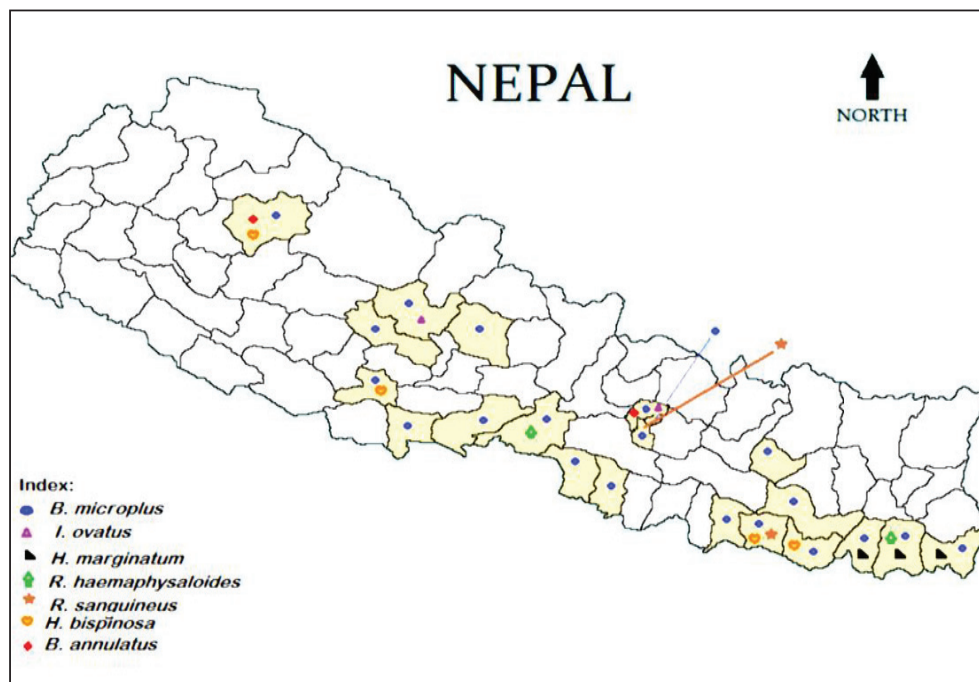


Figure 1. Map of sampled districts and different ticks found in those districts

In-vivo trial

At 1st day after treatment (DAT), all the treatments were significantly different as compared to the standard (Cypermethrin 0.02%). Among the plant extracts, the highest percentage reduction in number of ticks was recorded in 20% Bozo extract solution with a mean reduction of 58.05% and the least was recorded in 5% Neem extract solution with a mean reduction of 21.52%. No statistically significant difference in the percentage reduction in ticks in cattle treated with Bozo 20% extract, Neem 20% extract and Titepati 20%.

Table 2. Ticks species identified

Tick species	Districts	Livestock species
<i>Boophilus microplus</i>	All	Cattle, buffalo, goat, rabbit, pig
<i>Boophilus annulatus</i>	Jumla, Kathmandu	Cattle
<i>Hyalomma marginatum issaci</i>	Morang, Sunsari, Jhapa	Cattle
<i>Rhipicephalus haemaphysaloides</i>	Morang, Chitwan	Cattle
<i>R. sanguineus</i>	Dhanusa, Lalitpur	Cattle, Goat
<i>Haemaphysalis bispinosa</i>	Jumla, Argakhachi, Siraha, Saptari	Cattle
<i>Ixodes ovatus</i>	Myagdi, Kathmandu	Cattle, Goat

At 2nd DAT, all the treatments were significantly different as compared to the standard (Cypermethrin 0.02%) which has the highest percentage mean reduction in the number of ticks (93.10%). Among the different plant extracts, 20% Neem extract has the highest percentage reduction in number of ticks (mean 74.48%) at $P < 0.05$. There was no significant difference in percentage reduction in number of ticks between 20% Neem extract and 20% Bozo extract solution. The result of these two extracts was at par with 20% Titepati extract solution, 20% Sisno extract solution and 10% titepati extract solution. On the 3rd DAT, the highest percentage reduction in number of ticks was found in standard (Cypermethrin 0.02%), having mean reduction of 97.17%. Among the plant extracts, 20% Neem extract had the highest mean percentage reduction in number of ticks (91.46%). There was no statistically significant difference between 20% Neem extract (mean percentage reduction in number of ticks 91.46%) and 20% Bozo extract (mean percentage reduction in number of ticks 89.88%) at $P < 0.05$. Likewise, 20% Titepati extract solution (mean percentage reduction in number of ticks 85.85%) was significantly lower than the above two treatments. All the plant extracts exhibited mean percentage reduction in number of ticks by more than 50% until 3rd day after treatment. The study revealed that, the highest efficacy was found in 20% Neem extract and 20% Bozo extract (Table 3).

Table 3. In-vivo efficacy of different plant extracts

Treatment	Percentage reduction in number of ticks (Days post treatment)		
	Day 1	Day 2	Day 3
5% Neem Extract	21.52 ^h (27.56)	36.45 ^f (36.84)	64.89 ^f (53.75)
10% Neem Extract	31.20 ^{efg} (33.86)	49.18 ^e (44.51)	73.63 ^{de} (59.23)
20% Neem Extract	56.5 ^b (48.79)	74.48 ^b (59.79)	91.46 ^b (73.39)
5% Sisno Extract	25.86 ^{gh} (30.50)	50.49 ^e (45.27)	59.24 ^s (50.30)
10% Sisno Extract	35.50 ^{def} (35.33)	57.81 ^{de} (49.48)	67.04 ^f (54.95)

20% Sisno Extract	39.21 ^{cd} (38.74)	71.28 ^{bc} (57.59)	75.90 ^d (60.59)
5% Bozo Extract	28.73 ^{fg} (32.35)	52.54 ^e (47.52)	65.57 ^f (54.06)
10% Bozo Extract	40.52 ^c (39.50)	64.33 ^{cd} (53.33)	74.04 ^{de} (59.35)
20% Bozo Extract	58.05 ^b (49.62)	72.40 ^b (59.51)	89.88 ^b (71.67)
5% Titepati Extract	27.39 ^{fg} (31.40)	57.49 ^{de} (49.32)	68.90 ^{ef} (56.09)
10% Titepati Extract	36.87 ^{cde} (37.20)	67.39 ^{bc} (55.34)	73.81 ^{de} (59.20)
20% Titepati Extract	53.40 ^b (46.93)	71.59 ^{bc} (58.29)	85.85 ^c (67.88)
Negative control	4.02 ⁱ (9.660)	12.53 ^g (11.32)	11.86 ^h (13.12)
Positive control	80.75 ^a (64.04)	93.10 ^a (75.88)	97.17 ^a (80.95)
LSD (P=0.05)	3.843	5.149	3.337
SEM±	1.354	1.814	1.176
CV%	8.07%	8.07%	4.52%
Grand Mean	37.535	50.300	58.180

CV: Coefficient of variation, LSD: Least Significant Difference

Value with the same letters in a column is not significantly different at 5% by DMRT

The figures in parentheses are arcsine transformation.

DISCUSSION

Of the five genera, *Boophilus microplus* was the most abundant tick in all study districts and in all livestock species. It is likely that high infection rates of *B. microplus* ticks could maintain enzootic stability of Babesiosis in local cattle. In this study, almost in all animals, the number of tick infestation per animal was more than 100, *Hyalomma marginatum issaci*, the vector tick for theileriosis, was identified from three eastern Terai districts. Eastern Terai region is endemic for Theileriosis because of the presence of both pathogen and vector tick (Shrestha, *et al.*, 2005). *Haemaphysalis bispinoa*, a vector tick for *Theileria sergenti*, which is only common in Japan and Korea, may also transmit the disease, however, this particular protozoan parasite has not been identified from Nepal (Shrestha *et al.*, 2005). *Ixodes ovatus* tick was identified from Myagdi and Kathmandu from cattle and goat respectively.

Among the plant extracts, neem leaves extracts significantly had higher mean reduction of ticks. This finding is in line with Rahman *et al.* (2009) who found a mean reduction of 68% tick when 15% neem extract was sprayed on the calves in a study conducted in Bangladesh. 8% concentration of neem seed extract had the highest mortality compared to neem bark extract and 8% neem leaves extract, (Ghosh *et al.* 2010). Ismail *et al.* (2002) reported a high neem oil concentration of 40%, which was very much effective against *R. pulchellus* larvae. Choudhury (2001) reported 100% mortality of the *R. sanguinus* larvae at 8, 6, 4, and 2 hour after treatment with neem seed oil at the concentration of 20%, 40%, 60% and 80% respectively. In our experiment the 20% aqueous extract of neem leaves showed 91.46% reduction in tick 3 days after the application. Similar results with 92.4% mortality of ticks on third day post treatment with 8% concentration ethanolic extract of neem seed extract was recorded by Ghosh *et al.* (2010) in a study conducted in India. The 90–100% efficacy of cypermethrin was earlier reported by Khan (1996) against *B. microplus* in cattle along with residual period of 8–15 days. Main arthropodocidal neem compounds normally require a longer time to show their relevant properties. All the plant extracts showed a time and concentration dependent effect on the ticks. The reduction in the number of ticks may be either

from the death, from repellent property of the herbal drugs, from anaesthetization and dropping off from the body or combined effects of herbal drugs (Shrestha *et al.*, 2005).

Among 135 described compounds, Azadirachtin A (AZA) commonly referred to as azadirachtin stands out (Liu *et al.*, 2005). Azadirachta indica is considered the most phytochemical source of pesticides, and is widely used all over the world (Forster and Moser, 2000). In fact, the active compounds of neem show many modes of action against arthropods, such as antifeedant activity, growth regulation, repellency, reduction of fecundity and oviposition, changes in biological fitness, and blocked development of vector-borne pathogens (Mulla and Su, 1999; Schmutterer, 1990a). In a similar research Ghosh *et al.* 2010 found that the extract prepared from rhizome of *Acorus calamus* proved highly efficacious and 100% final mortality within 14 days post treatment. The extract was found safe and no reaction was observed when animals were treated with 50% concentration (Ghosh *et al.* 2010) which is 2.5 times the concentration used for in vivo studies in present research.

CONCLUSION

Neem leaves are used by majority of the farmers as a means of ethnoveterinary remedy to control ticks in Nepal. *Boophilus microplus*, *B. annulatus*, *Hyalomma marginatu issaci*, *Rhipicephalus haemaphysaloides*, *R. Sanguineus*, *Haemaphysalis bispinosa* and *Ixodes ovatus* are the major tick species found in Nepal with *B. microplus* present in all the districts and in all the livestock specie. 20% aqueous extract of Neem (*Azadirachta indica*) is found to be the most effective remedy for ticks in *in-vivo* trial followed 20% aqueous extract of Bozo (*Acorus calamus*). Hence, Neem in Terai and Bozo in both Terai and Hills can be used to control ticks without any harm to the animals. For further studies we suggest to analyse the chemical constituents of aqueous extracts and quantify them to use for tick control in livestock.

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Seroprevalence of *Chlamydia abortus* in Anestrous Cattle of Nawalpur and Chitwan District, Nepal

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ABSTRACT

Chlamydia abortus are gram negative coccoid, obligate intracellular zoonotic bacteria responsible for abortion and other reproductive problems in dairy cattle. This leads to sub-fertility and infertility in cattle thereby causing significant economic losses to dairy farmers. However, the status of *Chlamydia abortus* in Nawalpur and Chitwan districts, which are the dairy pockets of Nepal, are poorly understood. The objective of this study was to determine the seroprevalence of antibodies to *Chlamydia abortus* in anestrous dairy cattle of Nawalpur and Chitwan districts in relatively low risk winter season. In total, 92 cattle serum (Nawalpur (n= 27) and Chitwan (n= 65) samples were collected from April 2018 to May 2019 and tested using a *Chlamydia abortus* specific indirect ELISA kit to evaluate the seropositivity status of cattle. Results showed that only 2 (2.17%) cattle were seropositive for *Chlamydia abortus*. Both the positive cattle were Jerseys from Nawalpur area with a history of abortion. The ages of the seropositive cattle were 3 and 4 years. In conclusion, there was a low seroprevalence of *C. abortus* in dairy cattle of Nawalpur and Chitwan districts. The presence of this bacterium suggests that it is likely to be a cause of unidentified abortion in dairy cattle in Nepal. We suggest screening dairy animals for *C. abortus* infection to minimize this risk.

Keywords: Abortion; Cattle; *Chlamydia abortus*; iELISA; Seroprevalence

INTRODUCTION

In Nepal livestock and its product contribute 11.5 % of national Gross Domestic Product (GDP) (CBS, 2011), and 25.68% of the Agricultural Gross Domestic Product (AGDP). Among 7,302,808 cattle in Nepal, Chitwan and Nawalpur contain 71,864 and 96,113 cattle annually producing 14,947Mt and 18,451Mt milk respectively (MoLD., 2017) (VHLSEC, 2073/74). Nepal embodies dairy cattle production as one of the important contributors to the national economy. Anestrus has major economic consequences for the dairy industry worldwide (Dziuk, 1983). There are various causes of anestrous including nutrition, infectious disease (parasitic, bacterial, viral, and protozoal) and poor management practices. *Chlamydia abortus* is reported to be one of the bacterial causes of anestrous in dairy cattle. Members of the genus *Chlamydia abortus* are Gram negative coccoid, obligate intracellular bacteria within the family *Chlamydiaceae* and order *Chlamydiales*.

Chlamydia abortus (*C. abortus*) is endemic in ruminants throughout the world. It can efficiently colonize the placental trophoblasts and is one of the causative agents of abortion and fetal loss in sheep, goats, and cattle in many countries (Shewen, 1980). It is reported to be the causal organism for Epizootic Bovine Abortion (EBA) in milking cows which results in mastitis, reproductive issues such as endometritis, changes in length of open period and vaginitis, pneumonia, conjunctivitis, enteritis, polyarthritis and encephalitis. (Corner, 1968); (Twomey, 2003); (PRAGA-AYALA, 2014). *Chlamydia* can be shed and transmitted by almost all secretions and excretions (vaginal, ocular, and nasal fluids, semen and urine with fecal shedding being the most important route. Due to various pathogenetic peculiarities, chlamydial infections do not necessarily lead to clinical illness causing chronic inflammatory reactions and dysfunctionality of different organs. Subclinical chlamydial infections are probably economically more important than rare outbreaks of severe chlamydial disease. Generally cases become agent negative before they become serologically negative. So, serology is considered to be most important in the diagnosis of latent or chronic infection, as well as in confirming ongoing disease (Shewen, 1980). Infected animals may be a source of infection in humans and lead to severe outcomes including respiratory disorder and miscarriage (Walder, 2005); (Ortega, 2015).

Monitoring of this zoonotic pathogen in dairy cattle has become a very important issue in Nepal. Detailed reports of serosurveillance of Chlamydiosis within the national dairy cattle herd have not been conducted yet in Nepal. This research will help to assess the seroprevalence of *Chlamydia abortus* and its impact in causing anestrus in cattle of Nawalpur and Chitwan districts which are a major source of milk for Nepal. Likewise, this research will provide baseline information on the demographics and aetiology of subclinical forms of Chlamydiosis and its consequences for dairy herd fertility. So, the objective of the present study was to determine the prevalence of antibodies to *Chlamydia abortus* in anestrus and aborting dairy cattle in Nawalpur and Chitwan. The study was conducted in winter when the risk of infection is quite lower. The identification of age, breed, parity and previous history of abortion as contributory factors are all considered in the study.

MATERIALS AND METHODS

A cross sectional descriptive study on anestrus cattle of improved dairy cattle breeds (Jersey cross and Holstein cross) was carried out from November 2018 to May 2019 in Nawalpur and Chitwan districts of Nepal. Sample size was calculated using the given formula (Daniel, 1999).

$$n = Z^2 P (1-P) / d^2 \text{ where}$$

n= sample size,

Z = Z statistic for a level of confidence; at 95% level of confidence and 5% level of significance, Z value is 1.96

P= expected prevalence or proportion (in proportion of one; prevalence of Chlamydiosis in Nawalpur and Chitwan was 2.65% (0.02675). (NCRP, 2017/18)

d = Precision limit or proportion of sampling error which is 5% confidence limit (0.05)

Hence $n = (1.96)^2 * 0.02675 (1-0.02675) / (0.05)^2 = 40.005$

While the total number of samples collected was 92 (Nawalpur (n=27) and Chitwan (n=65)).

SAMPLING PROCEDURE

Purposive sampling was used. Blood samples from 92 cattle displaying signs or history of anestrus were collected from the jugular vein and transferred into 3ml clot activators. Samples were transported in icebox to the laboratory of the National Cattle Research Program. On the same day, serum was extracted by centrifugation of the clot activators 3000 rpm for 10 minutes and stored at 4°C until assay.

ELISA PROCEDURE

C. abortus-specific antibodies were determined in the sera according to the manufacturer's instructions. An indirect ELISA commercial kit The IDScreen® *Chlamydophila abortus* Indirect Multispecies ELISA from IDVet® (Montpellier, France) was used according to the instructions of the manufacturer. This assay reported a sensitivity of 100% and specificity of 99.7% (Pourquier, 2007). Briefly, 10µl each of negative control, positive control and serum samples were diluted in 90µl of dilution buffer in 92 well micro titre plates, incubated at 21°C for 45 min and plates finally washed three times by pipetting 300µl Wash Solution onto plates. For the detection of specific antibodies Conjugate 1X was added (100µl/well), plates incubated at 21 °C for 30 min, washed three times with Wash Solution (300µl/well) followed by the addition of the substrate solution (100µl/well) and incubation at 21°C for 15 min. The reaction was stopped by adding 100µl stop solution to each well. Optical densities of the samples were measured using spectrophotometry at a wavelength of 450 nm. Results were given as values (%) and calculated using the measured optical densities (OD) and the equation as follows:

$$\frac{S}{P} (\%) = \frac{OD_{450nm} \text{ test serum}}{(OD_{450nm} \text{ positive control})}$$

Sera with values $\geq 60\%$ were considered as positive for *Chlamydia abortus* antibodies. Sera with values of 50–60% were considered doubtful and $\leq 50\%$ were negative.

ESTIMATION OF TRUE PREVALENCE

If a test with less than 100% sensitivity and specificity is used to estimate prevalence of some characteristic, that estimate will invariably be biased. If the sensitivity and specificity of the test are known and if the apparent prevalence is greater than (specificity – 1), we can estimate the true prevalence with the **Rogan-Gladen estimator**

$$\text{True Prevalence} = \frac{\text{Apparent Prevalence} + (\text{Specificity} - 1)}{\text{Specificity} + (\text{Sensitivity} - 1)}$$

Where,

- The **true prevalence** is the proportion of all those which are tested and are actually positive
- The **apparent prevalence** is the proportion of those which are tested and rightly or wrongly, test positive

The iELISA used to detect *C. abortus*-specific antibodies in the present study had a sensitivity of 100% and specificity of 99.7% (Pourquier, 2007)

DATA ANALYSIS

The data analysis was conducted with the help of Microsoft Excel-10 and IBM SPSS Statistics 20 (IBM Corporation 1989, 2011). Statistical analysis of seroprevalence of *Chlamydia abortus* in dairy cattle of 2 districts was performed using IBM Statistics 20. Prevalence was studied among different age groups (less than 2 years, 2-5 years and 5 years and above), parity (1st, 2nd, 3rd 4th and 5th), history of abortion (aborted and non-aborted), and breed (Jersey cross and Holstein cross) using a chi-squared test to study the association between *Chlamydia* and different variables. The differences were considered statistically significant if $P < 0.05$.

RESULTS

Out of the total (92) samples (27: Nawalpur and 65: Chitwan) collected from the anestrus dairy cattle, 2.17% (2/92) were positive and 95.65% (88/92) were negative while 2.17% (2/92) yielded doubtful results which means weakly positive according to the kit manual for a *Chlamydia abortus* infection on Indirect Enzyme Linked Immuno-sorbent Assay (iELISA). So, the overall seroprevalence in this study is 2.17% while the overall true prevalence of *C. abortus* in our study is 2.26%.

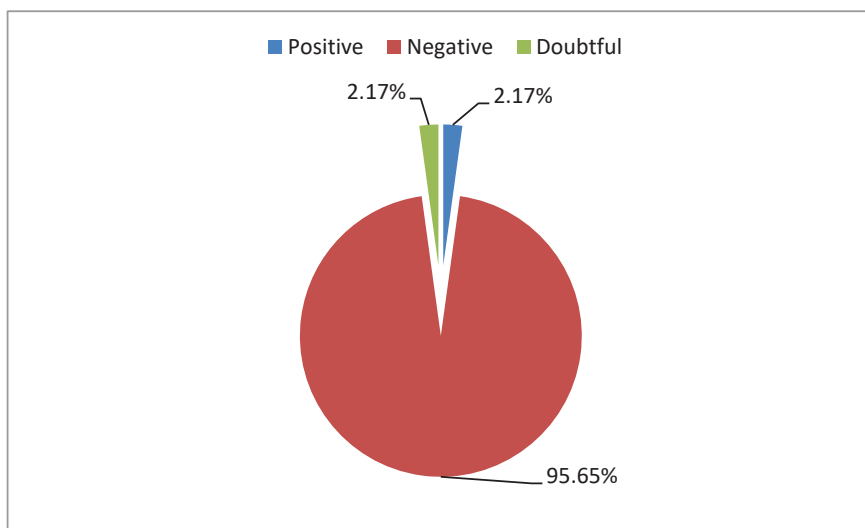


Figure 1: Overall seroprevalence of *Chlamydia abortus* detected in serum from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

District-wise seroprevalence

Among 27 cattle tested from Nawalpur, seroprevalence was (7.40%) and among 65 cattle tested from Chitwan there was no seropositivity. Some (3.08%) of the iELISA tests were inconclusive in samples collected from the District Chitwan. There was no significant difference between the district and seropositivity ($p=0.058$).

Table 1: Chi-square univariate analysis of relationships between location and the detection of chlamydia serum antibodies in serum from 92 dairy cows in Nepal (27 from district Nawalpur and 65 from district Chitwan).

Variable	Number of animals	Serum antibodies to <i>C. abortus</i>				P value
		Positive	Negative	Doubtful	Prevalence rate (%)	
District						0.058
Nawalpur	27	2	25	0	7.40	
Chitwan	65	0	63	2	0	

Age wise seroprevalence

No seroprevalence of *Chlamydia abortus* was found in dairy cattle less than 2 years old while one test was inconclusive (11.11%). Similarly, no seroprevalence was found in the age group above 5 years and above while one test was inconclusive (1/44; 2.27%). Seroprevalence was highest in the 2-5 years age group at 4.08% (2/39), although this agewise difference in seroprevalence was not significant ($p=0.139$).

Table 2: Chi-square univariate analysis of relationships between age and chlamydia serum antibodies detected in serum from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

Variables	Number of animals	Serum antibody to <i>C. abortus</i>				P value
		Positive	Negative	Doubtful	Prevalence (%)	
Age of animals						
<2 years	9	0	8	1	0	0.139
2-5 years	39	2	37	0	4.08	
≥5 years	44	0	43	1	0	

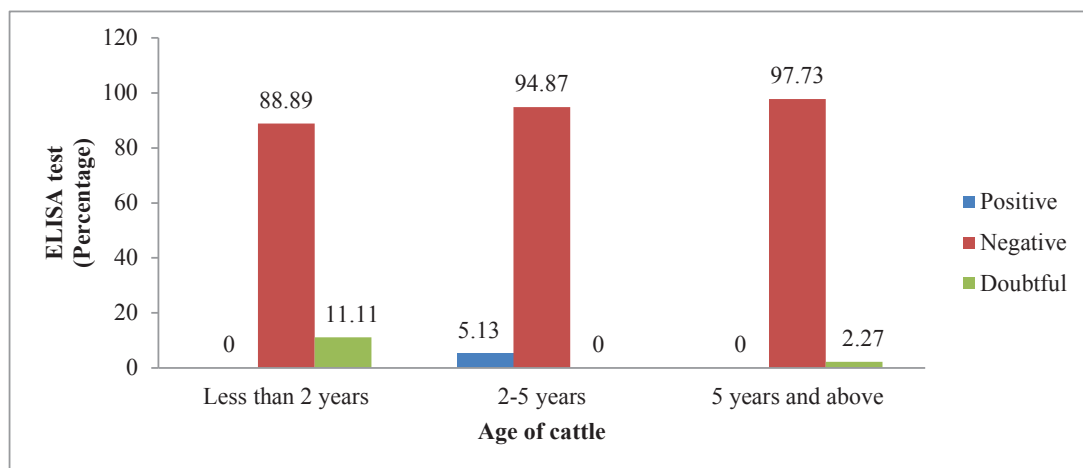


Figure 2: Age wise seroprevalence of *C. abortus* by ELISA test detected in serum from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

Abortion wise seroprevalence

Seroprevalence of *Chlamydia abortus* in cattle with a recent history of abortion was 11.11% while cattle with a normal calving and no abortions, none of them gave a positive result. Inconclusive results were observed in 2.70% (2/74) of the non-aborted cattle. The result showed that there was a significant difference in the prevalence ($p=0.012$).

Table 3: Chi-square univariate analysis of relationships between history of abortion and the expression of chlamydia serum antibodies in serum from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

Variable	N	Serum antibody to <i>C. abortus</i>			Prevalence (%)	P value
		Positive	Negative	Doubtful		
History of abortion						
Aborted	18	2	16	0	11.11	0.012*
Non-aborted	74	0	72	2	0	

*= $p<0.05$

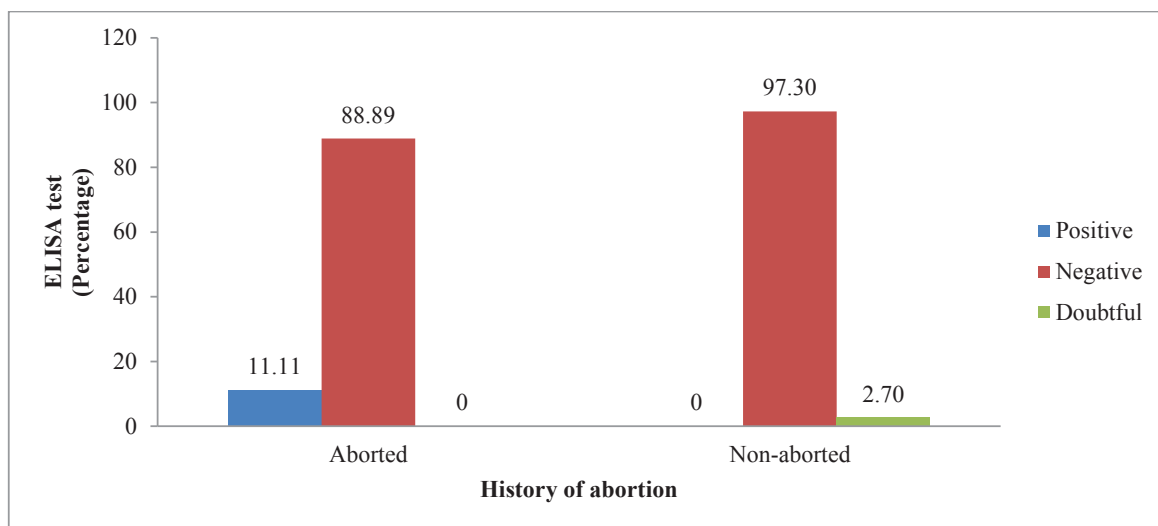


Figure 3: The relationship between the incidence of abortion and seroprevalence of *C. abortus* by ELISA test in serum from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

Breed wise seroprevalence

Out of the total of 92 cattle tested, 56 were Jersey cross and 36 were Holstein cross. Out of these seroprevalence in Jersey cross breed was found to be 3.57% while 3.57% yielded an inclusive result. Seroprevalence was completely negative in the Holstein cross breed but there was no significant difference in the prevalence ($p=0.261$).

Table 4: Chi-square univariate analysis of relationships between breed and chlamydia serum antibodies detected in serum from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

Variable	n	Serum antibody to <i>C. abortus</i>			Prevalence (%)	P value
		Positive	Negative	Doubtful		
Breed						
Jersey cross	56	2	52	2	3.57	0.261
Holstein cross	36	0	36	0	0	

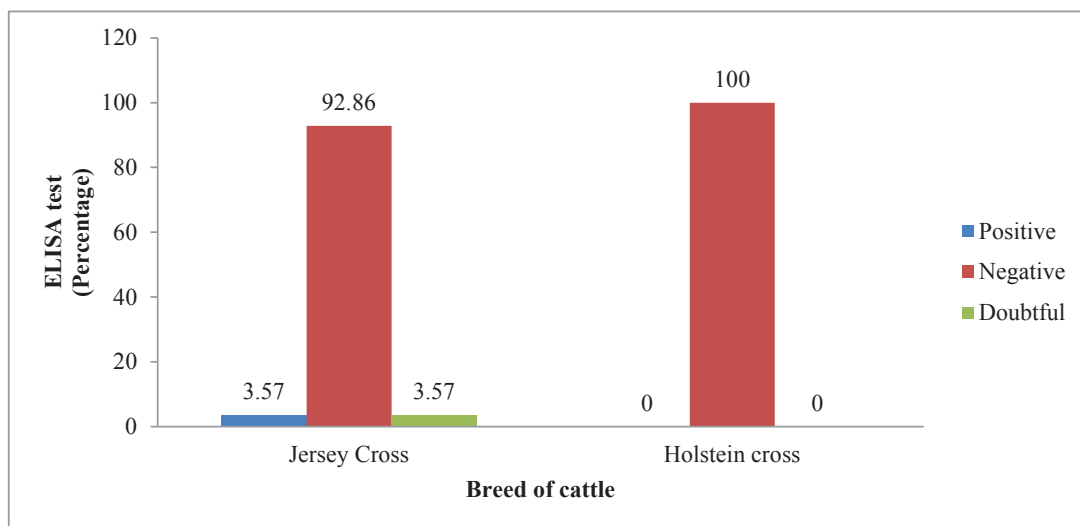


Figure 4: Breed wise seroprevalence of *C. abortus* by ELISA test in serum from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

Method of insemination wise

Out of the total of 92 cattle, 27 were serviced naturally and the remaining 65 were artificially inseminated. Seroprevalence was highest (7.40%) in naturally serviced cattle while none of the artificially inseminated cattle were seropositive to *Chlamydia abortus*. Of the artificially inseminated cattle 3.08% gave an inconclusive result to *C. abortus* antibody detection. The result showed that there was no significant difference between the two types of service used ($p=0.058$) Table 5: Chi-square univariate analysis of relationships between type of service used and chlamydia serum antibody expression from 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

Variable	n	Serum antibody to <i>C. abortus</i>			Prevalence (%)	P value
		Positive	Negative	Doubtful		
Artificial	65	0	63	2	0	0.058
Natural	27	2	25	0	7.40	

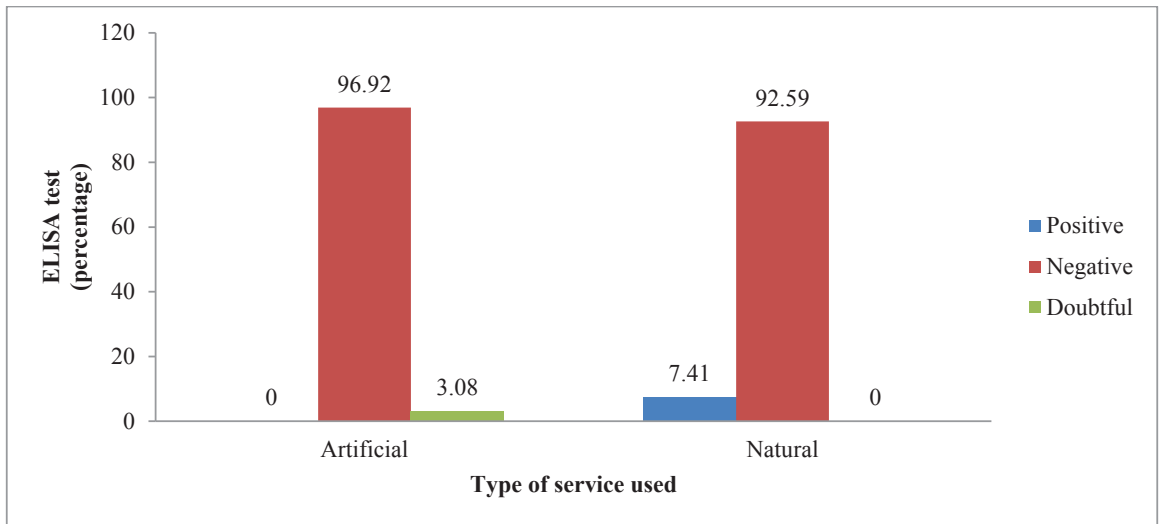


Figure 5: Seroprevalence of *C. abortus* according to type of service used detected in 92 dairy cows in Nepal (27: from district Nawalpur and 65 from district Chitwan).

DISCUSSION

The overall prevalence rate obtained from the present study exhibited a low seroprevalence (2.7%) of *C. abortus*. This suggests that this bacterial species is rarely present in dairy cattle of Nepal. A similar study conducted in Nepal by National Cattle Research Program in 2017/18 (NCRP, 2017/18) at the same study site using similar methodology has reported the overall seroprevalence of 2.65% which was slightly higher than reported here. The previous study was conducted in spring while our study was conducted in the early winter season. The disease is most commonly diagnosed in the lambing season during late winter or early spring (Essig, 2015). No evidence of vaccination against *Chlamydia abortus* in Nepal shows that seroprevalence has not been altered in association with the use of vaccination. However, occurrence of seropositive animals suggests previous contact with *C. abortus*, especially in herds in the district of Nawalpur. It has been reported that prevalence is associated with large mean herd size (Jiménez-Estrada, 2008). So, the low prevalence in our study area may be associated with the smaller mean herd size using different rearing strategies in a zero grazing system throughout the rearing period. Out of a total 92 samples tested (27: Nawalpur and 65: Chitwan), only 2 (2.17%) were positive and both of them were from Nawalpur. But the differences were not significant ($p < 0.05$) owing to the larger mean herd size in Nawalpur relative to that of Chitwan. Infected sheep and goats act as chronic carriers of *C. abortus* persistently shedding them in faeces, urine, uterine and vaginal discharge. None of the dairy cattle herds in the study area had close contact with sheep and other small ruminants to have enabled cross species transmission. In studies conducted in trachoma endemic regions in Nepal, trachoma was diagnosed in 63% of samples (serum and ocular swabs), collected from humans. These were the result of single or mixed infections caused by 5 zoonotic species of chlamydia; *C. trachomatis*, *C. psittaci*, *C. pecorum*, *C. suis* and *C. pneumonia*. Here, none of the samples yielded positive results for *C. abortus* where the test was conducted by detected by DNA Microarray Assay and by quantitative Real-Time PCR (Dean, 2013). This shows the lower prevalence of *C. abortus* species as a zoonotic pathogen in Nepal.

The iELISA used in the present study uses a synthetic antigen from a major outer-membrane protein (Momp) specific to *Chlamydia abortus*, which has high sensitivity (100%) and specificity (99.7%). This test reduces the frequency of non-specific reactions (Pourquier, 2007). It is a powerful tool to test both suspected *C. abortus* infected and healthy herds. Our findings here are consistent with a similar study carried out in Mexico by (Praga-ayala, 2014) where the total seroprevalence was found to be 0.73% using iELISA. The lower seroprevalence in Mexico was explained by lower interactions with other herds and particularly ovine herds and wild ruminants which exhibit a higher prevalence of *Chlamydia abortus*. The higher prevalence (80.7%) in placental samples by CFT in India reported by (Nanda, 1992) compared to our iELISA result can be explained by the fact that a bacteriologically positive result does not necessarily mean that the individual has developed antibodies. The active agent in the ELISA has a high affinity for reproductive tissues (Sachse K, 2009). Antigenic cross-reactivity between *C. abortus* and *C. pecorum*, which is endemic in small ruminants, as well as with some Gram-negative bacteria (e.g. *Acinetobacter*), can give rise to false-positive CF test results (OIE, 2018). A similar study conducted in Sweden among the dairy cows with reproductive disorders using rELISA showed a seroprevalence of 0.4% (Godin, 2008). The possible confounding factors for lower seroprevalence are more or less similar to our study. They are self-contained herds, little interactions with other species, little movement of animals and lower seroprevalence among the ovine population of Sweden. There are no published data on ovine seroprevalence in Nepal. We have identified a significant association ($p=0.01$) of seroprevalence of *Chlamydia abortus* with abortion in our study. Similarly there was a significant relationship between the presence of serum antibodies and a preceding abortion ($P < 0.001$) in a study carried out by (Wehrend, 2005) in dairy herds with fertility disorders in Hessen (Germany) using a genus specific ELISA. In our study prevalence of *Chlamydia abortus* was restricted to the Jersey cross cows and was not found in other breeds like Holstein Friesian crosses, local and pure breeds. This might be due to the larger sample size of the Jersey cross breed and a higher incidence of reproductive issues like mastitis, abortion and repeat breeding, among these Jersey cross cows. In a study carried out in Germany by (Kauffold J. H., 2007) prevalence of *Chlamydia abortus* was 2.5% (3/120) as detected by PCR in semen samples was reported indicating excretion of the organism in semen and possibly transmission as a venereal disease. Similarly, a higher seroprevalence (7.41%) compared to artificially inseminated cattle was found among naturally bred cattle in our study. Although the result was not significant, it might suggest possible venereal transmission from breeding bulls to cows. But prevalence of *Chlamydia abortus* among breeding bulls in Nepal is yet to be investigated.

The inconclusive results achieved by iELISA for infection with *Chlamydia abortus* may be related to a number of factors. Such results might have resulted from cross-reactivity with related antibodies using this test kit. Use of this kit has been reported to reduce non-specific reactions without eliminating them. Thus we could only test 92 serum samples. *Chlamydia abortus* is more prevalent in early spring to early summer while our study was conducted in early winter season when the risk of exposure was relatively lower. Likewise, we conducted our study for a month only while a more effective program detection would involve seromonitoring twice a year in the early and late monsoon seasons. Similarly the use of PCR technology would have provided more definitive results, however their cost prevented their use in the present study.

Since no vaccination is practiced in the study area, the seroprevalence observed was due to natural infection. The seroprevalence was higher in Jersey cattle of 3–4 years of age with recent history of

abortion. It also suggests that *Chlamydia abortus* is not related to the anestrous condition in the cattle on our study site. However the incidence of abortion is likely to be associated significantly with seroprevalence of *C. abortus* in our study. The seroprevalence was significantly higher in cattle with a recent history of abortion ($p < 0.05$).

CONCLUSION

Hence, the study suggests that there is a low seroprevalence of *Chlamydia abortus* in anestrous dairy cattle of Nawalpur and Chitwan districts of Nepal at the time of this study. We suggest that dairy cattle herd to be screened on a routine basis to rule out a possible causal relationship between the incidence of *C. abortus* infection and the prevalence of abortion. The issue would be resolved with the elimination of this pathogen from breeding herds.

Ethical statement

All animal owners provided their oral consent before the collection of the blood samples and agreed to answer the related survey questions. The cattle were sampled by a qualified veterinarian following all applicable guidelines for the care and use of animal.

Conflict of interest

The authors declare that they have no conflict of interest.

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Pattern of poultry diseases brought at Veterinary Teaching Hospital (VTH), Rampur and National Avian Disease Investigation Laboratory (NADIL), Bharatpur, Chitwan, Nepal during 2017/18

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ABSTRACT

*A study was conducted to analyse the case flow pattern of poultry diseases brought at Veterinary Teaching Hospital (VTH), AFU, Rampur and National Avian Disease Investigation Laboratory (NADIL), Bharatpur during the year 2017/2018 AD. All the poultry cases from record books of respective institutions were analyzed using MS-Excel 2016. A total of 398 and 4,132 cases were brought at VTH and NADIL respectively. At VTH, almost all cases (92.7%) were from western Chitwan, whereas at NADIL, the highest number of cases were from Western Chitwan (44.4%) followed by eastern Chitwan (25.2%), western to Chitwan (i.e. Nawalpur and Parasi) (17%) and others. In total, the highest numbers of cases were brought in April and the lowest were in October. Most of the cases were of broilers (69.8%), followed by layers (23.2%), parent stocks (3.5%), Giriraja (2.3%), and others (1%). The highest number of cases were from flock size ≤ 500 (32.8%), followed by 501-1000 (32.6%), 1001-3000 (27.7%) and >3000 (6.9%). In total, bacterial, mycotoxicity, viral, mycoplasma, metabolic, protozoal, parasitic and miscellaneous diseases were diagnosed in 43.4%, 19%, 18.6%, 13.8%, 13.6%, 1.5%, 1.3% and 3.7% poultry cases, respectively. Among bacterial diseases, *E. coli* infection was the most prevalent (27.4%) followed by *Salmonella* infection (2.4%), necrotic enteritis (1.5%) and others. Likewise, among viral cases, infectious bursal disease (IBD) was the most prevalent (12.2%) followed by low pathogenic avian influenza (2.1%), Newcastle disease (1.6%) and others. *E. coli* infection was most prevalent in summer (32.8%) followed by autumn (29.6%), winter (29.1%) and spring (28.6%). Likewise, IBD was most prevalent in summer (17.5%) followed by spring (16.6%), autumn (11.4%) and winter (8%). In conclusion, poultry cases flow at VTH were less than one-tenth of NADIL. Most of the poultry cases brought at these governmental diagnostic centers were from smaller farms. Typical seasonal pattern of major poultry diseases warrants for special measures for their prevention and control during particular seasons.*

Keywords: Case flow pattern; Flock size; Poultry disease; Season;

INTRODUCTION

Poultry business is considered an important agricultural practices in Nepal and its farming is in increasing trend (Bhattarai, 2017). This is because of easiness for rearing, less human resources, and less investment. In spite of having high growth rate of poultry production of commercial sector

(17%-18%) and 4% contribution in national GDP of Nepal, poultry consumption in Nepal is below global average which accounts just 4.1 kg chicken meat and 44 eggs annually (Nepal country poultry statistics data, 2015), whereas globally, 12 kg chicken meat per person and 153 units eggs are consumed annually (FAO, 2017). The commercial poultry farming exists in 64 districts in Nepal with 21,956 farms out of which 20,483 (93.29%) are broiler farms, 1,337 (6.09%) layer farms, 128 (0.58%) hatchery and (0.04%) Giriraja/Kuroiler farms throughout the country (Nepal country poultry statistics data, 2015).

Poultry farming is increasing in Nepal in the recent decades and Chitwan is considered as the poultry hub (Thapa, 2016) with 15,289,019 poultry number, 9,973 metric ton poultry meat and 5,879,848 units of eggs (Statistical information on Nepalese agriculture 2016/17). The marketing and transportation of chicks and adults for production and selling, and prevalence of disease pattern is changing each year. Veterinary Teaching Hospital (VTH), Rampur and National Avian Disease Investigation Laboratory (NADIL), Bharatpur are two non-private and largest poultry disease diagnostic centers in Chitwan. Thus, case flow of poultry disease in these centers may reflect the pattern of poultry diseases in and around Chitwan district. This may help to make strategic plans for prevention and control of poultry diseases in these areas. In past, case 236 cases in fiscal year 2014/2015 and 357 cases in fiscal year 2015/2016 were brought to the VTH, Rampur (Gautam, 2017) whereas at NADIL, 2,229 cases were observed from Mid-December 2014 to Mid-June 2015 (Sharma, 2015) and 1,688 cases were recorded from August 1, 2016 to January 31, 2017 (Shrestha, 2017). However recently, there have been no studies to analyse the case flow pattern at these diagnostic centers. Also, there were no comparative studies regarding case flow pattern between these two poultry disease diagnostic centers. The objective of this study was to analyse the recent case flow pattern of poultry diseases at both diagnostic centers, VTH and NADIL.

MATERIALS AND METHODS

One-year registered data of poultry diseases (April 2017 to March 2018) from VTH, Rampur and NADIL, Bharatpur were collected. The collected data were entered in MS- Excel 2016 and presented in table, bar diagram and pie chart. In NADIL, the cases were brought from Chitwan and surrounding districts such as Nawalpur, Parasi, Makwanpur, Tanahu, Gorkha, Bara, Parsa and Rautahat. Dead or sick birds were brought by the poultry producers for disease diagnosis. The diseases were mainly diagnosed through clinical signs and history of flock, postmortem lesions, rapid diagnostic test kit, plate agglutination test and cultures. Commercially available rapid test kits were used for diagnosis of Avian Influenza (AI), Newcastle disease (ND), Infectious Bursal Disease (IBD) and Infectious Bronchitis (IB). Similarly, plate agglutination tests were commonly used for detection of *Mycoplasma* and *Salmonella* cases. Cultures and antibiotic sensitivity tests were routinely used for diagnosis of suspected bacterial diseases. At VTH, the disease diagnosis was mainly through postmortem lesions and clinical signs and history of flocks. Microscopic examinations were done for suspected parasitic cases.

RESULTS

In total, the total number of poultry cases from April 2017 to March 2018 were 4,530 out of which 398 cases were from VTH Rampur and 4,132 cases were from NADIL Bharatpur.

Location-wise case flow pattern

Table 1 shows the percentage of case flow at VTH and NADIL during one year period from six different geographical locations namely Eastern Chitwan, Western Chitwan, Southern Chitwan, Midhills Area (Tanahu and Gorkha), Eastern to Chitwan (Makawanpur, Bara, Parsa and Rautahat) and Western to Chitwan (Nawalpur and Parasi). Overall, both at VTH and NADIL, maximum number of cases were brought from Western Chitwan. At VTH, almost all cases (92.7%) were from Western Chitwan, whereas at NADIL, the highest number of cases were from Western Chitwan (44.4%) followed by Eastern Chitwan (25.2%), Western to Chitwan (17%) and others.

Table 1. Location wise case flow pattern of poultry disease at VTH & NADIL.

Location	VTH	%	NADIL	%
Eastern Chitwan	5	1.9	1032	25.2
Western Chitwan	242	92.7	1812	44.4
Southern Chitwan	1	0.4	90	2.2
Mid-hills Area	1	0.4	171	4.2
Eastern to Chitwan	7	2.7	282	7
Western to Chitwan	5	1.9	692	17
Total	261	100	4079	100

Month-wise case flow pattern

Fig. 1 shows the month-wise case flow pattern of poultry diseases brought at VTH and NADIL during one year period. Overall, NADIL had quite higher number of cases than VTH throughout the year. At NADIL, the highest number of cases were brought in April and lowest were in October.

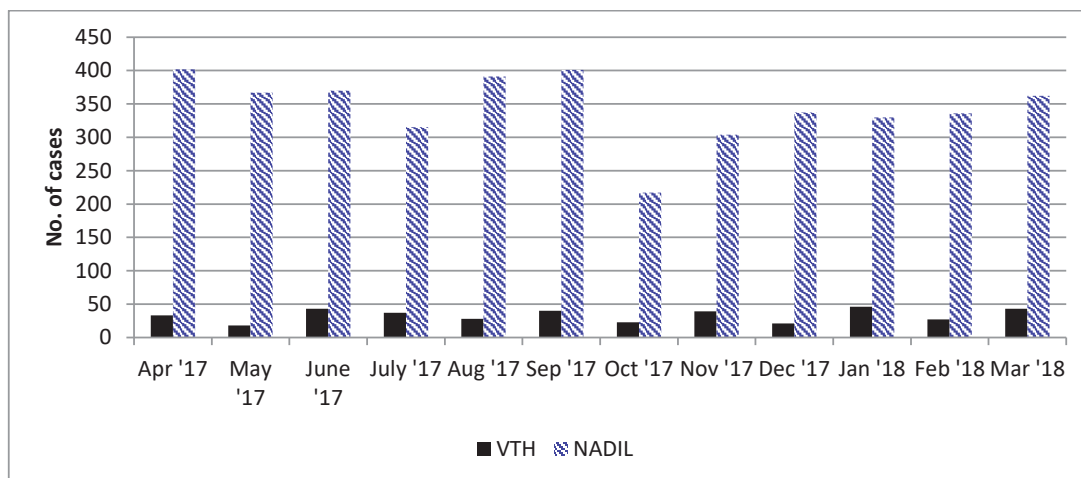


Figure 1. Month-wise poultry disease case flow pattern at VTH and NADIL

Case flow pattern according to flock size

Table 2 shows the case flow pattern of poultry diseases according to flock size at two poultry disease diagnostic centers. Overall, the highest numbers of cases were brought from small poultry farms. There were very few cases from poultry farms with a flock size over 3000 birds accounting to 7% only.

Table 2. Poultry disease case flow pattern according to flock size

Flock size (number)	VTH (%)	NADIL (%)
≤500	39	32
501-1000	31	33
1001-3000	23	28
3000>	7	7

Since the number of cases at VTH was quite a few, the data of VTH and NADIL were combined for further analyses.

Case flow pattern according to type of birds

Fig. 2 shows the case flow pattern of poultry diseases according to type of birds. The highest numbers of the cases were of broilers (69.8%) followed by layers (23.2%), parent stocks (3.5%), Giriraja (2.3%), and others (1%) including local domestic birds, duck and turkey.

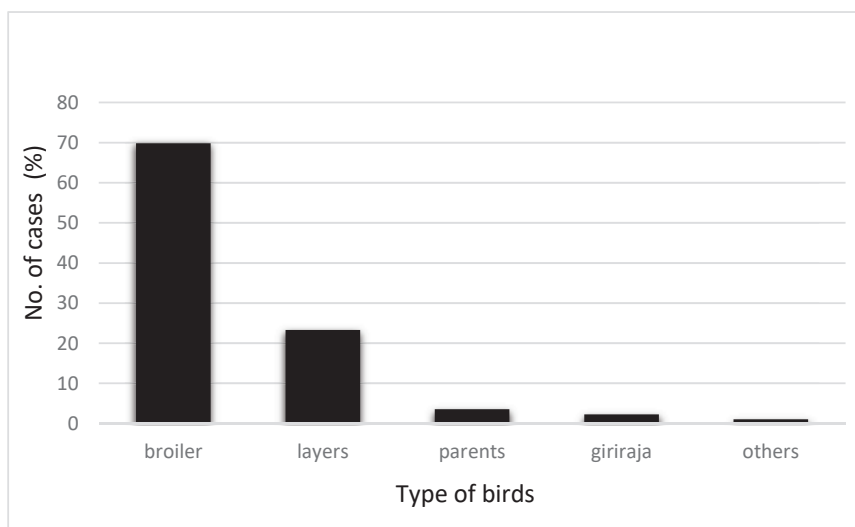


Figure 2. Case flow pattern of poultry disease according to type of birds

Age-wise case flow pattern in broiler and layers.

Fig. 3 and Fig. 4 show the case flow pattern of main commercial poultry, broiler and layers according to age at VTH and NADIL. Among broilers, the highest number of cases were from growing age (15-28 days) accounting to 46% of total broiler cases. Layers had the highest case flow at the age of laying (>18 weeks) which was 65%.

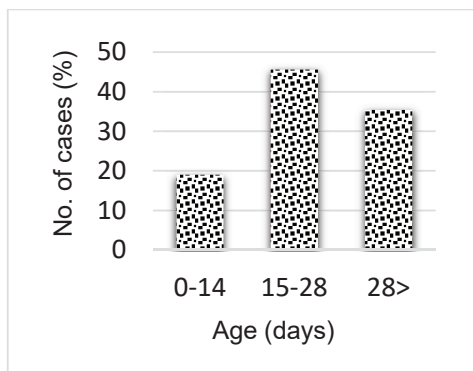


Fig. 3. Age-wise case flow pattern of broilers

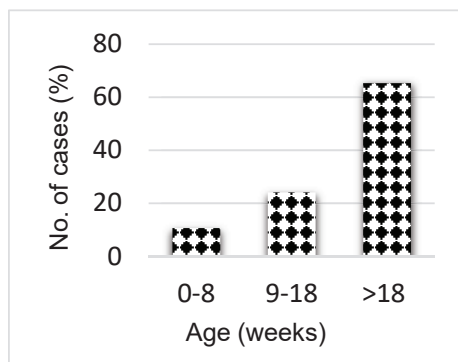


Fig. 4. Age-wise case flow pattern of layers

Etiology-wise case flow pattern

Table 3 shows the broiler cases brought at VTH and NADIL on the basis of etiological agents with or without the mixed diseases. Bacterial infections (43%) either single or in a mixed condition were the most frequent cases followed by viral infections and toxicity.

Table 3. Etiology-wise case flow pattern in broilers

Type of disease	Single disease (%)	Mixed with other (%)	Total (%)
bacterial	25	18	43
metabolic	8	6	14
mycoplasma	4	10	14
parasitic	1	<1	1
protozoan	1	1	2
toxicity	9	9	18
viral	12	7	19
miscellaneous	2	2	4

Table 4 shows the etiology-wise case flow pattern in layers. The highest number of cases were of bacterial infection (37%) followed by toxicity, viral infection, mycoplasma and metabolic diseases.

Table 4. Etiology-wise case flow pattern in layers

Type of disease	Single disease (%)	Mixed with other (%)	Total (%)
bacterial	20	17	37
metabolic	7	5	12
protozoan	<1	1	1
parasitic	<1	<1	<1
mycoplasmas	4	10	14
toxicity	9	9	18
viral	10	6	16
miscellaneous	1	1	2

Trends of various diseases in broilers and layers

Fig. 5 shows the trends of common diseases in broilers brought at VTH and NADIL during one year period of time. In commercial broilers, the *E. coli* infection (30%) was the highest encountered case followed by mycotoxycosis (20%), IBD (17%), complicated chronic respiratory disease [cCRD] (10%), chronic respiratory disease [CRD] (7%), gout (7%), ascites (6%), stress (2%), *Salmonella* infection (2%) and others.

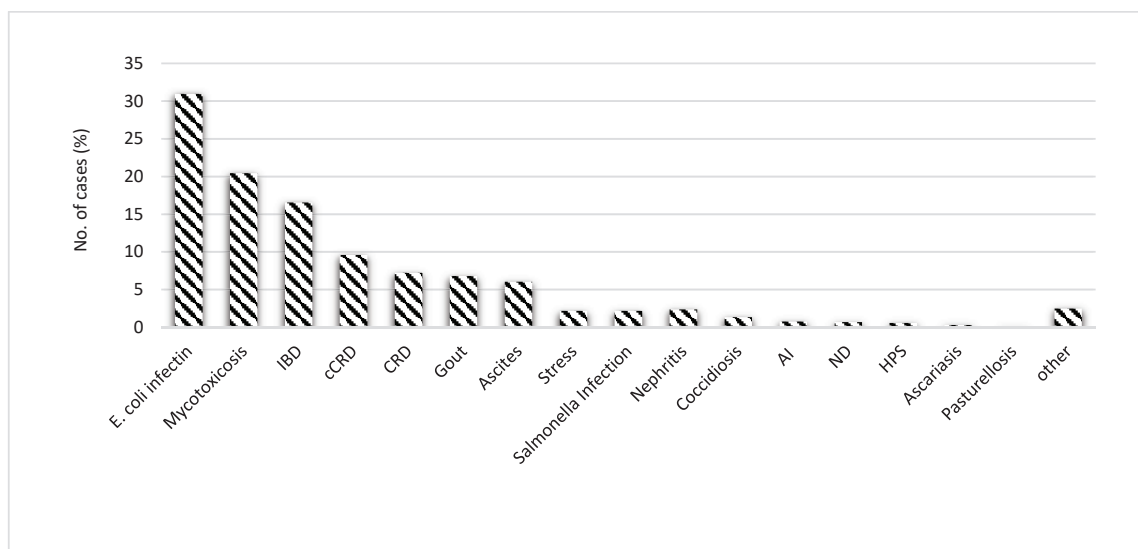


Figure 5. Trends of various diseases in broilers

Fig. 6 shows the trend of case flow of various diseases in layers at VTH and NADIL. In commercial layers, the *E. coli* infection (34%) was the highest encountered case followed by mycotoxycosis (17%), stress (9%), CRD (8%), AI (6%), IBD (6%), ND (5%), gout (4%), fowl cholera (4%), *Salmonella* infection (3%) and others.

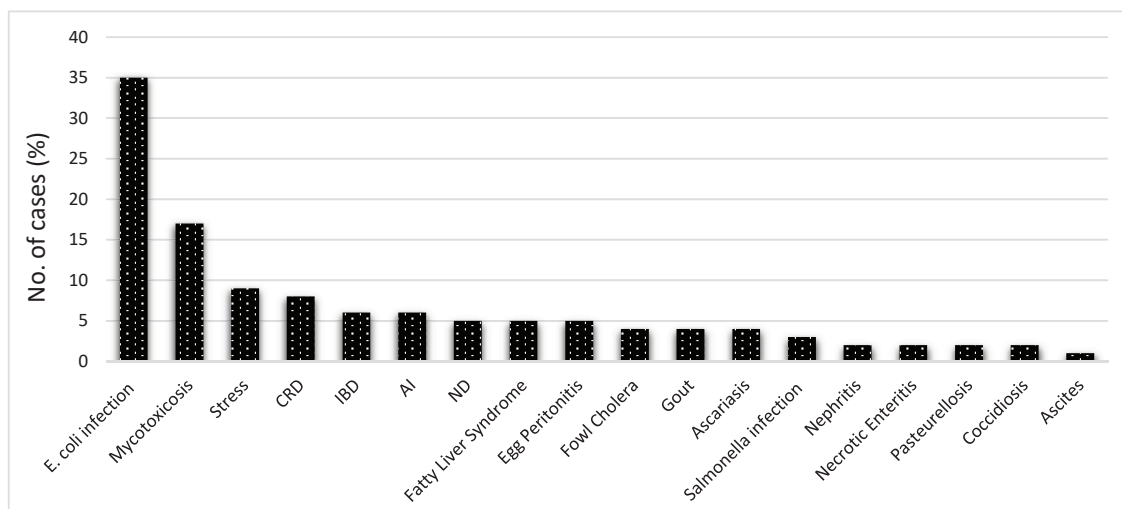


Fig. 6. Trends of various diseases in layers

Seasonal case flow pattern

Among all poultry cases, the highest number of cases were brought in spring (27%) followed by summer (26%), winter (24%) and autumn (23%).

Seasonal case flow pattern of major bacterial diseases

Table 5 shows the major bacterial diseases brought at VTH and NADIL during different seasons. *E. coli* infection was the most common bacterial case in all seasons. CRD was observed second most encountered bacterial disease and was seen more common in spring (18%) than in autumn (13%), summer (12%), and winter (14%). *Salmonella* infection, necrotic enteritis and fowl cholera cases were below 5% of total in all seasons.

Table 5. Season-wise case flow pattern of major bacterial diseases

Major bacterial diseases	Spring (%)	Autumn (%)	Summer (%)	Winter (%)
<i>E. coli</i> infection	29	30	33	29
CRD	18	13	12	14
<i>Salmonella</i> infection	2	2	4	2
Necrotic enteritis	1	2	3	1
Fowl cholera	2	2	2	1

Seasonal case flow pattern of major viral diseases

Table 6 shows the season-wise major viral case flow pattern during last one year. Among major viral diseases, IBD was the most encountered case throughout all seasons and the highest cases were observed in summer (18%) followed by spring (17%), autumn (11%) and winter (8%). AI cases were reported in three seasons: spring (7%), winter (3%) and autumn (<1%).

Table 6. Season-wise case flow pattern of major viral diseases

Major viral diseases	Spring (%)	Autumn (%)	Summer (%)	Winter (%)
IBD	17	11	18	8
AI	7	<1	0	3
ND	2	1	3	1
HPS (Hydropericardium syndrome)	<1	<1	<1	1

Seasonal case flow pattern of major metabolic diseases

Table 7 shows the seasonal case flow pattern of major metabolic diseases of poultry during last one year. Among metabolic diseases, gout was the most encountered case in autumn (12%) whereas ascites was the most prevalent during winter (13%).

Table 7. Season-wise case flow pattern of major metabolic diseases

Major metabolic diseases	Spring (%)	Autumn (%)	Summer (%)	Winter (%)
Gout	4	12	3	6
Ascites	3	2	1	13
Fatty Liver Syndrome	1	1	1	1

DISCUSSION

This study showed that the number of cases brought at VTH was around one-tenth of that brought at NADIL. This difference might be due to the availability of only few diagnostic facilities at VTH Rampur compared to that available at NADIL. In addition, the location of the laboratories might have played a role. NADIL is located at city area and possess larger case coverage whereas VTH is located 10 km away from city area. Also, these data signifies that VTH Rampur needs to upgrade their diagnostic facilities and have to advertise the facilities among farmers of surrounding area.

The number of poultry disease cases did not vary greatly among the months and seasons of the year. This result is different with the findings of Shrestha et al. (2017) who reported that higher numbers of cases were brought in spring and summer seasons compared to that in autumn and winter seasons at NADIL. Broiler birds constitutes the highest number of cases (70%) brought which is similar with Joshi et al. (2013) where out of 558 cases brought at VTH, 72.22% cases were broiler followed by layers, Giriraja, parents and others. Broilers are more popular because of high economic return in short period of time and more farmers are engaged in broiler farming as compared with layers and other birds. In addition, broilers are more susceptible to disease than layers and other species. The case flow pattern according to flock size indicates that the farmers

with small number of poultry (< 500 birds/household) often visited diagnostic centers as compared with larger flock size rearing farmers. This might be due to their low investment in business and they may not afford to bring sick birds in private clinics or cannot hire doctor and thus might have preferred to bring in these two public diagnostic centers. In addition, small scale poultry raisers are not much familiar with poultry rearing advanced tools and techniques, biosecurity and management practices. This theory is supported by Yemane et al. (2016); the farmers which are now established as a large scale producing farmer were small scale producing farmer in the past so that we can say that the inexperience or first attempt bring difficulties and problems in business too. On the other hand, there are many big poultry farms but the case flow is relatively low in comparison with small farmers. This might be because of larger poultry farmers have their own diagnostic facilities and hire private doctors to diagnose and treat their birds. In addition, the large scale producing farmers are experienced, biosecurity measures are relatively better and disease incidences might be comparatively lower.

Age of poultry and expected flock performance is considered important in poultry farming. On age-wise case flow pattern, in broilers, the highest number of cases were of 15-28 days age whereas in layers the highest number of cases were of laying stage (i.e. > 18 weeks of age). These findings are similar with the findings of Shrestha et al. (2017). In case of broiler, the reason might be due to fact that the middle age poultry can be susceptible to variety of diseases because of faster growth rate at that age which in turn would be responsible for cellulitis and various non-infectious diseases (Companion in World Farming Trust, 2005). But, in the later life of broiler people prefer to sell their birds immediately instead of treating the sick birds in most of the cases. On the other hand, high poultry case flow of layers after 18 weeks of age indicates that the layer cases near to their laying to whole laying period encounter more disease condition which is also supported by the study carried out by Uddin et al. (2011). In contrast, Bhattarai et al. (2017) reported that the higher mortality of layers were observed before the age of eight weeks and the reason behind this might be due to prevalence of Newcastle disease (ND) and chronic respiratory disease (CRD) in that area.

Bacterial diseases were more common which is similar with the finding of Shrestha et al. (2017) that analyzed the poultry disease case flow pattern at NADIL during 2016/17. In case of broiler, bacterial following toxicological, viral, mycoplasma and metabolic were major problem to those farmers who brought poultry in diagnostic centers. On the other hand, in case of layers bacterial following viral, toxicological and metabolic were major diseases. The obtained data signified that toxicity was more among broiler after bacterial whereas viral was more prevalent among layers. The mycoplasmas infection was more common in broiler than in layers. The high number of bacterial infection in poultry might be due to unhygienic practice inside the poultry farm and weak biosecurity inside the farm. More cases of broilers were suffered from mycotoxicity than the layers. This is supported by the fact that the broilers are highly susceptible to mycotoxin than the layers (Kamalzadeh A. et al., 2009; Suksombat W. et al., 2011).

Both in broilers and layers, *E. coli* infection was the most common disease. This might be due to organism's nature that is organism is naturally present in gut of birds (Pan D. et al., 2013) and if they suffer from stress or some contamination from water and feed sources the bacterial concentration increases causing symptoms (Kim J.Y., 2017). Mycotoxicosis was the second major disease both in broilers and layers. Following that, the IBD, cCRD, CRD, gout and ascites were

major diseases in broilers. In case of layers, the stress was the third most common disease/problem. Research shows that layers at the age between 24 weeks to 28 weeks are more susceptible to heat stress because of heavy body making which makes heat exchange difficult compared to younger birds and possess high metabolic rate due to peak lay and this ultimately demands more nutrient, particularly amino acids and energy generating more metabolic heat and hence more susceptible to heat stress. In addition to that, temperature higher than 19.5 °C predisposes the layers to the heat stress (Pereira D. F. et al, 2010) and the temperature is almost higher than that in our environment except during winter season. Following that, IBD, ND, Gout, *Salmonella* infection and ascariasis were major prevailing diseases in layers.

Season-wise, etiological analysis of diseases showed important information about the case flow pattern. Among bacterial disease, highest number of cases were observed in summer except for CRD, which was more common in spring. This result is different from finding of Islam A. et al., (2009), who reported that bacterial diseases were more prevalent during winter and rainy seasons as compared to that in summer season in Gaibandha district of Bangladesh. On the other hand, viral cases varied season to season. The most threatening disease, AI was seen only in winter, spring and autumn seasons. The reason behind this might be due to an outbreak in a certain region and as we know because of its high spreading capacity it was prevalent mainly during two seasons winter and spring possess cooler environment with low humidity in comparison with other seasons which favors the survival of influenza virus (Foster H., 2014). Another common viral disease, IBD was prevalent in all seasons but summer was found to be more common for this disease and this is similar with the case of ND too. Similarly, ascites and gout were most prevalent in winter. This might be possibly due to management defects in farms as there is water deprivation during winter and also, the farmers cover whole shed during nights which promotes low oxygen and thus causes ascites. Also, high energy diet for the optimal growth of chickens favoring high metabolic rate accounts ascites in poultry (Dahal G.K., 2011; Alice M., 2015).

CONCLUSION

In conclusion, poultry case flow at VTH Rampur was about one tenth of that at NADIL Bharatpur. Most of the poultry cases brought at these governmental diagnostic centers were from smaller farms. Typical seasonal pattern of major poultry diseases warrants for special measures for their prevention and control during such particular seasons.

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Isolation and Identification of Gram Negative Bacteria from Chicken Meat collected from Retail Meat Shops of Kathmandu Valley

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ABSTRACT

*Various health related issues may arise due to the microbial contamination in meat that we consume. Due to lack of proper sanitation, tools and other resources, meat usually gets contaminated with the microorganisms that are capable of causing serious illness to humans. A study was conducted to isolate and identify Gram negative bacteria in chicken meat samples collected during rainy season from different sites of Kathmandu valley. Altogether 40 samples were collected and assessed. The Total Plate Count (TPC) and Coliform Count was determined and found to be highest from Asan area i.e. 9.2 ± 0.04 log cfu/gm and 7.7 ± 0.03 log cfu/gm respectively. The further isolation was done to compare the various biochemical tests of each isolates. Altogether 40 samples were collected and assessed. Altogether ten genera of Gram negative bacteria were isolated. The isolated gram negative microorganisms were *Proteus* spp (32%,22/68), *Pseudomonas* (15%,10/68) *Citrobacter* spp. (12%,8/68), *E. coli* (13%,9/68), *Serratia marcescens* (10%,7/68), *Salmonella* spp. (7%,5/68), *Enterobacter* spp. (4%,3/68), *Morganella morganii* (3%,2/68), *Klebsiella* (2%,1/68), *Shigella* (2%,1/68). *Proteus* spp was found to be predominant. From this research, it was concluded that meat is prone to contamination. Therefore, they need to be cleaned, and cooked properly before consumption.*

Keywords: Bacteria, Biochemical tests, Isolation, Meat

INTRODUCTION

Meat and meat products are important source of selenium, phosphorus, niacin, vitamins(B₆ and B₁₂), choline, riboflavin, zinc and iron along with vitamin k (Schurgers and Vermeer, 2000; Lawrie, 2006). The most common sources of meat are domesticated animal species such as cattle, pigs and poultry and to a lesser extent buffaloes, sheep and goats (Bertol, 2004). Meat is an ideal culture medium for many organisms because it has high moisture content, source of nitrogenous food, various minerals and growth factors. It contains fermentable carbohydrates which act as a harbor for most microorganisms. The inner flesh of meat is usually sterile or few numbers of microorganisms can survive there (Aruno *et. al.*, 2007).

Chicken meat refers to whole carcasses or part of carcasses or boned out meat of *Gallus gallus*. The consumption of white meat is increasing day by day because of its popularity, low price, easy access with fast digestion, low religious taboo, tasty, and low calorie food. Therefore, the high quality of poultry meat should be maintain as they are consumed more. Majority of the spoilage microorganisms comes from external sources during unhygienic bleeding, handling, processing,

exterior part of chicken, tools and equipment, water, the area around the slaughtering, mode of transportation, hands and aprons of the meat handlers also play great role for the contamination (Sheridan, 1998; Bertol, 2004). Bacteria of many genera are found in meat, among which some of the more important are *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Micrococcus*, *Streptococcus*, *Sarcina*, *Proteus*, *Flavobacterium*, *Escherichia coli*, *Campylobacter*, *Salmonella*, *Shigella*, *Enterobacter*, and *Morganella* etc. Those bacteria including Coliforms can cause various food related infections and diseases in human (Williams *et. al.*, 2010).

As chicken meat is a diet rich in nutrition, it is also a better place for organisms and various organisms reside and grows. Also, bacteria may enter in meat at the time of slaughtering, washing or during storage. Mainly in the rainy season, there is high chance of contamination of meat because of the low sanitation, water pollution, soil pollution and environmental pollution. Meat is eaten by lots of people so the maintenance of quality in the meat is in the priority of us. So we must be careful regarding its quality. Therefore, the study will help to know the quality of meat from different parts of Kathmandu Valley.

MATERIALS AND METHODS

Sampling site and sample size

A total of 40 raw chicken meat samples were collected (purposive sampling method) from different local markets of Kathmandu valley that consists of Kathmandu, Bhaktapur and Lalitpur districts. The research was conducted on Research Laboratory for Biochemistry and Biotechnology (RLABB), Sitapaila, Kathmandu. Meat samples were collected in clean polyethene bags in the morning hours as the public were offered for meat sale at different retail meat shops located in Kathmandu valley. The samples collected were then transported to RLABB maintaining aseptic conditions, and then processed immediately. The laboratory analysis steps included all the works related with the enumeration, isolation, and identification of the isolates.

Total Plate Count (TPC) and Coliform Count (CC)

25 gm of raw meat sample was aseptically transferred into blender and blended with 225 ml of sterilized buffer peptone water (BPW) at 15000 to 20000 rpm for 2 minutes and was successively diluted to the required dilutions. Pour plating was done on two different agar mediums i.e. Plate Count Agar (PCA) for Total Plate Count and Violet Red Bile Agar (VRBA) for Coliform Count. For pour plate, 1ml of meat homogenate sample from appropriate dilutions were transferred to sterile petridish to which molten, cooled medium (PCA and VRBA) about 18-20 ml was poured and mixed gently. The petridish were then incubated at 37°C for 24 hours. Total count of organisms was performed from plate count agar (PCA) and coliform count was performed using violet red bile agar (VRBA) media, both of which were incubated at 37°C for 24 hours (Sharma and Chattopadhyay *et.al.*, 2015).

10 ml of meat homogenate sample from buffered peptone water (BPW) was enriched into 2 sterile conical flasks, each containing 90 ml of alkaline peptone water (APW) and selenite F broth (SFB). The tubes were then incubated at 37°C for 24 hours (Sharma and Chattopadhyay *et. al.*, 2015).

Isolation and identification of bacteria

A loopfull of sample from APW and SFB was streaked on Thiosulfate-citrate-bile salts- sucrose (TCBS) and Xylose Lysine Deoxycholate (XLD) agars respectively with the help of sterile inoculating loop. The petri dishes were incubated at 37°C for 24 hours. The isolated colonies from TCBS and XLD were then again inoculated on Nutrient Broth (NB) for about 4 hrs and then to Nutrient Agar (NA) and MacConkey Agar (MA) plates and further incubated at 37°C for 24 hours (Frazier and Westhoff, 1988). The bacteria were then identified using microbiological techniques as described in Bergey's manual comprising of colony morphology, Gram's staining and biochemical properties. Gram's staining was performed for the presumptive identification of bacteria according to the standard technique. The only gram negative cultures were used for the further identification. Catalase test, Oxidase test, Indole production test, Methyl red (MR) test, Voges-Proskauer (VP) test, Motility test, Citrate utilization test, Triple sugar iron (TSI) Agar test, Urea Hydrolysis test, Oxidation-Fermentation test were performed for the final identification of gram negative bacteria (Happy *et.al.*, 2018 ; Shrestha, 2009).

Data Analysis

The results were carried out in triplicates and expressed as mean±S.D. using MS Excel 13. The one way Anova was performed and the p- value was observed to be statistically significant ($p < 0.05$).

RESULTS

In the present study, altogether 40 meat samples (chicken) were collected from different places of Kathmandu valley for isolation and identification of gram negative microorganisms.

Table 1: Total Count and Coliform Count in meat

Name of districts and local area		Total samples collected	Chicken meat microbial load (log cfu/gm)	
			TPC	CC
Kathmandu	1.Asan	15	9.2±0.04	7.7±0.03
	3.New buspark		7.5±0.08	4.3±0.16
	4. Kalanki area		6.8±0.00	4.9±0.00
Bhaktapur	1. Bhaktapur Durbar square	10	8.6±0.02	6.1±0.08
	2. Old thimi area		8.4±0.08	6.2±0.00
Lalitpur	1.Patan durbar square	15	8.5±0.01	6.3±0.03
	2. Godawari area		6.1±0.03	4.3±0.09
	3. Kirtipur		8.1±0.00	3.03±0.02

All the values were expressed as mean ± standard deviation and found to be statistically significant ($p < 0.05$)

The Total Plate Count (TPC) for chicken was lowest count from Godawari area (6.1±0.03 log cfu/gm) and highest from Asan area (9.2±0.04 log cfu/gm). Likewise, the Coliform Count (CC)

was lowest count from Kirtipur (3.03 ± 0.02 log cfu/gm) and highest was from Asan (7.7 ± 0.03 log cfu/gm).

Table 2: Biochemical properties of isolated organisms

Ox	Cit	Mot	Ind	Urea	Slope	Butt	H ₂ S	Gas	Species
-	-	+	+	-	Y	Y	-	+	<i>E. coli</i>
-	-	-	+	-	R	Y	-	-	<i>Shigella</i> spp.
-	-	+	-	-	R	Y	+	-	<i>S. Typhi</i>
-	+	+	-	+	R	Y	+	+	<i>Citrobacter</i> spp.
-	+	-	-	+	Y	Y	-	+	<i>Klebsiella</i> spp.
-	+	+	-	-	Y	Y	-	+	<i>Enterobacter</i> spp.
-	+	+	+	+	R	Y	+	+	<i>Proteus</i> spp
-	-	+	+	+	R	Y	-	+	<i>Morganella morganii</i>
+	+	+	-	+	R	R	-	-	<i>Pseudomonas aeruginosa</i>
-	+	+	-	+	R	Y	-	-	<i>Serratia marcescens</i>

Key: Ox=oxidase test, Cit=Citrate test, Mot=Motility, Ind=Indole test, H₂S=Hydrogen sulphide, R=Red-Pink (alkaline reaction), Y=Yellow (acid reaction),

This research focuses on isolation and identification of gram negative bacteria. Therefore, the isolates with gram staining test negative were used for further identification. Total 68 gram negative bacterial isolates were obtained. Of these, predominant Gram negative microorganism was found to be *Proteus* spp (32%), *Pseudomonas* (15%) *Citrobacter* spp. (12%), *E. coli* (13%), *Serratia marcescens* (10%), *Salmonella* spp. (7%), *Enterobacter* spp. (4%), *Morganella morganii* (3%), *Klebsiella* (2%), *Shigella* (2%) was isolated (Figure 1).

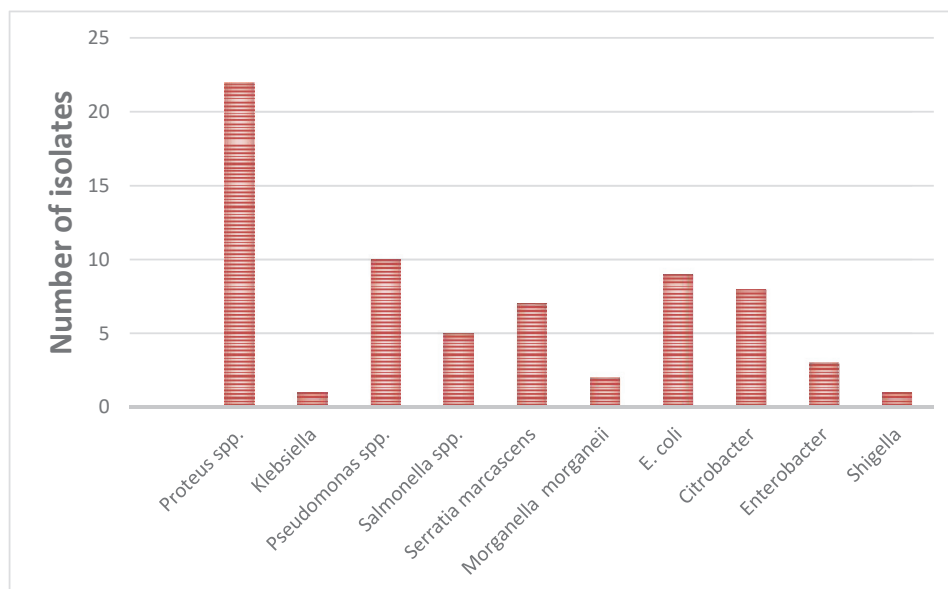


Figure 1: A bar diagram representing gram negative bacteria isolated from chicken meat

DISCUSSION

The TPC and CC was highest in the meat samples of Asan area i.e. 9.2 ± 0.04 log cfu/gm and 7.7 ± 0.03 log cfu/gm respectively. This shows that the hygiene practices were worst in Asan area compared to other areas. Therefore, the meat vendors need to be careful regarding the sanitation and hygiene. The slaughterhouse, the knives used for cutting chicken, the water, the feed for chicken, etc. should be clean and good.

A survey conducted on broiler chicken meat in different places of Chitwan i.e. Bharatpur, Ratnanagar and Institute of Agriculture and Animal Science (IAAS) vicinity were obtained as 11.1 ± 0.3 , 11.5 ± 0.3 and 12.2 ± 0.5 log cfu/gm for total plate count and 6.5 ± 0.3 , 7.6 ± 0.3 and 8.4 ± 0.5 log cfu/gm for coliform count respectively. Both total plate count and coliform count was higher compared to the result obtained by our study. Likewise, the coliform count from our study was closer to that of 5.12 log cfu /gm (Darshana et al 2014) and 5.85 log cfu /gm (Mukhopadhyay et al (2004). The TPC estimated by our research was much higher than that of TPC reported by Prasai (2000) and Poudel (1999). Prasai (2000) reported as high as 5.39 to 6.5 log cfu /gm and Poudel (1999) reported upto 7.13 to 7.7 cfu/gm. Likewise, 3.88 to 4.71 log cfu /gm and 4.69 to 6.9 log cfu /gm TPC were reported by Karki (1995) and Zobegow (2008) in chicken meat respectively.

The bacterial load may be due to contaminated feed, water used for washing the chicken and the other parameters like slaughtering houses, vehicles used for the transportation and food provided to chicken might be more contaminated and unhygienic. Bone crushes are provided to chicken which is also a reason for the high load of bacteria in chicken. A cross-sectional study of raw meat samples from the local meat market of Kathmandu, *Salmonella* spp was found in 11.4% of meat samples (Joshi *et. al.*, 2006). Likewise, Ahmed MUD *et. al.*, (2013) in his study reported that *E.*

coli was isolated from 9(45%) samples, and *Salmonella* from 7(25%) samples. The *Salmonella* may be present due to inappropriate method of storage of meat, poor handling, transportation etc. (Aftab *et. al.*, 2012; Anihouvi *et.al.* 2013).

Saika and Joshi, (2010) isolated *E.coli* by 98% from raw chicken meat samples and Odwar *et. al.*, (2014) reported 78% of the collected samples were contaminated with *E.coli*. The presence of Coliform and *E.coli* in chicken and meat product is the indication of defective techniques that is being used during preparation, handling, processing and temperature of holding, water. (Vanderlinde *et.al.*, 1998). Coliforms are present in gut of warm blooded animals and are indicator for faecal pollution and unsanitary condition of meat and meat product and their presence is the indication of enteropathogenic and toxigenic microorganisms which constitute public health hazard and economic loss (Morshidy and Roushdy, 1983). In the study conducted by Sharma and Chattopadhyay *et.al.*, (2015) the pathogenic microorganisms isolated were *E. coli*, *Staphylococcus aureus*, *Pseudomonas* spp., *Salmonella* spp., *Klebsiella pneumoniae*, *Enterococcus*, *Citrobacter* spp., *Proteus* spp. Which was similar to this study done.

CONCLUSION

The Total Plate Count and Coliform Count were done along with the isolation and identification of gram negative bacteria. All together 10 genera of Gram negative bacteria (*Salmonella*, *Escherichia*, *Shigella*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Citrobacter*, *Serratia*, *Enterobacter*, *Morganella*) were isolated, and identified. From the result of this study, it has been concluded that the meat of various places of Kathmandu valley was contaminated with health hazardous pathogens and the meat from such places was not of good quality. Meat should be cooked properly and adequately before consumption. Also the regular monitoring of quality of meat and meat market is necessary to prevent the outbreak of food borne illness caused by them as well as to prevent large economic crisis.

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Assessment of management and reproductive variability among the buffalo farms in Chitwan based on the scores of production efficiency

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ABSTRACT

Buffalo is one of the key contributors of the Nepalese farming system, mainly for milk and meat production. However due to low productivity, buffalo commercialization is getting difficult in the country. The present study was carried out to identify the management and reproductive variability among the buffalo farms based on production efficiency for assessing the constraints that affect in the production efficiency of the farms. The study was conducted from February 2019 to October 2019. A total of 50 households at Chitwan district having at least one milking buffalo were randomly selected for the study. The management, production and reproduction information were recorded with the use of questionnaire. The farms were categorized into three clusters; low efficient, medium efficient and highly efficient farm groups on the basis of production efficiency. The efficiency scores for these farms were found 0.52, 0.72, and 0.92 respectively. The education level and share to household are positively related to production efficiency; however feed consumption and cost of production are negatively related to production efficiency of buffalo farms. Similarly, management practice of deworming was associated with production efficiency. Reproductive parameters of age at first calving, calving interval, number of farmer's observation to see if a buffalo was in heat, adoption of fixed time artificial insemination protocol, and duration between heat detection and insemination were not associated with the production efficiency even though there was a tendency of association of low season breeding potentiality of buffaloes in relation to better production efficiency. In conclusion, the identified variability may be considered to increase the production efficiency of the buffalo farms.

Keywords: Buffalo; Management; Production efficiency; Reproductive; Variability

INTRODUCTION

There are 5.3 million buffaloes in Nepal which have increased by 0.58% (MOALD, 2020). Livestock farming prevails in all regions of the country, including the Mountain, Hill and Terai belts, with variations based on climate, topography, and socio-economic factors (Pradhananga et al., 2015). Buffaloes are considered as the 'black gold' due to its importance in Nepalese agricultural economy. Buffalo contributes 68.68% of the total milk and 58.30% of the total meat production in the country (MOAD, 2017). All buffaloes in Nepal are riverine type (Rasali et al 1998). Lime, Parkote and Gaddi are the major native breeds of buffalo that range from Terai belt to mid Hills of Nepal. The need for buffalo development has been identified as a driving force for food security, self-sufficiency and sustainable development (Soliman, 2008).

Milk production of Murrah and their crossbred is approximately 1500 liter/lactation which still is lower than the potential average productivity of 2300 liter/lactation (NARC, 2015). Though different breed characteristics have been described, the different production characteristics in terms of milk, meat and other outputs have not been reliably measured (Joshi, 1992). Animal production and productivity tends to be low on small, rural farms in Nepal on account of nutrition (Pant et al. 1993), disease (mainly parasitic) (Thakuri et al. 1992), genetics (Shrestha et al. 2003; Pokharel and Neopane 2006), lack of preventative care, and management practices (Shrestha et al. 2006). Thus there are opportunities for intervention leading to improved production. However, knowledge and understanding of prevailing systems is essential for improving production systems by identifying the problems and constraints that limit production (Devendra 2007). Nepalese buffalo farming is largely consisted of subsistence farming system under which farmers raise a small number of livestock on small land holdings (Pradhananga et al., 2015).

Basic information related to household, buffalo farm management and milk production, and access to animal health is still lacking. With variable data in production performance such as average lactation yield, age at first calving and calving interval, generating valid conclusions regarding the productivity of buffaloes seems difficult under various environmental and management regimes (Oil & Morel, 1985; Shrestha et al, 1988 and Joshi et al, 1992). There is a wide variability in production efficiency among buffalo farms (Shrestha et al, 2019). Efficient farm productivity is directly linked to farm economy. Factors affecting the poor reproductive efficiency of Nepalese buffaloes haven't been properly documented yet. This is probably the first research of its kind focused on identifying the factors that help to increase the productivity of buffaloes of Nepal by looking at the variability in production efficiency (PE) among buffalo farms and attempts to identify the constraints that render low efficiency.

MATERIALS AND METHODOLOGY

Data collection

A randomized survey was conducted from February to May of 2019 through face-to-face interviews with 50 farming households in different villages of Chitwan namely Bijaynagar, Madi, Jagatpur and Divyanagar. Each of the sampled household had at least 1 milching buffalo. Most of the households had adopted similar type of feeding and management practices. Buffaloes were reared in 24-hour tie-stall with limited access to grazing. It used a structured questionnaire to collect farm level physical, demographic, financial, management and reproduction information as well as farmers' intentions and perceptions of buffalo production. Characteristics such as age at first calving and calving interval were used to measure reproductive performance. Characteristics such as lactation yield, dry period and daily milk yield were used to measure productive performance of different buffaloes. Socioeconomic characteristics included age, sex, education, experience in buffalo farming of respondents and income from buffalo farming. Households were asked about visual, cow behavior and bull behavior under the observation of signs of estrus in their buffaloes. Interval between heat detection and insemination was grouped into two types i.e insemination upto 12 hours after heat detection and next is 12 hours or more after heat detection.

Calculation of net profitability

After completion of field survey, all the variables of the interview were entered in MS Excel to facilitate tabulation. Income from annual farming operations was obtained by summing up the returns from annual sale of buffalo products (milk sales), annual sale of live calf (animal sales) as by product. We only included milk revenues in this study as that is the main contributor of income on these farms. The farms only sell buffalo when they become unproductive. This is considered when replacement costs were included. Both physical and financial variables are included in the data. The total cost of each farm operation was based on variable costs. The variable costs included in this study are feed cost, labour cost, veterinary cost and breeding cost. The expenses made for medicines; deworming, vaccination and prevention come under veterinary costs. Insemination of buffalo either by bull or artificial means is the breeding expenses. Number of insemination depends upon the skills and success of insemination. Other inputs are calculated as the real value of total expenditures on fuel, machinery/building repairs, utilities and miscellaneous expenses, interest paid, taxes, water, electricity, transport and insurance payments. Following formula was used to calculate the profitability of each farm.

Net Profitability,

$$\pi = TR - TC$$

Where,

TR= Total milk produced per dairy buffalo per lactation multiplied by per liter buffalo milk price of the study area

TC= Sum of all needed costs of inputs for buffalo rearing

Farm clustering and calculation of PE

Farms were grouped using a cluster analysis technique, K-Means technique, in STATA 12.1 (www.stata.com). The K-Means technique was present to three groupings for the analysis and it generated three different means based on PE scores within the farm data. Each of the 50 farms was grouped separately using the Euclidean distance method which determined minimum distance between the group mean and the farm data. The three farm groups thus generated were categorized as; i) low efficient farm group; ii) medium efficient farm group and iii) high efficient farm group on account of the PE scores, farm size and production. Data envelope analysis (DEA) assigns an efficiency score ranging from 0 to 1 to each DMU in the analysis group and can be used to determine how inputs and outputs should be adjusted to obtain DEA efficiency (Stokes et al, 2007).

Efficiency score, $\hat{e}_x = (px / revx) / \hat{e}_{max}$

Where px = profit of farm x ; $revx$ = total revenues of farm x and $\hat{e}_{max}$ = maximum efficiency among farms in the sample.

Management and reproductive variability

In technical adaptations; use of technology like artificial insemination (AI), fixed time AI (FTAI), deworming practices, vitamin and mineral supplementation and vaccination practices and in breeding practices; adoption of pregnancy diagnosis, number of observations made to examine whether the buffaloes are in heat, breeding season and duration between heat detection and

insemination were included under management aspects. The productive performances analyzed were daily milk yield and number of dry months and the reproductive performances studied were age at first calving and calving interval of buffaloes of different farm groups.

Statistical analysis

Descriptive statistics included frequency to investigate socio-economic characteristics of the respondents. A multivariate regression was used to determine the factors affecting the efficiency of buffalo farms. Education level, experience in buffalo farming, daily milk yield, total cost of production, farm size, share to household, feed consumption and number of dry months are the variables used in this study as regressors in the regression equations. The model can be formulated as the following equation (Agha et al., 2012; Anas et al., 2013; Akan et al., 2015).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n + \varepsilon$$

Where, Y = Annual income (Rs.) from buffalo farming

β_0 = intercept of the regression equation

X_1 = education level of respondent

X_2 = experience of household head

X_3 = daily milk yield of the farm

X_4 = farm total milk production

X_5 = total cost of production

X_6 = farm size

X_7 = share to household

X_8 = feed consumption

X_9 = number of dry months

ε = error term

Similarly, the non-parametric data were analyzed by chi-square test and mean value was analyzed by one way ANOVA using STATA 12.1. Chi squared test was used to show the degree of association between efficient farms and technical adaptations adopted by farmers. Chi square test was also utilized to analyze parameters of breeding practices. One way ANOVA was used to detect significant differences ($P < 0.05$) existed in a group of data in case of analysis of productive and reproductive traits. The statistical analysis was set at 95% of confidence where $P\text{-value} < 0.05$ was considered as significant, and $0.05 > P \leq 0.1$ was considered to have tendency.

Where,

Gross return = Sum of gross return from milk, manure and calf

Total variable cost = Sum of cost of all variable ite

RESULTS AND DISUSSION

The result reveals that the education level and share of income from buffalo farming to household are positively related to PE, however feed consumption and cost of production are negatively related to production efficiency. The low efficient farm groups have lowest share to household income. Similarly, the cost of production incurred in highly efficient farm group is lower than that

of low efficient and medium efficient farm group. The last variable (Cons) represents the constant which is the predicted value of production efficiency when all other variables are zero.

Among the socio-economic characteristics, education was found to influence significantly on PE of the farms. It is evident that the more number of farmers of highly efficient farm group had attained tertiary education in our study. Similar finding was reported by Timsina (2010) in large farm category in Phulbari village of Chitwan. This might be due to more awareness about education and more household income in large category as compared to other categories of households. The results from the study of farmer education and productivity in Nepal agriculture also suggest that the increase in conventional input and investment in education will substantially improve agricultural productivity (Hu Bai and Nainabasti A, 2008). Pudasaini (1979) also reported the higher contribution of college education's in Bara district of Nepal. This indicates that higher education has a significant role in a modernizing environment where new inputs are continually introduced and where economic disequilibria arise as a result of changing technology, perhaps because higher education enables farmers to analyze, understand, and introduce new inputs in modernizing environment (Schultz, 1975).

Table 1: Farm clustering

Farm clusters	No. of households	Average PE
Low efficient	9	0.52
Medium efficient	20	0.72
High efficient	21	0.91

Table 2: Income from buffalo per month farming in different farm groups

Farm group	Mean	Std. deviation	Frequency
Low efficient	15885.83	14615.412	9
Medium efficient	14207.5	14333.64	20
Highly efficient	31619.048	20675.562	21
TOTAL	21822.45	18981.436	50

Table 3: Multivariate regression estimates of factors affecting production efficiency in different farm categories

Variable	Regression coefficient	p> t
Education	.0577673	0.010*
Experience	.0019849	0.955
Milk yield	-.000022	0.646
Total milk production of the farm	.000014	0.099
Total cost of production	-.0000206	0.000*
Farm size	-.0140922	0.826
Share to household	.2115662	0.005*
Feed consumption	-.0002246	0.050*
Number of dry months	-.0263149	0.479
Cons	.8686771	0.000

*indicates significance level at 5%

Table 4: Education level of farmers of different groups

Education level	Low efficient	Medium efficient	Highly efficient
Primary	4	11	10
Secondary	5	8	6
Tertiary	0	1	5

The cost of milk production is largely shared by labour and fodder. Labour accounts for about 70-80 percent of the total cost and fodder (green and dry) accounts for 15% to 21% on different farm categories (Ghulam et al, 2004). The lower cost of production in highly efficient farm groups is mainly due to controlled amount of feed ($p<0.001$) offered to the buffaloes. Ghulam et al (2004) had also shown higher increase in gross revenues of large farms with respect to small farms. The higher cost of production in low efficient farms might be due to small number of animal units and lower milk in young stocks. The highly efficient farm are cost efficient than the low efficient farms. Farmers of low efficient farm groups are found spending higher cost in market and electricity than their highly efficient farm counterparts. This might be due to the fact that less bargaining power of small dairy farmers, this is due to unorganized or scattered markets they faced (Diru et al, 2019). Moreover, small dairy farmers disburse 3% more mean feed cost per cow per year than their large counterparts. This might be due to the economics of scale (Diru et al, 2019). The more number of services per conception in low and medium efficient farm groups have increased the cost of rearing animal and decreases profitability (Tarabany et al, 2015).

Feed consumption is another important factor that negatively influences the PE. Majority of the farmers of highly efficient farm group made provision of grasses and legumes to their buffaloes. This might have promoted consumption of quality roughages thus improving the efficiency of feeding at lower cost resulting in low milk production cost (NPEC, 2014). The poor plane of nutrition and deficiency of green fodder and forage may be responsible behind poor breeding efficiency in low economic group. A strong association of true anestrus to poor body condition score (BCS) was observed in Nepalese buffaloes suggesting the effects of dry weather resulting shortage of feed and fodder availability during winter and spring months might have played a major role in causing higher incidence of true anestrus in the winter and spring months (Devkota et al., 2012). The low share of buffalo income to household income of low efficient farm group might be due to consumption of milk more for home purposes rather than selling through cooperatives or at local market.

Table 5. Farm level reproduction and production data of different farm group

Parameters	Low Efficient	Medium Efficient	Highly Efficient	p-value
No. of dry months	2.44±0.53	2.5±0.51	2.42±0.60	0.788
AFC (years)	3.5±0.35	3.23±0.34	3.37±0.33	0.983
CI (months)	13±0.87	12.85±0.59	12.61±0.86	0.216

DMY (L)	5.69±1.26	5.54±1.26	5.60±1.30	0.987
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Table 6. Farm level adoption of some reproductive and management practices

Technical intervention	Farm Group			p-value
	Low Efficient	Medium Efficient	Highly Efficient	
Use of AI	0	4	5	0.285
Use of FTAI	0	1	4	0.177
Deworming practices	6	20	21	0.001**
Practice of vitamin-mineral premix	3	4	7	0.589
Practice of vaccination	3	12	16	0.083*

The higher practice of deworming in highly efficient farm group might be an effort to lower the incidence of parasitism in their buffalo herds. The pasture contaminated due to feces of affected buffaloes and close confinement/interaction of buffaloes within a dairy shed also enhance the multiplication of parasites in low efficient farm group where herd size is greater (Sharif et al, 2014). Parasitism results reduced feed intake thereby decreasing the efficiency in feed utilization (Akanda et al, 2014). The extra expenses in treating secondary diseases probably because of parasitism might have increased the cost against its treatment. Gastrointestinal parasites decrease the growth rate of growing dairy buffalo calves, causes anemia, general weakness and debility, reduce the resistance to other secondary diseases and cause complications (Sharif et al, 2014). The adoption of vaccination practice in low and medium efficient farm might be attributable to poor infrastructure and farmer's perception towards vaccination that vaccine administration could lead to reduced milk yield, swelling and fever. There was frequent contact of farmers of highly efficient farm group with extension services. Knowledge and awareness of farmers of that group about possible economic losses due to reduced performance and sudden outbreak of diseases was good against other groups. Very few farmers of these groups had proper access to market and veterinary drugs retailer. Singh et. al. (2018) obtained best BCR in vaccination of both the adult and young population at the beginning of the program followed by an annual vaccination of the replacement calves.

Table 7. Breeding management practices adopted by buffaloes farmers

Practice	Category	Low Efficient	Medium Efficient	Highly Efficient	p-value
PD	Yes	2	5	11	0.12
	No	7	15	10	
Number of observation to see in heat	Once a day	6	14	2	0.588
	Twice a day	1	2	6	
	3 and above	2	4	8	
Breeding season	Active	9	15	13	0.092
	Low	0	5	8	
Duration between heat	Less than 12 hours	6	8	7	0.231

detection and insemination	12 hours and above	3	12	14
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The comparatively higher breeding efficiency in highly efficient farm group is mainly due to good level of training on heat detection in that group. In the buffaloes in Southern Nepal, silent estrus was a common problem round-the-year, although its higher incidence was observed during low breeding month (Devkota et al, 2012). The pregnancy rate is very poor despite of high ovulation rate in low breeding season (Devkota et al 2009). The awareness of farmers of this group to provide vitamin supplementation and improved concentrated feeds during low breeding season might have enhanced the breeding efficiency of buffaloes.

CONCLUSION

The education level and income from buffalo to household are highly significant to increase production efficiency; however feed consumption and cost of production significantly decrease the production efficiency. Similarly, management practice of deworming was associated with production efficiency. The highest income from buffalo farming in highly efficient farm group suggests that more income can be generated if cost of production is minimized. Adoption of novel reproductive technologies like FTAI and low season breeding protocol seem to be difficult at current level due to poor knowledge of farmers. It seems imperative to conduct further research and studies on more number of sample sizes, include different regions and production scales and focus on breed effect and ultimately expanding to pocket areas of buffalo in Nepal.

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Methanolic extract of sikari laharo (*Periploca calophylla*) has analgesic properties

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ABSTRACT

Pain is a manifestation of adverse pathophysiology during various clinical presentations. *Periploca calophylla* is a herbal plant used traditionally for multiple malaises in Nepal. However, studies on the analgesic property of this plant are scanty except empirical evidence reported by the traditional healers. This study aimed to determine the analgesic property of this plant, which is widely used for multiple conditions. Adult albino mice animal model was used for the in vivo assessment of the analgesic property. Three different doses of 80 % methanolic extract of the vine of *P. calophylla* (1.5 mg/kg, 2 mg/kg and 2.5 mg/kg), were administered intra-peritoneally to the test groups, once. The positive control group received indomethacin (25 mg/kg) and a negative control group received distilled water (3 ml/kg), via the same route of administration. The analgesic property was evaluated by the hot-plate test method, tail-flick test, acetic acid-induced writhing test and formalin-induced hind paw licking test. Extract of *P. calophylla* (1.5 mg/kg bd weight) significantly ($p < 0.01$) inhibited pain sensation in all the pain reduction evaluation of animal models. The data obtained from this study indicate that the phyto-extract of *P. calophylla* possessed the analgesic property which rightly corroborated its traditional use.

Keywords: *Periploca calophylla*, methanolic extract, analgesic, pain, animal

INTRODUCTION

The International Association for Study of Pain defines pain, as ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Tadiwos *et al.*, 2017). The substance which alleviates painful sensation by elevating the threshold to external stimuli is defined as an analgesic (Khanum *et al.*, 2019). Non-steroidal anti-inflammatory drugs (NSAIDs), opioid analgesics and co-analgesics such as corticosteroids, neuroleptics, benzodiazepines, local anaesthetics, capsaicin and clonidine are the commonly used analgesics (Sriraj *et al.*, 2018). Regular use of NSAIDs causes adverse side effects such as inflammation of gastrointestinal tract, renal failure and liver toxicity (Khanum *et al.*, 2019). Studies have shown that opiate analgesics cause physical dependence, tolerance and addiction (Hijazi *et al.*, 2017). New analgesic

compounds would be beneficial as most analgesics drugs available in the market possess multiple limitations such as efficacy and undesired effects, which constraints their usefulness (Tadiwos *et al.*, 2017). Phytoactive natural compounds from plants have been used in complementary and alternative medicine (CAM) to treat pain as well as other diseases that exacerbate pain since time immemorial. Traditional herbal medicine, the knowledge of which is handed down from generation to generation has been used for over centuries and has a strong potential for alternative medicines. Herbal medicines are a better choice due to their enhanced absorption rate, less toxicity and availability. It is noteworthy that most of the synthetic analgesic drugs such as aspirin and morphine are derived from plant products (Koech *et al.*, 2017). Cragg *et al.*, 1997 mention that approximately 70% of the analgesic compounds are synthetic drugs and most common ones like aspirin were also prototyped from the phytoactive chemicals. This emphasizes the importance and needs of studies on plants believed to have analgesic phytoactive natural compounds or used for such purposes (Nandani *et al.*, 2018).

The usage of herbal medicine is gradually gaining recognition and momentum worldwide because these products have minimal or no side effects and are relatively cheaper than chemical compounds (Koech *et al.*, 2017). *Periploca calophylla*, (Wight) Falconer of family *Asclepiadaceae* is a trailing shrub having lanceolate, long acuminate, leathery and shiny stalked leaves, 3.5-8.5 cm long and 0.3-1.7 cm wide. Flowers are pinkish in lax cymes and fruit is cylindrical. It flowers in April-May and fruits in November-January. It propagates by seeds and is distributed in shady places around the altitude of 1500-2000 meters above sea level throughout Nepal as well as northern India, Bhutan, Tibet and Central West China. Throughout the altitude range of 500-900 masl in Nepal, the *P. calophylla* (locally named as Sikari laharo) is one of the commonly used plants for treating multiple ailments (Bhujju 2005). In Nepal, the paste made from whole-plant of *P. calophylla* is used to treat bone fractures and muscle pains (Aryal *et al.* 2016). The objective of this study was to evaluate the analgesic properties of the stated plant. Despite its widespread usage, the evidence of proof is missing so this study would help evaluate the scientific merits of this empirical evidence.

MATERIALS AND METHODS

Collection and identification of the test plant

Vine along with bark of locally available Sikarilahara (literally translates as hunter vines in English) was collected from Ghalegaun of Lamjung district at 1800 meters above sea level in Nepal. A specimen was submitted to National Herbarium, Godawari, Lalitpur, Nepal for species identification. It was identified as *Periploca calophylla* by the taxonomists (Ref No 076/77, Dispatch No: 142, National Herbarium and Plant Laboratory, Godavari, Lalitpur, Nepal).

Methanol extraction

The collected plant parts were washed with clean water and air-dried in shade, powdered and soaked in 80 % methanol for 48 hours in a capped conical flask inside a rotatory incubator. The plant-methanol mixture was sieved by using a double layer of fine muslin cloth and Whatmann® filter paper no. 1. The filtrate was evaporated dry at 55°C in a rotary evaporator to get the methanolic extract. The extract was used as a trial drug for the downstream applications.

Animal experiments

Healthy male Swiss albino mice, with an individual weight of about 25-30 grams were used in this study. These animals were maintained as recommended by Acharya *et al.*, (2019) but with slight modifications. They were housed at 23-25⁰ C at a relative humidity of 60-70% with 12 hours of alternating light and dark cycle in standard-sized plastic cages at the lab animal house, Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Chitwan, Nepal. They were fed *ad libitum* on a commercial pellet diet and sterile filtered water. All the animal experiments were approved by the Animal Ethical Committee, Agriculture and Forestry University, Rampur, Chitwan, Nepal.

Experimental design

The animal model experiments were carried out in a completely randomized design (CRD). Experimental animals were randomly divided into three different treatment groups with five animals in each group and one positive and negative control groups with the same number of mice. Five replications were made for each treatment regimen. The animal equivalent dose was calculated based on body surface area. Animal equivalent doses (mg/kg) for rat and mouse were calculated by multiplying human equivalent dose as recommended by the traditional healers (mg/kg) by factor 6.2 and 12.3 respectively (Nair and Jacob, 2016). The extract of *P. calophylla* (*Pc*) was tested at doses of 1.5, 2.0 and 2.5 mg/kg given intraperitoneally (I.P), respectively. Indomethacin at the rate of 25 mg/kg, I.P. was used as the positive control, while only distilled water (3 ml/kg, I.P.) was used for the negative control. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) used as a prescription drug to reduce fever, pain, stiffness, and swelling due to inflammation. It inhibits the production of prostaglandins, (which cause the inflammatory responses) by inhibiting cyclooxygenase.

Evaluation of analgesic activity

Hot-plate test

The ‘hot-plate’ (thermal) analgesic test method using an analgesiometer (with adjustable temperature), described in an earlier publication by Acharya *et al.* (2019) was implemented with slight modification. The hot-plate was maintained at 55±0.5⁰C and individual mouse’s response like the licking of the forepaws and inclination to jump out of the beaker was noted by placing the animal in a beaker on top of the hot plate. The animal’s response as shown by jumping out of the beaker was taken as an indicator of pain. The time taken for each mouse to jump out of the beaker (in seconds, referred to as reaction time) was recorded. Each mouse was taken as its control. Pre-treatment reaction time was estimated twice at an hourly interval. The mean of these two determinations constituted the ‘pre-treatment reaction time’. The mean reaction times of all the mice in the experiment were pooled to obtain the final, ‘control’ mean reaction time (T_c). Each test mice were then treated intra-peritoneal (I.P.) with distilled water, different doses of plant extract or indomethacin. The reaction time was again evaluated once, twenty minutes post-I/P injection. This reaction time was pooled for all the mice used in each treatment group, and the final ‘test’ mean reaction time value (T_t) for each treatment group was calculated to represent ‘post-

treatment reaction time' for each group of treated mice. This Tt was finally used to determine the percentage of thermal pain relief for protection using the formula,
 Percentage protection against thermal pain = $[(\text{Test mean, (Tt)} - \text{Control Mean, (Tc)}) \times 100 / \text{Control Mean, (Tc)}]$

Tail-flick test

Mice were held firmly and the tail was extended out. The tail end (approx. 5 cm) was then dipped into a hot water bath maintained at $55 \pm 0.5^\circ\text{C}$. The time (in seconds) taken for withdrawal of the tail out of the water was recorded as the reaction time. The readings were taken after one, two, three and four hours of treatment as suggested by Palanichamy and Nagarajan, 1990; using the formula,

Percentage analgesic activity = $(\text{Tt} - \text{Tc}) \times 100 / \text{Tc}$, where, Tc, the average of the two values is the initial reaction time and Tt is the reaction time after the treatment.

Acetic acid-induced writhing test

This method recommended by Acharya *et.al.* 2019 was used to access the visceral pain. Intraperitoneal injection of 0.6% acetic acid at a dose of 0.1 ml/10 gram body weight elicits the writhing response. Visible abdominal constriction and total extension of the hind limbs indicates a positive writhing response. Test drugs were administered one hour before acetic acid injection after which the number of positive writhes was recorded for 20 min. The percentage of analgesic activity was calculated as,

Percentage analgesic activity = $(\text{N} - \text{N1}) \times 100 / \text{N}$, where, N represents the average number of the writhes of the control group and N1 is the average number of the writhing of the test group.

Formalin induced hind-paw licking test

The formalin test has two distinctive phases, indicating different types of pain. In this study, the method proposed by Hunskaar and Hole (1987) and Hossain *et al.*, (2015) was used with some modification. Mice were injected intra-peritoneally with treatment doses of the plant extract and after thirty minutes, 20 μl of 1% formalin was injected intra-dermally into the dorsal surface of the right hind paw. Mice were kept in the individual chambers and observed. The time (herein referred to as licking time) spent in licking the injected paw was recorded. Mice were observed immediately after treatment for five minutes (early phase) and at 20 minutes after treatment (for 10 minutes; late phase). The inhibition of licking, in percentage, was calculated based on,

Mean vehicle-treated group time – Mean drug-treated group time $\times 100 / \text{mean vehicle-treated group time}$

Data analysis

Data collection, management and analysis was done using Microsoft Office Excel 2010 and M-STAT version 3C. Descriptive statistical analysis was done using one way ANOVA. Data obtained from experiments were expressed as mean standard deviation ($\pm\text{SD}$). $P < 0.05$ were considered significant.

RESULTS

Hot plate test

Inhibition percentage of pain in treatment groups in comparison to the control group of the same hour was statistically significant (Table 1). Inhibition percentage of pain in the group treated with indomethacin was statistically non-significant with the group treated with *Pc* 1.5 mg/kg but statistically significant with the groups treated with other doses of *Pc* at all the hours. Similarly, inhibition percentage of pain in the group treated with *Pc* 1.5 mg/kg was statistically non-significant with the group treated with other doses of *Pc* at 1, 3 and 4 hours. But, at 2 hours, inhibition percentage of the pain of the group treated with *Pc* 2.5 mg/kg was statistically non-significant with the group treated with other doses of *Pc*.

Table 1. Effect of *P. calophylla* (*Pc*) extract on inhibition percentage of pain in hot plate test

Treatments	Inhibition percentage			
	1 (hr)	2 (hr)	3 (hr)	4 (hr)
<i>Pc</i> (1.5 mg/kg)	85.39 ^{ab} ± 5.87	93.09 ^{ab} ± 3.10	86.05 ^{ab} ± 2.45	85.96 ^{ab} ± 3.37
<i>Pc</i> (2.0 mg/kg)	83.38 ^b ± 6.21	89.14 ^b ± 6.21	84.16 ^b ± 3.96	83.87 ^b ± 1.48
<i>Pc</i> (2.5 mg/kg)	80.35 ^b ± 3.14	84.20 ^c ± 3.14	82.03 ^b ± 4.50	82.02 ^b ± 3.19
Indomethacin (25 mg/kg)	90.93 ^a ± 6.33	97.21 ^a ± 6.33	91.02 ^a ± 5.89	90.12 ^a ± 8.24
Control	0.00 ^c ± 0.00	0.00 ^d ± 0.00	0.00 ^c ± 0.00	0.00 ^c ± 0.00
LSD	6.542	4.445	5.163	5.648
CV (%)	7.29	4.63	5.70	6.26
F- Value	296.981**	738.235**	484.513**	401.270**

** $p < 0.01$ compared with control at the same hour. Treatment means superscripted with the same letter of the same column are not statistically significant.

Tail flick test

Pain latency increased with the intraperitoneal application of *Pc* across the entire treatment groups. The pain latency period at all hours was statistically significant in the groups treated with different doses of *Pc* and indomethacin, at 0 hours of the same group. Similarly, the latency period of the groups treated with various doses of plant extract at 4 hours and indomethacin was significantly different from other hours of the same treatment. Regarding the control group, the latency period at all hours was non-significant to each other (Table 2).

Table 2. Effect of *P. calophylla* (*Pc*) extract on pain induced by tail immersion test

Time (hr)	Latency period (in seconds)				
	<i>Pc</i> (1.5 mg/kg)	<i>Pc</i> (2.0 mg/kg)	<i>Pc</i> (2.5 mg/kg)	Indomethacin (10 mg/kg)	Control
0	2.56 ^c ± 0.29	2.78 ^d ± 0.13	2.74 ^c ± 0.26	2.80 ^c ± 0.07	2.68 ^a ± 0.39
1	5.10 ^a ± 0.16	4.88 ^b ± 0.13	4.84 ^a ± 0.11	5.34 ^a ± 0.05	2.70 ^a ± 0.32
2	5.26 ^a ± 0.21	5.08 ^a ± 0.13	4.98 ^a ± 0.16	5.26 ^a ± 0.11	2.74 ^a ± 0.15
3	5.08 ^a ± 0.15	4.96 ^{ab} ± 0.18	4.84 ^a ± 0.11	5.14 ^a ± 0.11	2.72 ^a ± 0.18

4	4.760 ^b ± 0.11	4.68 ^c ± 0.13	4.54 ^b ± 0.11	4.80 ^b ± 0.16	2.58 ^a ± 0.33
LSD	0.2538	0.1866	0.2168	0.2044	0.3801
CV (%)	4.24	3.18	3.73	3.30	10.72
F- Value	171.091**	227.743**	163.157**	238.008**	0.234**

** $p < 0.01$ compared to the same group at 0 hours. Treatment means with the same superscript letter within the column are not statistically significant.

Maximum inhibition percentage of pain was recorded for the group treated with indomethacin, at all the hours followed by the group treated with *Pc* 1.5 mg/kg. However, the difference in inhibition percentage of pain in the groups treated with indomethacin and *Pc* 1.5 mg/kg was not statistically significant from two hours onwards (Table 3). The inhibition percentage of pain treated with all doses of *Pc* and indomethacin were statistically significant at all hours compared with the controls. At one hour, the inhibition percentage of the pain of the group treated with indomethacin was significantly different from the pain of groups treated with all the doses of *Pc*. Inhibition percentage of the pain of the group treated with *Pc* 1.5 mg/kg was also significantly different from the groups treated with its remaining doses. The inhibition percentage of the pain of the group treated with indomethacin was significantly different with the inhibition percentage of the pain of the group treated with *Pc* 2.5 mg/kg at two, three and four hours.

Table 3. Effect of *P. calophylla* (*Pc*) extract on inhibition percentage of pain in the tail-flick test

Treatments	Inhibition percentage of pain			
	1 (hr)	2 (hr)	3 (hr)	4 (hr)
<i>Pc</i> (1.50 mg/kg)	88.89 ^b ± 5.86	91.97 ^a ± 7.57	86.76 ^a ± 5.45	84.50 ^a ± 4.42
<i>Pc</i> (2.00 mg/kg)	80.74 ^c ± 4.83	85.40 ^{ab} ± 4.76	82.35 ^{ab} ± 6.68	81.40 ^{ab} ± 5.05
<i>Pc</i> (2.50 mg/kg)	79.26 ^c ± 4.22	81.75 ^b ± 6.00	77.94 ^b ± 4.19	75.97 ^b ± 4.42
Indomethacin (25 mg/kg)	97.78 ^a ± 2.03	91.97 ^a ± 4.16	88.97 ^a ± 4.19	86.05 ^a ± 6.13
Control	0.00 ^d ± 0.00	0.00 ^c ± 0.00	0.00 ^c ± 0.00	0.00 ^c ± 0.00
LSD	5.263	6.809	7.092	5.963
CV (%)	5.75	7.35	6.96	6.89
F- Value	489.190**	292.820**	326.395**	332.544**

** $p < 0.01$ compared with control at the same hour. Treatment means followed by the same superscript in the same column are not statistically significant.

Acetic acid-induced writhing test

Acetic acid-induced writhing was significantly inhibited in all treatment groups as compared to control. Inhibition percentage of the writhing of the group treated with Indomethacin was statistically non-significant only in comparison with the group treated with *Pc* 1.5 mg/kg. Inhibition percentage of the pain of the group treated with *Pc* 1.5 mg/kg was statistically non-significant with the group treated with *Pc* 2 mg/kg but significant with 2.5 mg/kg. Inhibition percentage of pain was observed at maximum in the group treated with Indomethacin followed by *Pc* 1.5 mg/kg (Table 4).

Table 4. Effect of *P. calophylla* (*Pc*) extract on the acetic acid-induced writhing

Treatments	No. of writhing	Inhibition percentage
<i>Pc</i> (1.5 mg/kg)	7.2 ^{bc} ± 1.30	84.75 ^{ab} ± 2.76
<i>Pc</i> (2.0 mg/kg)	8.2 ^{bc} ± 0.84	82.63 ^{bc} ± 1.77
<i>Pc</i> (2.5 mg/kg)	9.4 ^b ± 1.14	80.08 ^c ± 2.42
Indomethacin (25 mg/kg)	6.0 ^c ± 1.58	87.29 ^a ± 3.35
Control	47.2 ^a ± 2.95	0.00 ^d ± 0.00
LSD	2.277	3.112
CV (%)	11.07	3.52
F- Value	2.980**	1264.597**

** $p < 0.01$ compared with control at the same hour. Treatment means followed by the same superscript in the same column are not statically significant.

Formalin induced hind paw licking test

Inhibition percentage of pain in treatment groups as compared to the control group of the same phase was statistically significant. Inhibition percentage of pain in the groups treated with *Pc* 1.5 mg/kg and 2 mg/kg were statistically non-significant with other groups in both phases. Whereas, inhibition percentage of pain in the groups treated with *Pc* 2.5 mg/kg and indomethacin were statistically non-significant. In both phases, maximum inhibition of pain was observed in the group treated with *Pc* 1.5 mg/kg (Table 5).

Table 5. Effect of *P. calophylla* (*Pc*) extract on inhibition percentage of pain in formalin-induced hind paw licking test

Treatments	Time of licking (in seconds)		Inhibition percentage	
	First phase	Second phase	First phase	Second phase
<i>Pc</i> (1.5 mg/kg)	12.80 ^d ± 2.59	21.20 ^d ± 2.59	84.50 ^a ± 3.13	92.13 ^a ± 0.96
<i>Pc</i> (2.0 mg/kg)	21.00 ^c ± 5.52	37.80 ^c ± 6.14	74.58 ^b ± 6.69	85.97 ^b ± 2.28
<i>Pc</i> (2.5 mg/kg)	21.90 ^c ± 3.01	53.60 ^{bc} ± 3.51	69.01 ^{bc} ± 6.44	80.10 ^c ± 1.30
Indomethacin (25 mg/kg)	30.40 ^b ± 5.46	58.20 ^b ± 7.29	63.19 ^c ± 6.61	78.40 ^c ± 2.71
Control	82.60 ^a ± 8.20	269.4 ^a ± 25.62	0.00 ^d ± 0.00	0.00 ^d ± 0.00
LSD	7.064	16.33	6.974	2.297
CV (%)	15.87	14.06	9.07	2.59
F- Value	136.891**	342.271**	200.825**	2383.794**

The values are shown as mean ± SD

** $p < 0.01$ compared with control at the same hour. Treatment means followed by the same superscript in the same column are not statistically significant.

DISCUSSION

P. calophylla (locally called Sikari laharo) has been used extensively since the historical times in Nepal for treating different ailments in animals and humans by traditional healers and village elders. The extracts of this plant are used as a concoction in joint pains and aches (Sapkota, 2009). Acetic acid-induced writhing reflex is a model for the assessment of visceral pain and commonly

used in the screening of peripherally acting analgesic drugs (Acharya *et al.*, 2019). The constriction response of the abdomen involves the release of arachidonic acid (AA) from tissue phospholipids via cyclooxygenase (COX), elevated levels of prostaglandin E₂ (PGE₂) and prostaglandin F₂-alpha (PGF₂ α) and lipo-oxygenase (LOX) products in the peritoneal fluids. Prostaglandins (PGs) activate and sensitize the peripheral chemo-sensitive nociceptors, causing inflammatory pain and abdominal constriction (Tadiwos *et al.*, 2017). The acetic acid administered intraperitoneally provokes abdominal contractions, total-body movements, twisting of the dorso-abdominal muscles, and suppression of motor activity and coordination in mice (Bars *et al.*, 2001). Acetic acid elevates the release of endogenous stimulus via mediators to activate the nociceptive neurons. It is sensitive to NSAIDs, narcotics as well as some centrally acting drugs (Reichert *et al.*, 2001). The release of cytokines, such as TNF- α , interleukin-1 β and interleukin-8, by resident peritoneal macrophages and mast cells contribute to the nociceptive activity of acetic acid (Ribeiro *et al.*, 2000). The administration of indomethacin, that suppresses the COX-1 and COX-2, also inhibits the algogenic action of acetic acid by scavenging the inflammatory mediators of pain in peripheral tissues (Tadiwos *et al.*, 2017).

In this study, the test drug, at all doses attenuated the writhing response of mice inflicted by intraperitoneal acetic acid administration. Inhibition percentage of the test extract at 1.5 mg/kg was statistically non-significant as compared to the indomethacin. Inhibition of local peritoneal receptors, AA pathways, involving COX and/or LOX could have mediated the observed analgesic effect. Although different agents ameliorate the pain due to acetic acid, substances that inhibit the writhing response preferably work by inhibiting the synthesis of PG (Muhammad *et al.*, 2012). Thus, it could be inferred that our plant's extract possibly inhibited the pain through the peripheral action, involving inhibition of PG synthesis. Some flavonoids have been shown to produce analgesic action by interfering with the PG synthesis (Tadiwos *et al.*, 2017). The results of our test indicate that the anti-nociceptive action of *Pc* in the acetic acid-induced writhing test could be due to the inhibition of the release of TNF- α , interleukin-1 β and interleukin-8 and the analgesic effect is probably mediated via its peripheral effect. The central analgesic action of the plant extract could also play an additional role in preventing the pain in this model because it has been reported that analgesics with central actions diminish the writhing response (Tadiwos *et al.*, 2017).

The test drug was evaluated by the hot plate test to access its anti-nociceptive effect via the supraspinal mechanism. Heat-induced nociceptive pain in the paw of mice is a highly sensitive model for evaluating the therapeutic agents for the analgesic property (Acharya *et al.*, 2019). During the inflammation process arachidonic acid (AA) pathway significantly contributes towards the generation of inflammatory response such as pain. AA generated from membrane phospholipids by the action of phospholipases, is, later on, degraded by cyclooxygenase (COX) to potent inflammatory mediators such as the prostaglandins. This hot-plate test is used in elucidating the centrally mediated analgesic effects of the test drug. Generally, heat induces low pain reaction threshold (latency time) that is elevated by the use of analgesic agent (Jan and Khan, 2016). In our study, the test extract (at all doses) elevated the mean reaction time and the pain was inhibited significantly. While comparing the pain inhibition percentage of test drug at the dose rate of 1.5mg/kg with indomethacin, it was statically non-significant.

In the tail-flick test that assesses the response to a thermal stimulus, increased reaction time is regarded to be an important criterion for evaluating central analgesic property (Rujjanawate *et al.*,

2003). This test can differentiate between central and peripheral analgesics (Asongalem *et al.*, 2004). Increase in the reaction time in this experiment indicates the central analgesic property of the test extract. Inhibition of thermal pain induced by hot plate test by the plant extract is characteristic of the central analgesic effect while peripheral analgesia is known to be inactive on this kind of painful stimuli (Yin *et al.*, 2003).

The formalin-induced hind paw licking test is useful for assessing analgesic drugs as well as in elucidating the mechanism of analgesia (Zeashan *et al.*, 2009). The inhibition of both early and late phases is due to the action on central analgesic effect while the inhibition of the late phase (only) is due to peripheral analgesic effect just like the action of non-steroidal anti-inflammatory drugs (Hossain *et al.*, 2015). In our experiments, the effect of test extract on the late phase of the formalin test indicates that the result is due to its peripheral action when compared with indomethacin activity. The anti-nociceptive effect of test agent could be attributed to inhibition of prostaglandin release and other mediators and also by modulation of pain transmission via μ and K receptors (Perianayagama *et al.*, 2004).

In all the analgesic test models, the lower dose (1.5 mg/kg) was found to be more effective than higher doses. This possibly shows that 1.5 mg/kg dose of the extract is an optimum dose for inducing both central and peripheral analgesia. Lack of increase in potency at higher doses of the extracts suggests that at the dose beyond 1.5 mg/kg is not necessarily effective for the required analgesic property. This phenomenon is explained by the hypothesis that some of the active constituent(s) of the plant extract at high concentrations may exhibit pro-inflammatory (Rezazadeh *et al.*, 2005). On the other hand, some explain that the plant extract has multiple compounds acting differently via anti- or pro-inflammatory responses (Tadiwos *et al.*, 2017).

CONCLUSION

The analgesic effect of *P. calophylla* has been evaluated using a mouse model. This study validates the traditional use of plant parts as an analgesic agent and provided scientific evidence of its effectiveness. The pain was significantly inhibited by 86.76 ± 5.45 , 82.35 ± 6.68 and 77.94 ± 4.19 percentage at the dose level 1.5, 2.0 and 2.5 mg/kg of *Pc* respectively in the tail-flick test. The analgesic property of *Pc* in superficial pain was also supported by the similar results of significant inhibition of pain in hot plate test. Inhibition percentage of pain (84.75 ± 2.76) at the dose level of 1.5 mg/kg in acetic acid writhing test at 1.5 mg/kg suggests the centrally acting mechanism of pain inhibition, which was also supported by formalin-induced paw licking test. However, the active phytochemicals present in the extract needs to be purified and further tested for their exact physiological mechanism of action at the cellular level along with the sub-acute and chronic toxicity as well as the LD₅₀ and ED₅₀ of the plant.

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Conflict of Interests

All the authors declare that there are no conflicts of interests that might affect the findings of this study.

Authors Contribution

JA and NP conceptualized, designed, executed the experiments, collected & analyzed the data and drafted the manuscript, ST located the plants and provided the experiments guidelines, MKS and SS provided the mentorship on animal experiments, JA and NP designed the experiments, and NP finalized the draft. All authors have read and agreed upon the submission of this manuscript.

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The situation of Animal Rabies in Nepal from 2013 to 2017

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ABSTRACT

Rabies is endemic in Asia and Africa, including Nepal. About 100 to 300 livestock and 10 to 100 humans are estimated dying of rabies in Nepal annually, but these numbers are likely underestimated. The cross-sectional study was conducted to analyse the pattern of animal Rabies using five years (2013-2017) historic data. The animal rabies case, human-dog bite case, data related to demography, and Nepal's socioeconomic have been collected from concerned government agencies. The animal, time, place, and exposure factors like human dog bite cases, literacy rate, and forest area were considered independent variables for an epidemiological study. QGIS 3.4.9 was used for mapping and GIS plotting. Microsoft Excel and STATA 14 were used as statistical tools for univariate and multivariate analysis. During this period (2013 to 2017), a total of 343 outbreaks (mean 5.84 and SD 9.76) with 1,121 animal rabies death (mean 14.55 and SD 24.24) cases were reported to the veterinary epidemiology section and central veterinary laboratory, Nepal. Among the 77 districts, 55 districts had found at least one or more outbreak(s) during this period. Out of total cases, 87.4% of cases were clinically diagnosed. The association ($p < 0.05$, 95%CI) between animal rabies and human-dog bites was significant. While analyzing the five years' data, it can be concluded that rabies is almost evenly distributed throughout the country, including ten different animal species. Our study may provide valuable information for review of the surveillance plan, control, and eradication strategies of rabies in the future.

Keyword: Animal rabies, Cross-sectional study, Nepal

INTRODUCTION

World Health Organization (WHO), World Animal Health Organization (OIE), Food and Agriculture Organization (FAO), and the Global Alliance for Rabies Control (GARC) have set a global target of "zero dog mediated human rabies deaths by 2030" (FAO, WHO, and Global Alliance for Rabies Control 2018). Globally, rabies causes the deaths of 59,000 people per year. Asia and Africa continents share 95% of human rabies (DoHS 2018; Guo et al. 2013). The global economic loss due to canine-mediated rabies is about \$8.6 billion. The most significant component of economic loss is due to premature death (55%) followed by PEP (15.5%), dog vaccination (1.5%), and additional costs to communities from livestock losses (6%) (Hampson et al. 2015).

It is known to be present in more than 150 countries and territories on all continents except Antarctica. Dogs are the primary source of human rabies deaths contributing up to 99% of all

rabies transmissions. Forty percent of people bit by suspected rabid animals are children under 15 years of age (WHO 2015). Dog-mediated human rabies disproportionately affects poor rural communities of Asia and Africa, particularly children. (Khan et al. 2019). Out of the annual global deaths due to rabies, 45% of these deaths occur on the Indian subcontinent, and approximately 33% take place in India alone (Rahman and Isloor 2018). The majority (84%) of these deaths occur in rural areas (Rabies in Asia Foundation 2013; Yao et al. 2015).

Rabies virus, prevalent in Nepal, has been characterized at the molecular level, and it was found that the virus resembled the South Asian type (Pant 2010). Rabies has been diagnosed in Nepal for a long time, but the information on its epidemiology, impact, and control remains scattered (VEC 2015). Official reports show that each year 100-300 livestock and 10 to 100 humans die of rabies in Nepal, but these numbers very likely underestimate the actual rabies burden (EDCD 2019; VEC-2018). This review could be supportive of the control and eradication plan of rabies in Nepal. This study's main objective was to explore the distribution of animal rabies in Nepal from 2013 to 2019 and to measure the possible association of animal rabies and other risk factors.

METHODOLOGY

A cross-sectional study of secondary data of animal rabies in Nepal from 2013 to 2017 was conducted. The five years (2013-2017) district level animal rabies data and human dog bite cases data of Nepal had accessed, and based on these data, tried to explore the status of animal rabies in Nepal. The ecozone, human-dog bite cases, low literacy rate, and high forest area of district were taken as independent variables for epidemiological analysis of disease. The Microsoft Excel and Epi-info as statistical tools were used for data analysis. QGIS 3.4.9 was used for mapping and GIS plotting. During the data analysis, concerning risk factors were considered, spatial distribution was conducted by the district, province, and ecozone concerning outbreaks and animal deaths. Similarly, temporal distribution was analyzed by months, seasons, and years. Data were collected from 75 districts of Nepal by Veterinary Epidemiology Section (VES), but the same data were analyzed as per the new administrative division (77 districts and seven provinces) of Nepal. Data were categorized in the spreadsheet. For data analysis, seven provinces, three ecological zones (mountain, hill, and terai), and four seasons (winter, autumn, spring, and summer) with other relevant risk factors were considered.

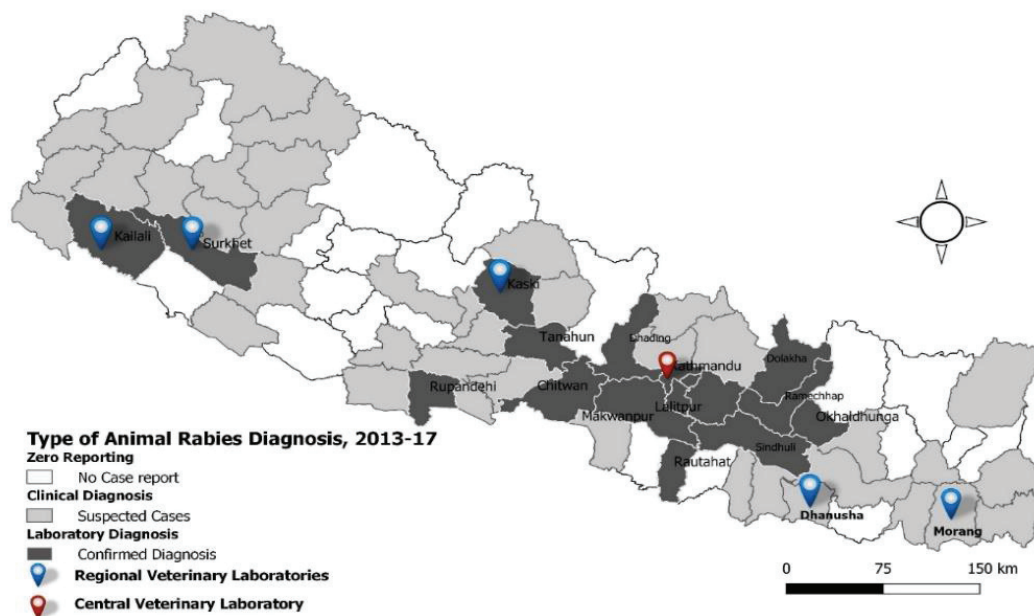
The animal rabies and animal population data have been collected from the VES, Central Veterinary Laboratory (CVL), and Department of Livestock Services (DLS) of Nepal. The animal rabies data were the cumulative data of monthly epidemiological reports of District Livestock Service Offices (DLSOs) and CVL. Similarly, human rabies and dog bite case data were collected from the Department of Health Services (DoHS), Nepal. DoHS collect the data by the Health Management Information System (HMIS), which is the online database system of DoHS.

For the descriptive study suspected case was defined as Municipality level animal rabies cases reported by the DLSO to VES, diagnosed based on the clinical sign of animal rabies (abnormal behavior, difficulty in swallowing, hyper-salivation, excitable, bark and snap at imaginary objects, development of muscular incoordination, disorientation, and seizure and paralysis) by the district veterinary officer between 2013 to 2017. Municipality or Village Development Committee (VDC) level animal rabies cases reported by CVL to the VES from 2013 to 2017, confirmation was made by Fluorescence Antibody Test (FAT) was defined as a confirmed case. All collected data were

verified and validated from the authorized organization after cleaning. Animal rabies data were broadly classified into the confirmed and suspected case. Spatial distribution of animal rabies and dog bite cases were done by using district centroid. The temporal distribution of the animal rabies and dog bite cases were presented in graph and tables.

RESULTS

From 2013 to 2017, there were 343 animal rabies outbreaks (mean 4.45 and SD 6.81), and 1121 (mean 14.55 and SD 24.24) cases were reported from the 77 district. The number of animal rabies outbreaks and cases was found to be in increasing trend from 2013 to 2017. Among 1121 cases, 141 (12.6%) cases were diagnosed with FAT by CVL, and the remaining 980 (87.4%) cases were found clinically diagnosed as animal rabies by the DLSOs. Among the 55 districts of rabies cases, only 17 districts had positive laboratory diagnosis, most of them were from Bagmati, which shared 90.7% (127). Map 1 showed that most of the laboratory-confirmed cases were represented from the vicinity of RVL and CVL.



Map 1: Type of diagnosis of animal rabies by district from 2013-17

During the study period, ten animal species were found positive with rabies, including wildlife (Jackal). Cattle, dog, buffalo, and goat were found to be the main species with a high number of cases, respectively. The least number of cases were reported in yak (1), jackal (2), and cat (3). District coverage of rabies was high in the dog (38 districts) followed by cattle (37) and buffalo (32) out of the 77 districts. The mean value of animal rabies cases of cattle was found to 10.2 with SD 14.6, followed by the dog (mean 8, SD 12) and buffalo (mean 7.7, SD 7.7) (Table 1).

Table 1: Species-wise descriptive statistics of animal rabies case from 2013 to 2017

	Buffalo	Cattle	Dog	Goat	Equine	Pig	Cat	Jackal	Sheep	Yak	Total
Animal rabies case	245 (21.85%)	377 (33.6%)	304 (27%)	164 (14.6%)	11 (0.9%)	5 (0.4%)	3 (0.2%)	2 (0.1%)	9 (0.8%)	1 (0.1%)	1121
Mean	7.7	10.2	8.0	6.07	3.67	2.50	1.50	1	4.50	1.00	14.6
Median	5.5	6.0	4.0	4	1.00	2.50	1.50	1	4.50	1.00	5.0
SD	7.7	14.6	12.0	7.05	4.62	0.71	0.71	0	0.71	N/A	24.2
District count	32	37	38	27	3	2	2	2	2	1	55

Source: VES & CVL, Nepal, 2019

Out of total 141 confirmed case, two in jackal (100%), three in cat (100%), 102 in dog (34%), 27 in buffalo (11%), 6 in goat (4%) and one in equine (9%) found positive. All reported cattle, pig, sheep, and yak cases have been diagnosed based on clinical signs (

Figure 2).

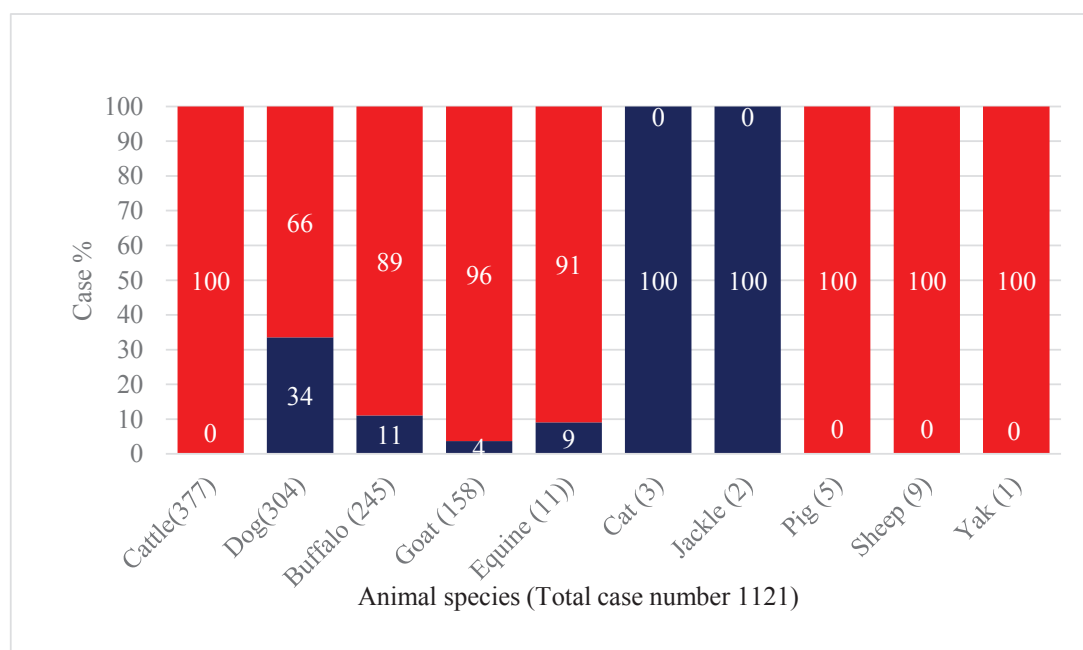


Figure 2: Animal rabies cases by species (Blue indicates laboratory confirmed while red indicates clinically suspected cases)

From 2013 to 2017, the number of human-dog bite cases was ranged from 20,151 to 37,226, with a mean of 31,445 per year. Similarly, total district-wise human dog bites cases were 153742 with mean 1996 and SD 1644 (Figure 3). The number of animal rabies cases was found 111 (2013), 225 (2014), 233 (2015), 251 (2016), and 301 (2017). Similarly, the highest number of outbreaks was 81 (2017), followed by 75 (2016), 71 (2015), 63 (2014), and 53 (2013). The animal rabies deaths increased by about three times in 2017 (301) than in 2013 (111 deaths). Similarly, human rabies death was in increasing trend from 2013 (10) to 2017 (32) (Figure 2Error! Reference source not found.).

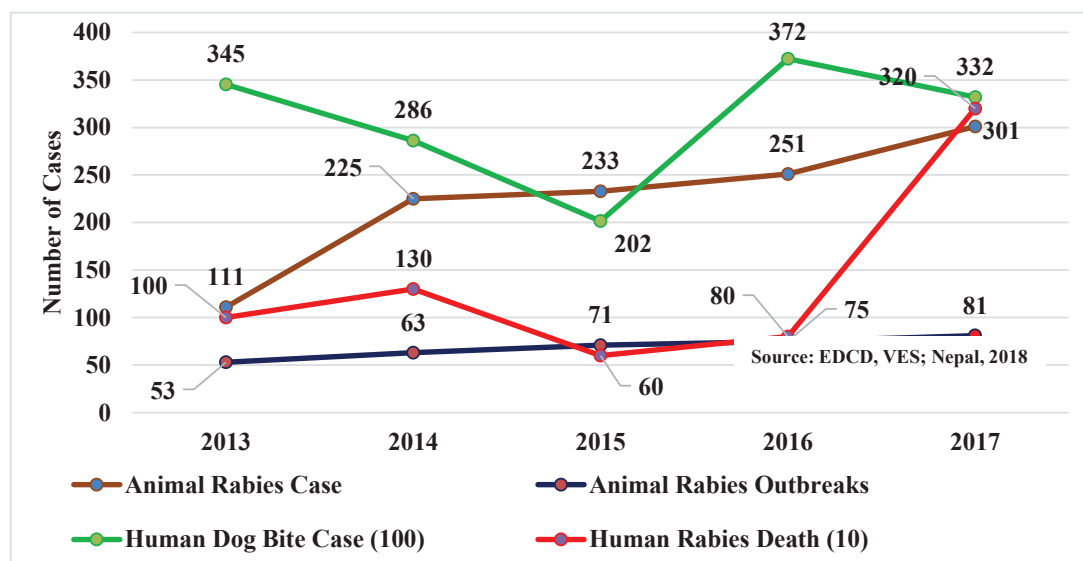


Figure 3: Human dog bite, animal rabies, and human rabies death from 2013-17

The high number of animal rabies cases were reported in spring season 347(30.95%), but summer 331(29.52%) and winter season 311 (27.28%) had slightly lower animal cases. However, the least number of deaths was occurred in autumn 132 (11.77%). However, the number of outbreaks was found higher in summer season 110 (32.1%), followed by Spring 98 (28.57%), winter 95 (27.69%), and the least number of outbreaks seen in autumn 40 (11.66%). The highest number of animal rabies cases were found in April (168), followed by January (143), September (116), February (102), and March (105), and the least number of animal cases was reported in November (55). Similarly, the highest number of outbreaks were reported in January (36), followed by March (36), April (36), September (35), and February (30), but the least number of outbreaks were found in November (17) and June (21). The average number of animal rabies cases is presented in Figure 4.

Spatial distribution of animal rabies cases form 2013-17

Among the 77 districts, only 55 districts had rabies outbreaks, and 22 districts found zero reporting from 2013 to 2017. Dailekh district had the highest number of cases (150), followed by Siraha (78), Kathmandu (71), Jajarkot (56), Ramechhap (56), Kanchanpur (49), and Bajhang (32). Kathmandu (34) district had the highest number of outbreaks, which was followed by the Dailekh

(30), Chitwan (26), Siraha (20), and Kanchanpur (20). The 22 districts including mountain, hill, and terai (Bajura, Bara, Bardiya, Bhojpur, Dang, Darchula, Dolpa, Gorkha, Gulmi, Mustang, Myagdi, Panchthar, Pyuthan, Rolpa, Rukum_East, Rukum_West, Sankhuwasabha, Saptari Sarlahi, Sindhuli, Solukhumbu, and Terahathum) did not have any case recorded during this period. Out of the 77 districts, the highest district coverage of animal rabies cases was found in 2014 (29 districts), which was followed by the 2015 (25 districts), 2016 (25 districts), 2017 (22 districts), and 2013 (17 districts) (Map 2; **Error! Reference source not found.**).

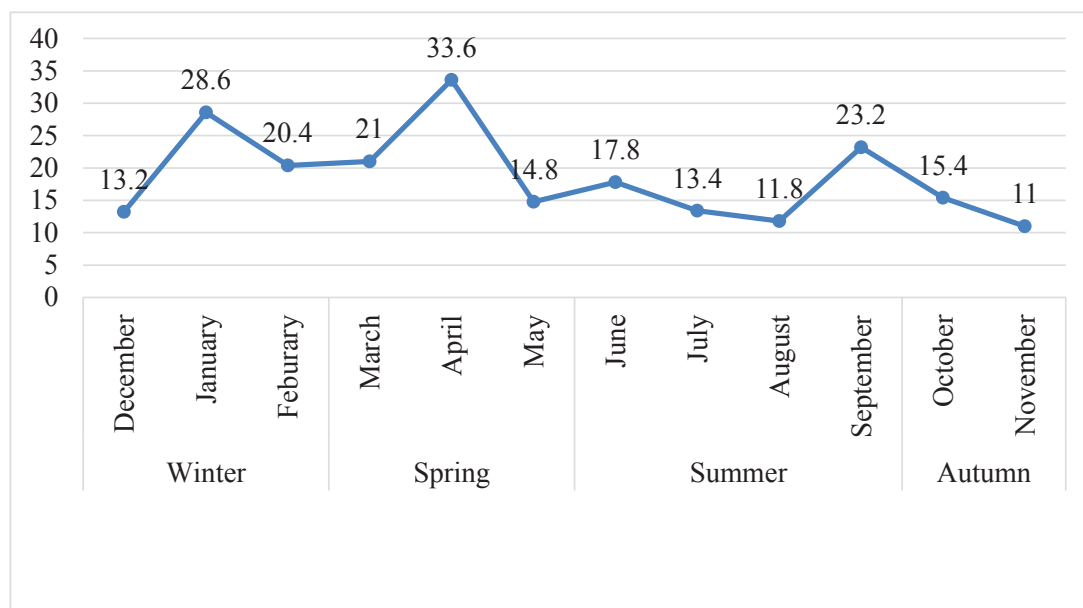
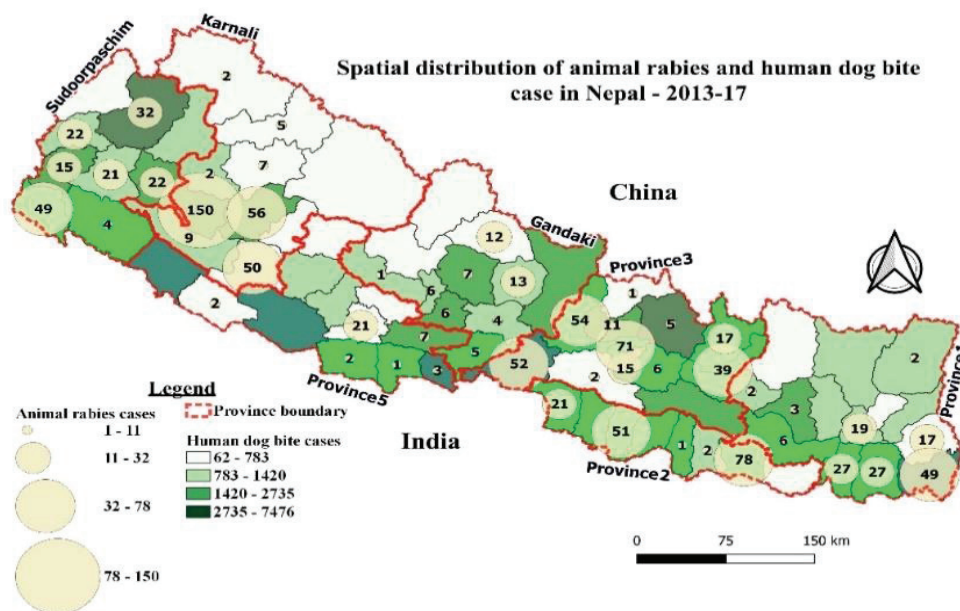


Figure 4: Month-wise average of animal rabies case from 2013-17



Map 2: Spatial distribution of animal rabies and human-dog bite cases from 2013-17

Karnali and Bagmati had more animal cases compare with other provinces. It was found that Karnali Province had 281 (25.1%) animal rabies deaths with 49 (14.28%) outbreaks followed by Bagmati with 280 (25%) deaths and 119 (34.69%) outbreaks, Sudurpashchim province with 165 (14.5%) deaths and 50 (14.58%) outbreaks. Similarly, province 2 had 153 (13.6%) deaths and 40(11.66%) outbreaks, province 1 had 152(13.6%) deaths, and 43(12.54%) outbreaks, Gandaki with 54(4.8%) deaths and 13(3.79%) outbreaks and least number of outbreaks and deaths was found in Lumbini 13 (3.79%), 36(3.2%) respectively (

Table 2 and **Error! Reference source not found.**3). The hilly region had a significant number of animal rabies outbreaks (54.2%), and cases (59.1%) than the terai region with 36.44% outbreaks and 33.4% cases and the mountain region had 9.32% outbreaks and 7.6% deaths (

Figure 5).

Table 2: Province-wise animal rabies and human-dog bite from 2013 to 2017

Name of Province	Number of District	Animal Rabies Outbreaks	Animal Deaths	Human-Dog-bite (2012/13-2016/17)
Province 1	14	43 (12.5%)	152 (13.5%)	23324 (15.2%)
Province 2	8	40 (11.8%)	153 (13.6%)	30417 (19.7%)
Bagmati	13	119 (34.7%)	280 (25%)	31839 (20.7%)
Gandaki	11	29 (8.45%)	54 (4.8%)	17360 (11.3%)
Lumbini	12	13 (3.8%)	36 (3.2%)	21786 (14.2%)
Karnali	10	49 (14.3%)	281 (25%)	8607 (5.6%)
Sudoorpaschim	9	50 (14.6%)	165 (14.7%)	20409 (13.3%)
Grand Total	77	343	1121	153742

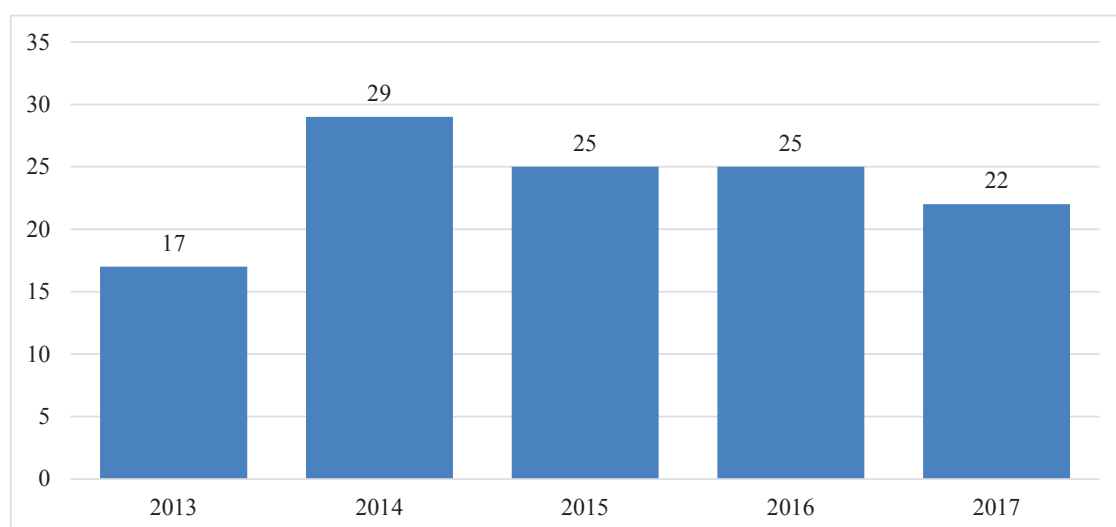
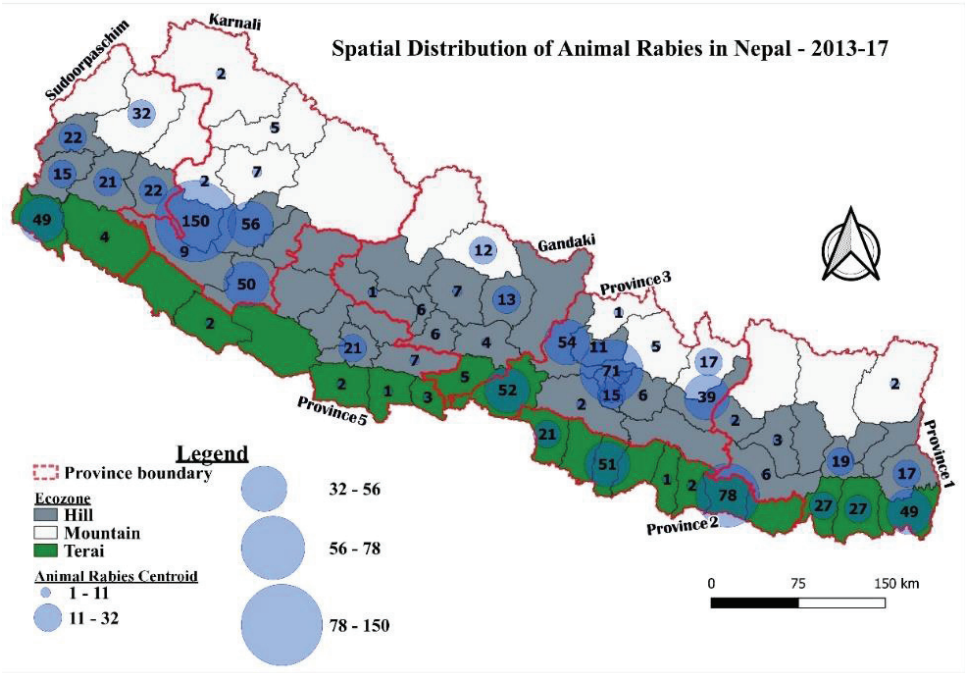


Figure 4. Number of districts from where Rabies were reported by year

Among 77 districts of Nepal, 37 districts not share the border any country, 25 districts shared border with India, 13 with china, and remaining two district shared border with both India and china. All border districts with China and India had 558 (49.78%) animal deaths and 178 (51.89%) outbreaks, while non-border districts with any countries had 563 (50.22%) animal deaths and 165 (47.81%) outbreaks. The only border with china had 128 deaths, and the only border with India had 428 deaths out of 1121 total deaths (

Figure 56).



Map 3: Spatial distribution of animal rabies case in Nepal, 2013-17

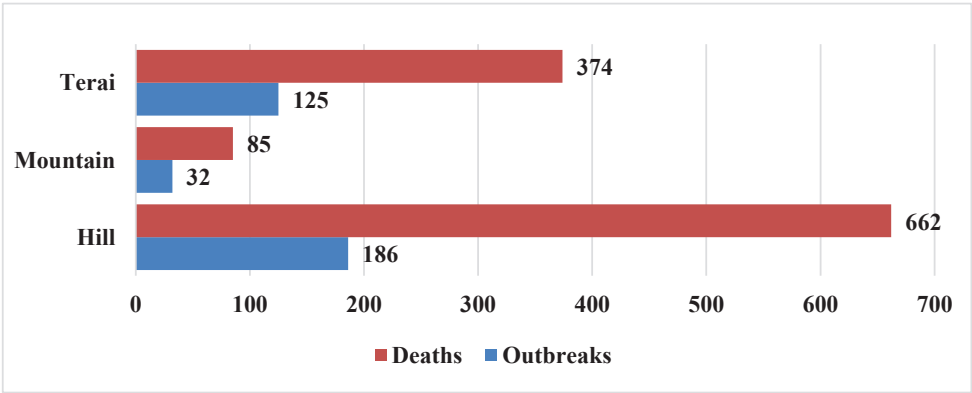


Figure 5: Ecozone-wise animal rabies outbreaks and cases from 2013-17

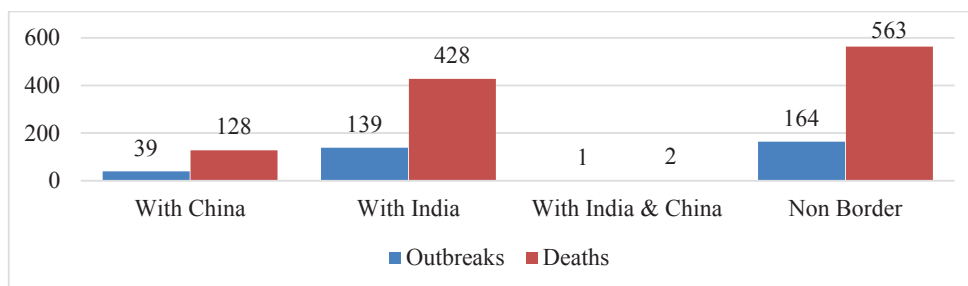


Figure 6: Border district-wise animal rabies outbreaks and deaths from 2013-17

DISCUSSION

A total of 1121 rabies cases were found from 2013 to 2017, which was relatively higher than cases reported (1713) from 2000 to 2009 in Nepal (Yadav 2012). This study showed that out of 77 districts of Nepal, 55 had rabies outbreaks remaining did zero reporting or no reporting, which means districts' coverage was like an endemic (S. Karki and K. C. Thakuri 2010). 980 (87.4%) cases of rabies were clinically diagnosed by DLSOs, and only 141 (12.6%) cases diagnosed by laboratory confirmation, but as per the previous study before 2009 there were almost suspected cases were clinically diagnosed (Yadav 2012). This study showed 90.7% of total laboratory-confirmed cases (141) from Bagmati, where CVL is located. It might be due to access to transportation and the Central Veterinary Laboratory. Among the ten rabies reported species, there were no confirmed cases from cattle, pig, sheep, and yak, and it may be due to the difficulty in sampling and long-distance from the laboratory. During the study period, ten animal species (cattle, dog, buffalo, goat, equine, sheep, pig, cat, jackal, and yak) had a death due to rabies. Out of the 1121 cases, the highest cases found in cattle (33.63%) may be due to the religious reason, and street cattle, which is similar to previous findings (Yadav 2012) conducted between 2000 to 2009. In the same period, rabies was reported other than yak, jackal, and sheep species (Spickler 2009).

Studies in China showed that rabies cases are high in the hot season because people tend to wear less and have outdoor activity (Yao et al. 2015). This study also revealed that spring (30.95%) and summer season (29.52%) had higher animal rabies death. Some authors said that breeding seasonality of dogs and wild carnivores is also associated with the increase of rabies cases (Devleeschauwer et al. 2016). This study showed an April had 168 animal deaths followed by January (143) and September (116). The previous study in Nepal also showed that breeding season of dogs (August or September) has higher contact rates, and they are often seen in groups (Yadav 2012), which is responsible for increase the chances of getting infected with the rabid dog or other animals.

Dailekh district had the highest number of death (150), followed by Siraha (78) and Kathmandu (71). There was no animal rabies reported from 2000 to 2009 from 7 districts (Argakhachi, Bajura, Dolpa, Gulmi, Mustang, Myagdi, Solukhumbu) of Nepal (S. Karki and K. C. Thakuri 2010; Yadav 2012). That might be due to several reasons like under-reporting and laboratory facilities. Hilly region has a higher rabies death 662 (59.1%); the previous study also showed hilly regions had higher rabies cases, 988 (58%), followed by 625 (36%) in the terai and 100 (6%) cases in the mountain region (Yadav 2012).

Similarly, in logistic regression, odds of animal rabies outbreak were found higher with increasing the human-dog bites (OR=3.41) and dog population density (OR=2.08) of exposure population. The highest literacy rate is reported in Kathmandu district (86.3 %) and the lowest in Rautahat (41.7%) (McKenney 2012). It was found that there were 15 districts that had the number of outbreaks and forest area was more than average. Similarly, there were only 12 districts that had below-average literacy rates with an outbreak above the average. Pro-poor rural areas, low literacy rates, and ignorance compounds are the problems of rabies (Talha Burki 2008; Yadav 2012), but our study did not find an association between animal rabies and a literacy rate of the districts (OR 0.73, p-value 0.69).

Due to the lack of clinical rabies surveillance, most of the cases are deemed to be under-reporting. Still, these data did not reflect the exact picture of the animal rabies of Nepal. Most of the zero rabies reported districts had an equal chance of rabies outbreak than the neighboring districts. There were several limitation of this study. The absence of clinical surveillance and under-reporting of data, all cases of animal rabies by district and year might be less than actual prevalence. All passive surveillance data of the district cannot give the exact GIS location of every rabies cases, therefore centroid of district was used for mapping.

CONCLUSION AND RECOMMENDATIONS

While analyzing the five years' data, it can be concluded that rabies is almost evenly distributed throughout the country. Moreover, the number of animal rabies death is increasing every year. Most cases are reported as a suspected case; even there is no confirmed case reported from some species like cattle, pig, equine, etc. Most of the mountain and hilly region's districts are far from the central and regional veterinary laboratories; there is a possibility of under-reporting due to transportation, difficulty in the sample collection, and distance from the laboratory. Our study may provide valuable information for review of the surveillance plan and control and eradication strategies of rabies in the future.

Detail records of animal health surveillance should be kept in an online or electronic database system with individual cases or samples. The validation of the monthly epidemiological report is highly recommended. The strengthening or capacity enhancement of district or regional laboratories for disease diagnosis and disease surveillance. There should be the mandatory provision of detailed animal health data reporting provision to the veterinary epidemiology section. The coverage of laboratory services should be increased to increase the surveillance system's sensitivity, especially from the hilly and mountain regions. The strengthening and evaluation of the rabies surveillance systems, including wildlife, is highly recommended. The mandatory legal provision of rabies case reporting, dog population management, and vaccination should be inbuilt in local government legislation.

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Prevalence and identification of sub-clinical mastitis in the commercial cattle of Rupandehi District, Nepal

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ABSTRACT

Mastitis is a major enemy of dairy farmers causing loss of milk production and degrading quality of milk leading to great economic losses. This study was carried out to assess the sub clinical mastitis, its prevalence, causative organisms and their resistance pattern to major antibiotics used. A cross sectional study was conducted in the commercial cattle farms of Rupandehi district, taking 94 milking animals randomly from the herd along with questionnaire survey with owners. California Mastitis Test (CMT) was performed at the farmer's shed and 10 ml of milk sample from the infected quarter was collected. Milk samples were subjected to culture and causative organisms were identified based on colony characteristics, Gram's staining and biochemical tests. Antibioqram was performed to find out the resistance pattern of microbes. The preventive measures of sub-clinical mastitis (SCM) were found loosely followed in the farms. Out of total 94 animals screened for SCM, 62.77% were found positive in CMT. LF (39.36%) quarter has more prevalence followed by RF (30.85%), RH (29.79%) and LH (21.28%). In total, out of 376 quarters tested for SCM, 30.32% were found positive. Among positive cases, causative organisms were coliform (57.63%), Staphylococcus (11.86%) and Streptococcus (8.47%). In 22% CMT positive samples, organisms did not grow in the culture medium. Gentamicin (68.57%) was found more sensitive antibiotics on antibiogram followed by Amikacin (62.86%), Tetracycline (57.14%) and Ciprofloxacin (51.43%). 23.9 % of the etiologic agents were found multidrug resistant. It is very crucial to follow precautionary actions like milking order, teat dipping, farm biosecurity as well as early detection and treatment with judicious use of antimicrobials to minimize economic losses.

Keywords: Cattle, Prevalence, Quarter, Sub-clinical mastitis

INTRODUCTION

Dairy is an important agricultural sector which contributes about 12% to the agricultural gross domestic product (AGDP) and 62% to the livestock GDP (DLS 2014). Cattle and buffaloes are the main milk producing animals of Nepal which share 24.7% and 32.7% in milk and milk products respectively (Regmi et al., 2017). Rupandehi is one of the districts in the western region where large number (98,384) of cattle are reared for dairy purpose (MOLD, 2017). Total 43,118 Mt. milk is produced in this district, out of which 13,133 Mt. milk is contributed by 12,914 milking cows (SINA, 2017). This shows that Rupandehi district only contributes about 2.3% in the total national milk production where as 2% in the total cow milk production.

Mastitis is a multi-etiological and complex disease characterized by pathological changes in the mammary tissue followed by physical, biochemical and usually bacteriological changes in the milk. The sub-clinical form is 15-40 times more prevalent than the clinical form, and usually precedes the clinical form and is of long duration (Seegers et al., 2003). Detection of sub-clinical mastitis (SCM) is more difficult because cardinal signs are not readily apparent. It is also important to emphasize that the sub-clinically affected animals remain a continuing source of infection for herd mates (Islam et al., 2011).

SCM is financially important as besides causing loss in milk production and its quality, it also increases the chances of clinical mastitis. It can lead to a 10% to 20% decrease in milk production (Iraguha et al, 2015). In addition, it has an undesirable effect on the constituents and nutritional value of the milk, rendering it of low quality and less fit for processing (Iraguha et al., 2015). If the animal can be screened early for SCM and treated, it will be more cost effective than treating for clinical mastitis later. CMT is the most preferable test for SCM screening (Iraguha et al., 2017). Therefore, this study is aimed to find out the prevalence of SCM using CMT, identification of causative organisms and their antibiotic sensitivity pattern in the commercial cattle of Rupandehi district.

MATERIALS AND METHODS

Study Site

This study was conducted in Siddharthanagar municipality of Rupandehi district, which is the pocket area for milk production. The laboratory analysis of the collected milk sample was carried out at the Veterinary Laboratory, Pokhara.

Sampling Technique and Laboratory Examination

94 milking animals were randomly selected, out of which 50 were Jersey cross, 37 Holstein cross, 5 local cross bred and 2 were Hariyana. CMT was performed at the farmer's shed and ten ml of milk sample from each quarter was collected in a sterile bottle from the animal infected sub-clinically. They were numbered and marked as right front (RF), left front (LF), right hind (RH) and left hind (LH). All the samples brought to the laboratory were subjected to cultural examination on Nutrient agar, Mac Conkey agar and EMB media. They were incubated at 37° C for 24 hours. Cultural isolates were identified on the basis of colony characteristics, Gram's staining and different biochemical tests; IMViC, Oxidase, Catalase and Motility (Table-1). Finally, antibiotic sensitivity test (AST) was performed using commercially available Penicillin, Aminoglycosides, Quinolones, Tetracycline and Cephalosporin group of antibiotics.

Table 1. Biochemical test used for identification of cultural isolates

Bacterial isolates	Oxidase	Catalase	IMViC	Motility
<i>E. coli</i>	-	+	++--	+
<i>Klebsiella</i> spp	-	+	----	+
<i>Staphylococcus</i> spp	-	-	----	-
<i>Streptococcus</i> spp	-	-	----	-

RESULTS AND DISCUSSION

The husbandry practices were almost similar in all farms. Biosecurity measures were poor as well as other preventive measures of sub-clinical mastitis like teat dipping, milking order and calf weaning practice were not properly followed though all the farms had clinically infected mastitis animals (Table 2).

Table 2 Husbandry practices of five commercial cattle farmers

Husbandry practices	no of farms	no of animals
Biosecurity measures followed	1	51
Stall floor:		
Concrete	5	94
Concrete+mattress	1	51
Feeding method:		
Stall	5	94
Bedding:		
No bedding	4	89
straw	1	5
Use of disinfectant:		
Occasionally (Lime)	2	29
Calf suckling	3	44
History of clinical mastitis	5	94
Milking order	3	34
Teat dipping:		
Regular	3	24
Only to infected animals	2	56
Never	1	14

The prevalence of SCM was found 62.77 % which is lower than the findings of Lamsal (2018) in a study conducted in the commercial cattle of Chitwan district where the prevalence was reported 69%. Out of the total 376 quarters tested, 30.32 % were found to be affected with SCM. The highest incidence was in the LF quarter (37%) followed by RF (29%), RH (28%) and LH (20%) as shown in Table-3, which is similar to the findings of Jha et al. (1993). Khanal & Pandit, (2013) have also found highest incidence (26%) in the left fore quarter which is similar to our result.

Table 3 Screening of milking animals for SCM based on CMT

Screening Result; n (%)		Quarter wise distribution of SCM			
Positive	Negative	Teat Location	Positive	Negative	Total Prevalence n (%)
59 (62.77)	35 (37.23)	RF	29	65	29 (30.85)
		LF	37	57	37 (39.36)
		RH	28	66	28 (29.79)
Total: 94 milking cows		LH	20	74	20 (21.28)

Abbr.: RF, Right Front; LF, Left Front; RH, Right Hind; LH, Left Hind

The main causative organism causing SCM was coliform (57.63%) followed by *Staphylococcus* (11.86%) and *Streptococcus* (8.47%) as shown in Table-4. In the 22% affected milk sample, the organisms could not be grown that may be due to the antibiotics used to treat the decrease in milk production because of suspicion of SCM or it may be due to the organisms other than bacteria (fungus). Thapa & Kaphle, (2002) & Subedi & Dhakal, (2002) had also found the coliform as the main causative organism followed by *Staphylococcus*.

Table 4 Distribution of different types of subclinical mastitis on animal and quarter basis

Culture Report	No of animals (94) n (%)	No of quarters (376) n (%)
Mastitis Negative	35 (37.23)	262 (69.68)
Coliform mastitis	34 (57.63)	
<i>Staphylococcus</i> mastitis	7 (11.86)	114 (30.32)
<i>Streptococcus</i> mastitis	5 (8.47)	
No growth	13 (22)	

Out of the six important antibiotics used namely Amikacin, Ampicillin, Ciprofloxacin, Ceftriaxone, Tetracycline and Gentamicin (Figure 1), antibiogram revealed that majority of the isolates were sensitive to Gentamicin (68.57%) which is in concordance with Thapa & Kaphle, (2002) and Subedi & Dhakal, (2002), followed by Amikacin (62.86%), Tetracycline (57.14%) and Ciprofloxacin (51.43%). Coliform showed a mean antimicrobial susceptibility of 27.03% to Gentamicin, 21.62% Amikacin, 21.62% Tetracycline, 16.22% Ciprofloxacin, 9.5% Ceftriaxone and 4.05% Ampicillin (Figure 2). 23.9% isolated organisms were found multidrug resistant (Figure 3).

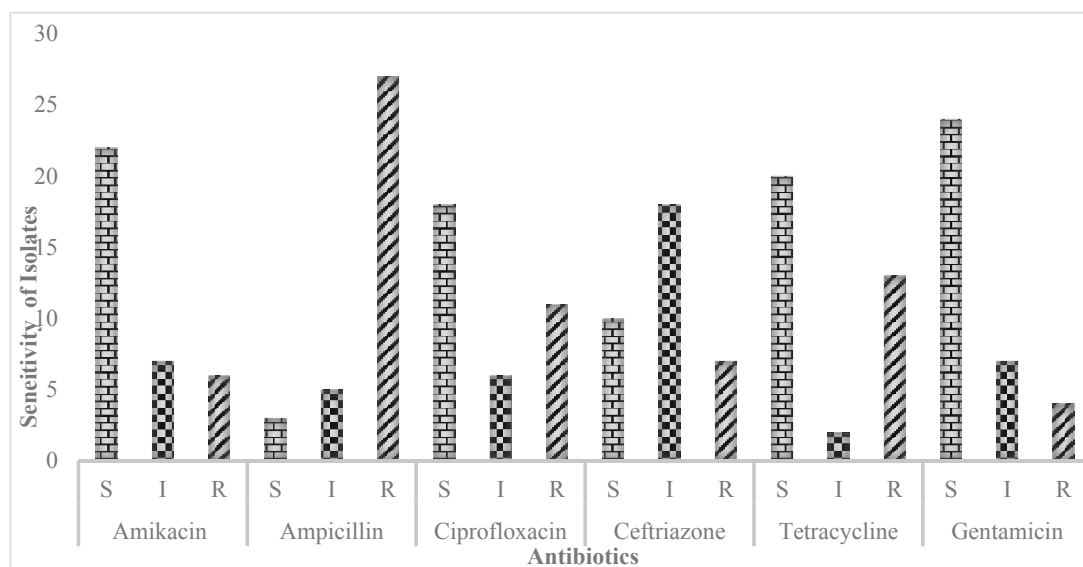


Fig 1 Antibiotic sensitivity Test (AST) of isolates (Abbr.: S, Sensitive; I, Intermediate; R, Resistant)

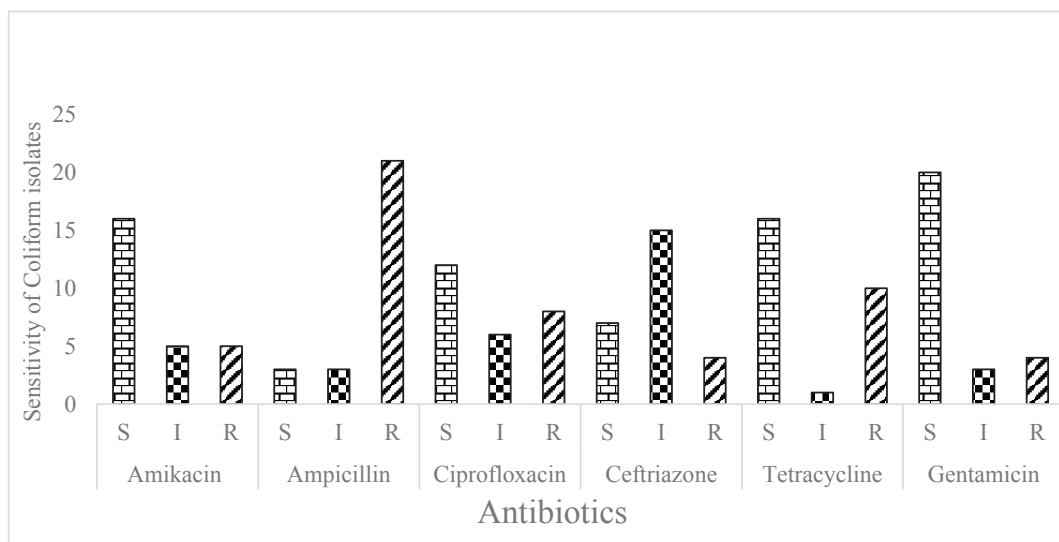


Fig 2 Antibiotic sensitivity Test (AST) of Coliform isolates (Abbr.: S, Sensitive; I, Intermediate; R, Resistant)

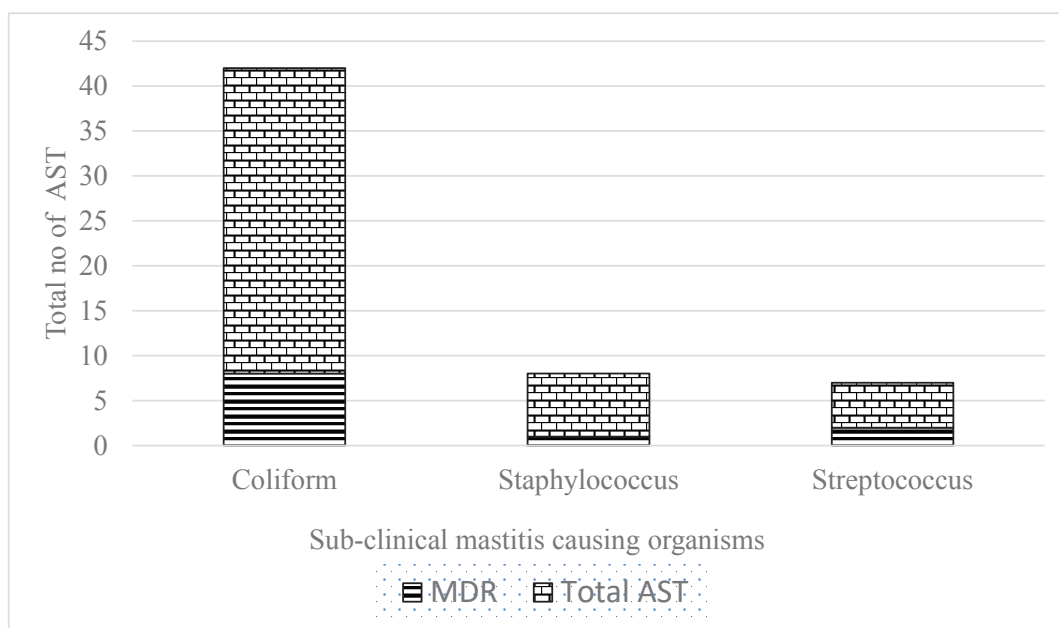


Fig 3 Multidrug resistance pattern of sub-clinical mastitis causing organisms

CONCLUSION

The prevalence of SCM was found 62.77% in the commercial cattle of Rupandehi district. This higher positivity of SCM certainly indicates that the animals are underdiagnosed and the treatment has not been initiated timely. Most of the causative organisms (23.9%) were found multidrug resistant. This shows that the dairy farmers are losing significant amount of money from this

disease. Therefore, preventive measures must be strictly followed in the farms. There is a need of regular screening of milking animals for the presence of sub-clinical mastitis. Either antimicrobial susceptibility test or at least recent knowledge of drug susceptibility ensures the right selection of antimicrobials for the successful treatment.

RECOMMENDATION

Adoption of preventive measures like milking order, teat dipping, farm biosecurity as well as early detection and treatment with judicious use of antimicrobials, will ensure the sub-clinical mastitis to control successfully.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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Exploration of problems, prospects and marketing potential of game poultry birds in Nepal

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ABSTRACT

This study was conducted from to June/July 2019 to Jan/Feb 2020 in 3 different places viz. Kathmandu, Chitwan and Biratnagar/Jhapa to explore the status of Pigeon, Turkey, Quails and Guinea fowl (Laukat) in the country. Productive, reproductive and commercial value of these game birds were explored. The method included survey, review of secondary data, interview, observation and focus group discussion (FGD). The questionnaire included both open and closed ended questions. Results showed that Turkey, Laukat, Quail and Pheasant are being introduced recently. A lot of farmers are rearing or planning to rear these species with limited knowledge. The Kadaknath chicken was found to fetch good price (Nrs 4000-5000/bird) in the market. Similarly, the market value of Laukat ranged from Nrs 1200-1500 and the body weight of those birds ranged from 1200-1500 gm. The body weight of the pheasants ranged from 850-900 gm. Ducks were found to be raised together with fish. The common constraint of farming was biosecurity management. In summary, if stakeholders in the value chains succeed to reduce the incidences of disease and ensure the supply of quality poultry and poultry products at reasonable price, the demand will significantly increase in the days to come.

Keywords: Alternate poultry meat; Commercial value; Game birds, Survey

INTRODUCTION

In last two decade, poultry sector have been booming up and is progressing towards industrialization. The major reasons for this boom might be related to the increasing human population, greater purchasing power and urbanization. Additionally, the development and transfer of feed, slaughter and processing technologies have increased safety and efficiency of poultry production. These developments have driven the poultry industry and the associated feed industry to scale up rapidly, to concentrate themselves close to input sources or final markets, and to integrate vertically. Nepal ranks 112th in chicken meat production, 92nd in egg production and the GDP contribution by poultry sector 4 %. Farmer's, middle man, wholesale, retailers and consumers are the key players for value chain in poultry production system of the country (FAO, 2012). The primary role of the farmer is to supply cheap and safe food to populations. However, the different studies have indicated that the demand for poultry and its products chicks in Nepal is not met by the internal supply. There is no or limited export.

The game birds are type of birds that is hunted in the wild for sport and/or food. Quail, turkey, Kadaknath poultry, pheasant, pigeon and guinea fowl are newly emerging game birds in the country. According to Yakubu et al (2013), game birds production is an important and highly profitable agricultural industry with a rising global demand for its products as they are adaptable to wide range of climatic conditions (Ogundipe and Dafwang, 1980). Karki (2005) stated that consumption of turkeys and broilers as white meat have been rising worldwide. The consumption of broilers and commercial layers are increasing however, there is also a shifting trend towards relatively new poultry birds in Nepal such as Turkey, Laukat, Quails Pheasants, Pigeons etc. and are providing alternative source of poultry meat. The recent trend indicates their potential to flourish in the Nepalese market. The popularity of these birds are increasing due to advertisements and publicity on gamey flavor of meat with lower fat content. Furthermore, rearing of these birds require few inputs (purchase of initial stock, sheds etc.) and are right sized for home consumption. It allows opportunities to utilise family labour force including older people, women and children. In addition to these, there is alarming issue of haphazard uses of antibiotic in commercial poultry birds which can be a potential threat to human health. Alternative birds as can be complement other commercial poultry birds. However, the situation of these gamebirds farming in the country is not well known. Therefore the study was conducted with the objective to explore problems, prospects and marketing potential of game poultry birds in Nepal.

MATERIALS AND METHODS

Research sites and farmer's selection

A study was conducted from June/July 2019 to Jan/Feb 2020 in 3 different places viz. Kathmandu, Chitwan and Biratnagar/Jhapa. The methods used included survey, review of secondary data, interview, observation and focus group discussion (FGD). The questionnaire included both open and closed ended questions. The questions included information about rates of fertility and hatchability, egg production, body weight and market rates. A total of 10 farmers from each site were selected to collect the information. The farmers were selected from game bird producing groups of the research sites. The selected study sites included experienced game bird producers like pigeon, turkey, quails and guinea fowl (Laukat). Both primary and secondary sources of data were used for the study. Primary data revealed the outcomes from questionnaire and the secondary data were retrieved from the literature review.

Analysis of data

The data collected on different parameters were analyzed using the Microsoft Excel.

RESULTS AND DISCUSSION

Game poultry birds in Nepali context

In recent days in the country, there is shifting trend towards alternatives. In order to maximize food production and meet protein requirements in the country, variable options need to be explored. Turkey, Laukat, Quail and Pheasant etc. are the best options for alternative protein source. Additionally, these birds are being introduced recently and there are various sectors of them to

be explored in the country. A lot of farmers are rearing these or planning to rear these birds with a limited extent and experience without having prior experience.

Few pioneer farmer of turkey reared around 500 turkey birds in the eastern region. Despite, there was little practice of farming and marketing of game birds. The turkey meat was sold in few Department store and butcher shop @ Nrs.1000-1200/ kg. Despite few people used to buy whole turkey @ Nrs 4000-6000. However, sale of turkey meat increased slight during Christmas, thanks giving day and newyear. However, the region had encouraging number of pigeons. The practice of raising pigeons in the rural part of the eastern region was since long time with no commercialization. Many people of these areas consumed the young ones of pigeon as medicinal value and they even sacrificed these to God. In some instances the young ones of pigeon were available in some major rural agro-market commonly known as “hatiyas”. The “Sombari Hatia”, “Sani Hatia”, Budhabari Hatia and Sonbarsha Hatia were some major marketing place for pigeons. The local vendors used to sell these in Nrs. 250-300 per pair. Sometimes there was slight increase in price in special occasions like Navaratri and other festivals. Little information could be collected regarding reproductive and productive traits. The breeds were identified as none descript ones. The size of eggs varied from 10-20 gms and total production of eggs varied from 40-50 as per questionnaire survey.

Similarly , Madi Krishi farm, Chitwan, Namuna Krishi farm , Bhaktapur , Siddhi Ganesh Hatchery, Sarlahi and Ali Poultry farm, Siraha were identified as pioneer farms of “Laukat “ in the country. Some detail description about these game birds have been given as under.

Kadaknath

Kadaknath chicken breed is famous for its meat quality, texture and taste (Parmar et al., 2007). The demand for Kadaknath chicken is growing day by day and spread across most of the Indian states due to their excellent medicinal values. Especially these birds bear great medicinal value in homeopathy and useful in treating a particular nervous disorder (Belsare and Narayankhedkar, 2004). The Kadaknath chicken eggs are also have good nutrition values and good for old people. The black meat contains vitamins B1, B2, B6, B12, C and E, niacin, protein, fat, calcium, phosphorus, iron, and nicotinic acid the breed is hardy and highly resistant for diseases (Belsare and Narayankhedkar, 2004). Additionally, a lot of advertisements and publicity are being made on gamey flavor of meat with lower fat content The size of the bird ranged 1.8-2 kgs and 1.5-1.8 kgs. No information could be explored regarding egg production Due to the relatively high consumption of the breed, its numbers have sharply declined. To save the breed from extinction, Because of the limited availability and increased popularity of the breed since the mid-2000s, there have been multiple types of scams in which both poultry farmers and consumers have been swindled (Parmar et al., 2007). Similarly, the meat of Kadaknath revealed the bird fetched good price (Nrs 4000-5000/bird) in the market.



Laukat (Guinea Fowl)

Guinea fowl (Laukat) are birds of the family Numididae within the Galliformes. These large birds measure from 40–71 cm (16–28 inches) long, and weigh 700–1600 grams or 1.5-3.5 pounds (Christopher, 2005) resembled the study. Consistent with the survey, the market value of Laukat ranged from Nrs 1200-1500. The body wt. of those birds ranged from 1200-1500 gm. The weight of male and female were almost similar with a variation of 50-100 gm. The egg production of those birds ranged from 50-60 gm. The breeds were identified was non- descript ones. However, The Laukat was known to measure in flocks with very less feed consumption around 50-60 gm/ birds with good FCR. Most of the Guinea fowl were raised on fodder basis. Berseem, Lucern and Oat were commonly used forage grasses. The birds were identified as hardy ones and thanks to these desirable trait, the birds were becoming more popular in Nepalese market. Additionally, the vendors and sellers of guinea claimed that the taste of those resembled of Pheasant. There are increasing demand thanks to limited availability and increased popularity of the breed. Especially, the rising agro-tourist the interest of the many farmers are directing interest.



Pheasants (Kalij)

The survey on pheasants revealed the itsfarming is newly emerging business in the country. Some pioneer farmers of Pokhara, Chalnakhel and Balkhu brought the eggs of these from Belgium and France. Some information regarding productive and reproductive parameters were collected. According to the survey, the body weight of these phesants ranged from 850-900gms. They produced 60 eggs per annum. Some special net housing system and wild foraging system was followed. However, the birds fetched high market price. According to the farmers, the price of per bird was Nrs. 5000. Additionally, some tourist resort from Tistung and Palung and other various places were selling the Pheasant meat.



Pigeons

The practice of raising pigeons within the rural a part of the eastern region was since while, however with no commercialization. Those areas consumed the young ones of pigeon as medicinal value and that they even sacrificed these to God. However, the young ones of pigeon were available in some major rural agro-market commonly referred to as “hatiyas”. In accordance with the census report published by CBS Nepal in 2001/2002 Nepal had a population of 1.8 million pigeons constituting but 1 percent (0.1) of the entire number of birds in Nepal. No



commercial farming of pigeons has been recorded. They will be spotted in sizable amount in several temples and holy sites throughout the country offered as a spiritual sacrifice by pilgrims. they're primarily reared for this purpose, though consumption of pigeons are often found consistent with a study entitled “Integrated characterization of 4 cross-border areas of Nepal within the border with India for the danger assessment of HPAI: a socio-economic perspective” conducted by the Society for Management and Development (FAO, 2010) approximately 10-12 percent of the households within the study districts (Jhapa, Parsa, Rupandehi and Banke) were found to stay pigeons

Ducks

The duck population is scattered throughout the country and generally raised in traditional farming systems alongside local chickens. The ducks are often raised around water sources and are more common in certain ethnic communities like Tharu, Newar and Rajbansi Ducks are kept in a traditional scavenging system and no commercial farming of ducks exists at present. Duck keeping practices can be categorized in two ways on the basis of the presence or absence of large water bodies. The ducks are raised together with indigenous fowls and livestock in the Terai districts, primarily in eastern Terai. Unlike in other countries, duck raising practices associated with rice paddy fields is not important in Nepal. The prime purpose of duck farming is for domestic consumption of meat and eggs along with its contribution to generating emergency cash to fulfill household requirement. Rural people have been encouraged to take up duck-raising as a source of additional protein and income and, in the case of fish farmers, in helping to produce more fish, through the nutrient supply from duck manure to the fish ponds. Ducks are also reared for religious and cultural significance. Every Tharu, member of an indigenous caste, rears ducks as it is necessary to offer ducks and duck eggs to gods. Ducks are used for cultural ceremonies. In the Jhapa district, the Rajbansi an indigenous community imports a large number of ducks from India during their “Siruwa” festival. It is reported that during important festivals like Dashain and Saun Sankranti, the Newar and Tharu communities consume a large number of duck eggs and use ducks as religious offerings, furthering illegal imports in the country



Quail and Turkey

There are dozens of varieties of quail, one of the most populous game birds, throughout the world. Quail are commonly hunted but are also commonly farmed. Farmed quail and quail eggs are available in many markets, butchers, and specialty food stores. Like all game birds, quail need to be cooked quickly and is well suited for grilling or roasting



with an addition of fat to avoid drying out. Small and plump, quail have a very mild gamey flavor. Now-a-days a lot of people have been rearing quail in their roof tops for their own consumption. Additionally, it was observed that the sales of quail eggs boom-up during the winter season. The survey on turkey revealed that meat were sold in few Department store and butcher shop at the rate of Nrs.1000-1200/ kg. Despite few people used to buy whole turkey at the rate of Nrs 4000-6000. However, sale of turkey meat increased slight during Christmas, Thanks giving day and New Year. The turkey farming is gaining popularity throughout the country.

Production system

The information about usage of production system and feed ingredients was challenging. Three types of production system was observed as per Dhaubhadel, (1992) who stated that the paucity of available data complicates classification of Nepal's poultry production systems following the FAO classification relating to bio-security levels and in Nepal classification is usually more related to farm size and is grouped into the following categories. i) Scavenging system ii) Semi-scavenging iii) Intensive 3.2 Industrial and integrated.

Scavenging system comprised few birds (3-10 adults per flock) under the giving the farmers extra income in the form of meat and egg sales and partly serves for their own domestic consumption. Around 30% percent of the total poultry populations including ducks were kept under this system. Similarly, local chickens and Kadaknath were the predominant species under this system. The chickens were kept with a low level of inputs, housing, and feeding, and disease control. Most of the farmers raised these game birds in semi-intensive system. The different grasses and commercial feed commonly used included Comfrey, Oat, White clover, commercial layer feed L1 and poultry L3 feed. The commercial feed, water and seasonal grasses were offered ad lib to almost all these different types of bird.

Semi-scavenging system

Some households with flock sizes of above 20 birds have built separate housing facilities. Moreover, some households are self-reliant in producing day old chicks through broody hens. Around 60% of the total poultry population including ducks was kept under this system. Similarly, local chickens and Kadaknath were the predominant species under this system. The poultry birds were kept with a low level of inputs, housing, and feeding, and disease control.

Intensive system

Quails and Pheasants were seen to be reared in this system. Some households with flock sizes of above 100 birds have built separate housing facilities even with cage facilities.

Marketing Channels

A field survey was conducted by the author to assess the causes of dropping out of poultry business in the four major poultry pocket districts, Kathmandu, Kaski, Chitwan and Biratnagar of the country. This was done via developing questionnaire and a total of 50 persons, ten persons from each district, were interviewed. The study concluded that the high mortality and high input prices were the major constraints of out of the poultry business. The general marketing channels of these game birds was also explored and have been given by the table below.

Table 1. Marketing channels for game birds in Nepal

Farm	→	Consumers			
Farm	→	Slaughterers	→	Consumers	
Farm	→	Wholesale	→	Slaughterers	→ Consumer
Farm	→	Middleman	→	Wholesale	→ Slaughterers → Consumer

In addition, an inventory of the Haat bazaars found that 85 operate at regular intervals in the eastern Terai, 34 in mid-Terai, 7 in western Terai, 5 in far-western Terai and 23 in the hill regions (FAO 2012/13). Most of those Haat bazaars are focused on the transactions of livestock including cows, oxen, male and female buffaloes, and male and female goats. Poultry trade either in small or big numbers also happens at those places. Haat-bazaars happen once or twice per week involving the transaction of hundred rupees to millions. The Jhapa districts has the largest number of haats (36) followed by the Morang (12), Sunsari (12), Saptari (10) and Siraha (8) districts. These Haat-bazaars are either owned or managed by local authorities (VDC/municipality) or private organizations. They have contributed to generating significant revenues, but only a small portion of the income is utilized for maintenance, infrastructure development and management improvement.

Similarly, the poultry value chain study sheds some light on the live bird markets in four Terai districts of Nepal. It mentions 30 permanent live birds markets with heavy concentration of wholesalers (25 trading commercial chickens and 5 village chickens) and slaughterers (more than 200) in Jhapa district. LBMs are located in Birtamod, Chandragadhi/Bhadrapur, Damak/Dhulabari, Surunga, Gauradaha, Budhabare, Kerkha, Charali, Sanischare, Garamuni, Rajghat, Maheshpur and Prithvinagara. Most of these points are collection points of both formal and informal (illegal) trade catering the neighboring and distant districts as well.

Parsa district has a total of 6 such live bird markets namely Birta, Murli, Chhapkaiya, Gandak, Naguwa and Meena Bazaar serving as well as wet markets and are situated in Birgunj Municipality. On an average, 10-40 live birds are sold from each market place either directly to the consumers or through slaughterers to consumers. The varieties of birds sold live include Kuroilers, Lohatans and village chickens. Informal birds imported from India contribute with almost 60 percent to the total broiler trade from these markets.

In addition to these, some constraints for game birds production were identified as well. Some major constraints have been illustrated as under.

- Illegal import of day old chicks
- Problems with consistent availability of quality and disease free chicks
- Lack of quality feed and lack of continuous monitoring feed ingredients
- Lack of awareness and training about poultry farming and bio-security related practices with ample amount of ignorance.
- Vaccination not effective due to poor quality and storage facility

- High mortality and high input prices were the major causes of dropping out of the poultry business
- Birds were either killed or slaughtered in the market or they may be brought home by the buyer to be slaughtered at home. There is no disposal mechanism for waste material in any of the locations, thus causing serious health hazards

Table 2. Farmer's response to questionnaire survey

S.N.	Response (Agreement) on particular Issue	Percentage
1	Input price of production is higher than output price	60%
2	Day old chicks related problems	50%
3	Price was high for day old chick	70%
4	Timely chick supply was not available	70%
5	Shortage of capital	50%
6	Adequate quantity of chick was not available	65%
7	High mortality of birds	80%
8	Low productivity of birds	50%
9	Low demand of products in local market	60%
10	Difficult to sell in distant markets	70%
11	Shortage of labour	60%
12	Poor quality feed /needs home-made formulation	70%

Biosecurity Management

Biosecurity management in all the farms were very poor. Unsatisfactory disposal of unhatched eggs, dead chicks and chickens in two of the three visited hatcheries. Rodent proof construction only in one of the few farms, however all have a strong fence or wall around their premises to prevent village chickens, ducks, dogs and cats from entering.

The strength and weakness of game bird farming have been given as under.

Strength

- Increasing trend in production and productivity heading towards self-sufficiency in the country.
- Increasing tendency of consumer favoring white meat;
- Popularity of poultry meat without regard to caste/creed/age.
- one of the cheapest source of animal protein but very low consumption compared to buffalo meat;
- Availability of meat and eggs in all urban centers and even in small market areas
- Quarantine act, meat act for quality control Organized private sector and good coordination between government stakeholders in opening slaughterhouse in near future;
- Rapid financial turnover;
- Increasing inflow of tourists, rapid urbanization, and changing food habits of urban inhabitants; and

- Availability of comparatively cheaper labor.

Weakness

- Irregular supply of quality chicks at competitive price;
- Lack of updated database regarding demand and supply statistics, manufacturer information, product pricing, feed, medication and veterinarians;
- Weak knowledge of poultry farmers about maintaining bio-security, production and marketing;
- Lack of adequate knowledge about quality standard of feed, medicine, day old chicks and vaccines;
- Improper handling of poultry products, medicines and vaccines;
- Not competitive in terms of pricing and quality standards to control informal imports;
- Several incidence of informal imports with possibility of immediate disease transmission threatening public health;
- Quality standard not fully adopted in all steps of production and distribution;
- Lack of slaughterhouses and processing companies delivering safe and hygienic poultry products;
- Lack of introducing new policies and adapting old through comprehensive guidelines and gap in effective implementation and enforcement;
- Weak enforcement of existing rules, regulation and guidelines ;
- Lack of grandparent stock farm in Nepal limiting the prospects to some degree in decreasing cost of chickens entire the value chain;
- Porous border, inadequate quarantine check post with minimal institutional arrangements to control informal imports;
- Weak association of poultry farmers across the value or supply chain;
- Maintenance of bio-security throughout the supply chain;
- Lack of regular sero-monitoring, disease diagnosis and prevention mechanisms.

CONCLUSION

In summary, gamebirds are increasingly getting popular in Nepal and are contributing as an alternate protein source to the people. However, there are several challenges such as disease problems owing to the poor biosecurity measures in the farm. If stakeholders in the value chains succeed to reduce the effects of disease and ensure the supply of quality poultry and poultry products at reasonable price, the demand will significantly increase in the days to come. Economic growth and rapid urbanization will also fuel the increase in the demand.

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Prevalence of Metabolic Disease in Cattle in Sylhet, Bangladesh

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ABSTRACT

Metabolic diseases are one of the major reasons for huge economic losses to the dairy farmers globally. This study aims to determine prevalence of metabolic disease in cattle in Sylhet, Bangladesh. The data on metabolic diseases were collected from Sylhet Veterinary Hospital from December 2015 to December 2016. In total 1252 animals results showed that during the summer season (March-June), Milk Fever (1.39%), Ketosis (0.23%), and Downers cow syndrome (0.47%) were the major metabolic disease observed where no case of lactation Tetany observed. In Rainy Season (July- October) Milk fever (1.58%), Ketosis (0.25%), Lactation Tetany (0.25%), Downers cow syndrome (0.25%) were observed. In winter season (November -February) Milk fever (1.31%), Ketosis (0.26%), Lactation Tetany (0.26%), Downers cow syndrome (0.52%). In summary, we found that highest occurrence of Milk fever was during Rainy season (1.58%), while occurrence of Ketosis, Lactation Tetany and Downers Cow Syndrome was observed more during winter season which was (0.26%) and (0.52%) respectively. Metabolic disorders were more frequent during in transition period in dairy cows and when there were sudden changes in diet and gaps between nutrient supply and demand. We suggest farmers to be aware during high risk periods to reduce the prevalence of metabolic diseases.

Keywords: Metabolic, Ketosis, Transition, Nutrient

INTRODUCTION

Bangladesh has 24 million cattle, out of which 6 million are dairy cattle of local and crossbreds (DLS, 2008). The majority of the dairy cattle are in the hands of smallholder dairy producers. The estimated numbers of dairy farms in Bangladesh is 1.4 million (T. Hemme, “IFCN Dairy Report”, 2008). Bangladesh is a populated, rural and agrarian country located in South Asia. Cattle are important component of the mixed farming system practiced in Bangladesh from a long time. In Bangladesh, livestock population is also high and near about 80% of population is employed in agriculture and livestock farming (BBS, 2008). Metabolic diseases continues to be a substantial

challenge to in the modern dairy industry, not only in Bangladesh but globally where modern dairy cattle breeds are managed and fed for high milk yields. The prevalence of metabolic diseases has been reduced globally in the last four decades; however there are still some of metabolic diseases that continue to negatively impact economics of farmers through reduction in production efficiency (Christy and Thirunavukkarasu, 2006). Disease outbreak among dairy cows constitutes a problem both in terms of financial losses (value of dead cow, decreased production and extra labor) and compromised animal welfare (suffering before death or euthanasia), (Thomsen and Houe, 2006). The nutritional management of dairy cows during the transition period has been extensively studied due to the severe adjustments that cows must face to meet metabolic challenge of high milk yield (Overton T.R. & Waldron M.R. 2004). The metabolic diseases or disorders of production are caused by an imbalance of intake of dietary feed nutrients that enter the animal organism (glucides, proteins, minerals, and water). Nutritional imbalances in livestock occurs because the supply of foods do not meet nutritional requirements for maintenance, growth, production, reproduction of the animals (Martinez *et al*, 2014). Cows in higher producing herds are at increased risk of Parturient paresis, Udder edema, and Ketosis. Cows that had higher previous yields will generally be at increased risk of Pre and post parturient Milk fever and ketosis. The imbalance of insufficient concentrations of calcium in the blood to allow normal bodily functions can cause Periparturient hypocalcemic paresis, more commonly known as milk fever. Early lactation is often associated with other common noninfectious disorders such as displacement of the abomasum and rumen acidosis. Dystocia, prolapsed uterus, other infertility, and abortion were not risk factors for any of the metabolic disorders; however, retained placenta, early metritis, traumatic reticuloperitonitis, acute and chronic mastitis, and foot or leg injury each were direct risk factors for some metabolic disorders. (Gröhn *et al*, 1989). The objective of this study was (i) to determine the prevalence of metabolic diseases of cattle, and (ii) to know the economic importance of metabolic diseases of cattle.

MATERIALS AND METHODS

Study area and time

The data were collected from daily patient register book of Veterinary Hospital, Sylhet and farms around Sylhet Agricultural University (Figure 1) from December 2015 to December 2016. The study was conducted under the Department of Medicine, Sylhet Agricultural University, Sylhet-3100.

Study population

The study was conducted on cattle population of Sylhet and record book of Veterinary Hospital.

Data collection procedure

The data were collected from the daily patient register book of District veterinary Hospital.



Figure 1. Study area in Sylhet, Bangladesh

RESULTS AND DISCUSSION

This study was conducted in Sylhet Government Veterinary Hospital, Sylhet in order to determine the prevalence of metabolic diseases in cattle and factors associate with the metabolic diseases. The following data were collected from monthly report (December 2015 to December 2016) of District veterinary Hospital, Sylhet.

Table.1: Prevalence of Metabolic Diseases of Cattle in Sylhet (Dec 2015 to Dec 2016)

Month /diseases	Total affected animals	Milk fever		Ketosis		Lactation Tetany		Downers cow syndrome	
		Cases	Prevalence (%)	Cases	Prevalence (%)	Cases	Prevalence (%)	Cases	Prevalence (%)
January	105	1	0.95	1	0.95	-	-	1	0.95
February	90	2	2.22	-	-	1	1.11	-	-
March	95	1	1.05	-	-	-	-	1	1.05
April	112	3	2.67	-	-	-	-	1	0.89
May	110	2	1.81	1	-	-	-	-	-
June	122	-	-	-	-	-	-	-	-

July	128	3	2.34	-	-	-	-	1	0.78
August	118	1	0.84	-	-	-	-	-	-
September	96	2	2.08	1	1.04	1	1.04	-	-
October	100	1	1.00	-	-	-	-	-	-
November	90	2	2.22	-	-	-	-	1	1.11
December	86	-	-	-	-	-	-	-	-
Total	1252	18	1.43	3	0.24	2	0.15	5	0.40

Source: District Veterinary Hospital, Sylhet

In the table shows the important metabolic diseases which were found in Sylhet. to Dec 2015 to Dec 2016 one year report indicate the Prevalence (%) of metabolic diseases of cattle are Milk fever (1.43%) , Ketosis (0.24%), Lactation tetany (0.15%) and Downers cow syndrome (0.40%). We found that most of incidence of the metabolic disease is in the transition phase, defined as the period between three weeks before to three weeks after parturition (Wittwer, F., 2000), is the most challenging and critical period in relation to the dairy cow's health status during the lactation cycle. Major physiological, nutritional, metabolic, and immunological changes occur within this time frame as the production cycle of the cow shifts from a gestational nonlactating state to the onset of copious milk synthesis and secretion (Wittwer, F., 2000, Drackley J.K., 1999).

Seasonal variation of metabolic disease of cattle

The seasonal variation of metabolic diseases of the year 2015-2016 was divided into three different seasons as follows- summer (March-June), Rainy (July-Oct.) and winter (Nov.-Feb.) season.

Table.2: Seasonal Variation of metabolic diseases of cattle (December 2015 to December 2016)

Seasons	Total affected animals	Milk fever		Ketosis		Lactation tetany		Downers cow syndrome	
		Cases	Prevalence (%)	Cases	Prevalence (%)	Cases	Prevalence (%)	Cases	Prevalence (%)
Summer (March-Jun),	429	6	1.39	1	0.23	0	0.00	2	0.47
Rainy (July-Oct.)	442	7	1.58	1	0.25	1	0.25	1	0.25
Winter (Nov.-Feb.)	381	5	1.31	1	0.26	1	0.26	2	0.52
Total	1252	18	1.43	3	0.24	2	0.15	5	0.40

In the table season wise prevalence (%) of different metabolic diseases of cattle are presented. The clinical prevalence (%) of different metabolic diseases of cattle observed in the summer season (March-June), Milk fever 1.39%, Ketosis 0.23%, Lactation Tetany 0.00%, and Downers cow syndrome 0.47%. In Rainy season (July- Oct.), Milk fever 1.58%, Ketosis 0.25%, Lactation tetany 0.25%, Downers cow syndrome 0.25%. In winter season (November-February) Milk fever 1.31%, Ketosis 0.26%, Lactation tetany 0.26%, Downers cow syndrome 0.52%. Here shows that highest occurrence of Milk fever in Rainy season (1.58%), Ketosis, Lactation Tetany, Downers cow syndrome in Winter season 0.26%, 0.26% and 0.52% respectively. As well as the present study shows that total prevalence of metabolic diseases is 2.22% in 2015-2016. The study correlated with the findings of the Rahman (2010) reported that seasonal differences, three major seasons-summer (mid-February to mid-June), rainy (mid-June to mid-October) and winter (mid-October to mid-February) were considered. The percentages of major diagnostic groups were parasitic (29.2%), general and systemic (28.7%) and metabolic disease (14.7%). Whereas, bacterial, congenital, fungal and surgical disease cases were found as 6.3%, 5.3%, 4.3% and 4.8% respectively.

The prevalence of different metabolic diseases in Sylhet was 2.22%. Findings of the present study were lower than the earlier findings of Dohoo and Martin (1984a) found that the prevalence of ketosis was 12.10% in cows. Ketosis 5.45% in cattle was evidenced. Ketosis in cattle was 5% in winter, followed by 9.52% in summer and 8.33% in winter whereas Downers cow syndrome was recorded in 7.27% cattle and it was 10% during winter followed by summer (4.76%) and (7.14%) rainy seasons (Lucky *et al.*, 2016). Milk fever was recorded as 14.54% in cattle. However, it was 20% during winter followed by summer (9.52%) and rainy (14.29%) seasons in cattle (Bar and Ezr, 2005) observed clinical hypocalcemia before, during or after calving caused by milk fever. (Thirunavukkarasu *et al.*, 2010) reported that among the 3774 cows in five milk sheds at the State of Tamil Nadu, 516 (13.67%) were affected with milk fever. Hutjens (2003) studied on Israeli Holstein cows and reported that 8% of the pretentious animals died and culled were 12% of them due to milk fever. Even in studies in which milk fever was not constrained to be a periparturient disorder, it is clear that the disorder is rare at other times. The median day of diagnosis was the day of calving or (Dohoo *et al.*, 1983) the day after calving (Grohn *et al.*, 1986), and 140 out of 143 cases were diagnosed within 15 day of postpartum (Erb *et al.*, 1984). The prevalence of metabolic diseases was showed that highest occurrence of Milk fever in Rainy season (1.58%), Ketosis, Lactation Tetany, Downers cow syndrome in Winter season 0.26%, 0.26% and 0.52% respectively. As well as the present study shows that total prevalence of metabolic diseases is 2.22% in 2015-2016.

CONCLUSION

The overall prevalence of metabolic diseases in cattle was relatively lower in study area. Milk fever is uncommon before the third parturition and incidence is highest at the fifth or sixth parturition. Thus, cows are most likely to develop milk fever during their most productive years. Economic losses due to milk fever occur due to expenditure on treatment of disease affected animals and reduction in quantity of milk. Therefore, implementing nutritional and health programs that reduce the risk of metabolic disturbances are expected to not only improve cow health, but also enhance fertility. Low nutrient intake coupled with high energy demand during the transition period will increase the risk of occurrence of metabolic disorders. Strategies to

manipulate peripartum metabolic health involve dietary formulation to minimize the degree and extent of negative nutrient balance, improve Ca homeostasis, and minimize the severity of negative energy balance. Some owners cull and slaughter older, high producing cows from a herd, because of a history of repeated case of milk fever. There are few cases of Downer Cow Syndrome.

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Effect of Using Saturated and Unsaturated Fats in Broiler Diet in its Performance and Blood Parameters

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ABSTRACT

A trial for 42 days on 360 Cobb-500 broiler was conducted on CRD design with six treatments, four replicates of fifteen birds per experimental unit consisted of isocaloric and isonitrogenous diets containing 21% C.P. and 3008 Kcal ME in starter, 19% C.P. and 3086 Kcal ME in grower and 18% C.P. and 3167 Kcal ME in finisher. The inclusion of 6% mustard oil (T1), 1.5 % tallow and 4.5% mustard oil (T2), 3% mustard oil and 3% tallow (T3), 4.5 % tallow and 1.5% mustard oil (T4), and 6% tallow (T5) were used, the control diet did not contain any fat (T0). The studied parameters were feed consumption, feed conversion ratio, body weight gain, carcass characteristics, blood parameters, economical analysis. Fat levels and sources significantly ($p < 0.05$) influenced the body weight gain, feed consumption, feed conversion ratio. Feed consumption decreased significantly ($p < 0.05$), FCR decreased significantly ($p < 0.05$), body weight increased significantly ($p < 0.05$) in all treatments except T0. The carcass characteristic did not differ significantly ($p > 0.05$) in all treatments. High benefit-cost ratio was showed by diet containing 3% tallow and 3% mustard oil. There was linear decrease in serum glucose, serum cholesterol compared to control (T0) diet. Albumin: Globulin ratio was lowest in all treatments except T0. It can be concluded that saturated and unsaturated fat not only increased the performance but also decreased the heat stress and increased the immune response.

Keywords: Blood parameters; Cobb-500; Mustard oil; Performance; Tallow

INTRODUCTION

The livestock and poultry sectors are an integral part of Nepalese economy and lifestyle. Livestock and poultry populations have continuously been increasing in the last decade in Nepal and are likely to follow that trend as the interests in this field is growing (Poudel et al., 2020). Chickens tolerate a very narrow range of climatic variation, and therefore, it is essential to determine the most suitable climatic area and weather for broiler production in open-house systems (Osti et al. 2017). Mainly Carbohydrates are used in poultry diet for energy supply but fats and oils give 2.25 times more energy than Carbohydrates. In poultry production system, energy is the most expensive item. Source of energy contributed about 60 to 80 % of total cost in ration. The biological impact and economic efficiency of a feeding system is in fact determined by dietary energy level and its relation with other nutrients. The possibility to increase its production to the level of potential output by improving technical efficiency which is done by ensuring high quality inputs both physical and technical to the poultry farms (Pradhan et al. 2019).

In tropical countries Environmental temperature is one of the major stresses for poultry (Reddy et al., 2002). It is also known that the apparent digestibility of fat is 95 to 100% if it is low in waxes and chlorophyll, and has boiling point less than 60 degree centigrade. Fat produces highest calories as compared to other nutrients. For instance, fat, protein and carbohydrates yields 9.455, 5.65 and 4.2 kcal/gm (Church and Pond, 1988). If adequate amount of fat is not present in the diet, the function of fat soluble vitamins will decrease and animal show fat soluble vitamins deficiency symptoms (Church and Pond, 1988). Poultry growth performance is not only inherited, but it is also greatly affected by the environment (Babinszky et al., 2011).

Saturated fatty acid has higher energy density but lower metabolisable energy than unsaturated fatty acid. Absorbability of saturated fatty is lower as compared to unsaturated fatty acid. If saturated and unsaturated fatty acid is combined, then metabolisable energy will be higher than the sum of the individual fatty acid. This is called as Fatty Acid Synergism. Mohammadi, *et al.* (2011) who reported that the chicks feed diet supplemented with 6 % tallow or soyabean oil and 3% tallow + 3% soyabean oil had the most body weight gain and the best value for feed conversion ratio ($P < 0.01$). The aim of this study was to evaluate the effect of the different inclusion level of mustard oil and tallow on the performance and blood parameters of Cobb-500 broilers.

MATERIALS AND METHODS

A feeding trial was conducted at Mahalaxmi Poultry Farm, Bhandara-09, Chitwan from 30th April 2017 to 10th June 2017. The experiment was aimed to evaluate the performance of Cobb-500 broiler fed diet containing saturated and unsaturated fat alone or in combination. A completely randomized experimental design with six treatments, four replicates of 15 birds per experimental unit was applied. Altogether 360 Cobb-500 broiler chickens were reared. The treatment consists of isocaloric and isonitrogenous diets containing 21% C.P. and 3008 Kcal ME in starter, 19% C.P. and 3086 Kcal ME in grower and 18% C.P. and 3167 Kcal ME in finisher. The inclusion of 6% mustard oil (T1), 1.5 % tallow and 4.5% mustard oil (T2), 3% mustard oil and 3% tallow (T3), 4.5 % tallow and 1.5% mustard oil (T4), and 6% tallow (T5) in basal diet were used, the control diet did not contain any fat (T0). Birds were provided with the general management of ad libitum feeds and water. Experimental diets were based on corn and soybean meal, and each oil source was added as above mentioned inclusion level except for the control diet, which did not have any fat included. Feeds were formulated to supply nutritional requirements of broilers according to the recommendation of the Cobb 500 manual guide, 2008.

Parameters like cumulative feed consumption (gm), average daily feed consumption (gm), mean cumulative body weight (gm), weekly body weight gain (gm), daily body weight gain (gm), dressing percentage, feed conversion ratio, economics of broiler production, blood parameters, etc. were collected. Data obtained from experimental trial were tabulated in Microsoft excel 2007. One way analysis variance was performed for significance test at 1 and 5 probability level. The data were analyzed using statistical program MSTAT-C version 1.5, treatments means difference were compared by Least Significant Difference (LSD $p < 0.05$) and Duncan's Multiple Range Test (DMRT $p < 0.05$).

RESULTS

Feed conversion ratio

Table 1. Mean feed conversion ratio of broiler fed diets with different levels of saturated and unsaturated fat alone or in combination at Mahalaxmi Poultry Farm, Bhandara-09, Chitwan from 30 April 2017 to 10 June 2017.

Treatment	Mean feed conversion ratio						Overall FCR
	1 st Week	2 nd Week	3 rd week	4 th Week	5 th week	6 th week	
T0	2.11 ^a	1.91 ^a	1.73 ^a	1.92 ^a	2.25 ^a	2.30 ^a	2.04 ^a
T1	1.86 ^{ab}	1.89 ^a	1.81 ^a	1.92 ^a	2.14 ^{ab}	2.16 ^a	1.96 ^a
T2	1.76 ^{ab}	1.84 ^{ab}	1.67 ^{abc}	1.80 ^{ab}	2.00 ^{ab}	2.01 ^{ab}	1.85 ^{ab}
T3	1.26 ^b	1.53 ^b	1.44 ^c	1.52 ^c	1.67 ^c	1.69 ^b	1.52 ^c
T4	1.84 ^{ab}	1.84 ^{ab}	1.69 ^{ab}	1.81 ^{ab}	1.92 ^b	1.82 ^b	1.82 ^{ab}
T5	1.41 ^{ab}	1.54 ^b	1.46 ^{bc}	1.56 ^{bc}	2.01 ^{ab}	2.01 ^{ab}	1.66 ^{bc}
F-value	1.948 ^{ns}	2.872*	3.819*	4.622**	6.447**	4.561**	4.826**
Probability	0.1360	0.0445	0.0156	0.0069	0.0013	0.0073	0.0057
CV %	26.40	11.69	9.30	9.31	7.81	10.26	9.59
LSD	0.67	0.30	0.23	0.24	0.23	0.30	0.26
SEM	0.23	0.10	0.08	0.08	0.08	0.10	0.09

Result of the study showed that overall mean feed conversion ratio of birds at the end of the experiment was significantly poor (2.24) of birds fed diet without supplementation of fats and oils (T0) and superior of birds (1.66) fed diet with 3% mustard oil and 3% tallow (T3). No significant difference in feed conversion ratio of birds was observed in between T3 and T5 of birds during the experimental periods. This results are in agreement with Mohammadi *et al.* (2011) who reported that the chicks feed diet supplemented with 6 % tallow *or* soyabean oil and 3% tallow + 3% soyabean oil had the most body weight gain and the best value for feed conversion ratio ($P < 0.01$). The better weight gain in unsaturated fat based diet might be due to the fact that vegetable oils contain high amount of polyunsaturated fatty acids which were more soluble (into micelles) and ultimately more digestible in the intestine than the saturated fatty acids from animal fat and there by unsaturated fats provides the highest dietary apparent metabolizable energy value in broilers.

Carcass characteristics

Table 2. The average dressing percentage and relative percentage of organs on different treatments of cob-500 poultry fed with saturated and unsaturated fat alone or in combination at Mahalaxmi Poultry Farm, Bhandara-09, Chitwan from 30th April 2017 to 10th June 2017.

Parameter	T0	T1	T2	T3	T4	T5
Empty Gizzard	2.37	2.66	2.83	2.89	2.50	3.23
Liver	2.20	2.15	2.55	2.30	2.27	2.40
Heart	0.57	0.47	0.61	0.59	0.61	0.61
Giblet weight	5.14	5.28	5.99	5.79	5.39	6.25

Spleen	0.08	0.08	0.14	0.07	0.09	0.09
Neck	5.57	6.58	6.17	5.24	5.90	5.17
Shank	5.06	4.27	4.77	4.92	4.39	5.00
Leg Piece	22.24	21.99	22.20	22.85	22.46	22.63
Back	12	12.35	11.28	11.87	12.60	12.08
Chest	22.40	19.69	20.04	20.57	20.01	20.41
Wings	8.72	8.78	8.50	9.02	8.45	8.69
Abdominal Fat	0.93	1.17	1.67	0.95	1.28	1.49
Head	2.75	2.44	2.61	2.71	2.57	2.76
Dressing %	74.9	75.83	75.85	76.42	76.2	76.19

The highest(76.42)dressing percentage was found in bird fed with diet T3 containing 3% mustard oil and 3% tallow and lowest (74.9) with diet T0 containing no added fat and oil. The lowest percentage of abdominal fat was observed in the bird fed with diet T0 (0.93) and the highest in the bird fed with diet T2 (1.67). There were no significant differences in the dressing percentage and its carcass characteristics.

These findings are in agreement with Mohammadiet *al.*(2011) where there was no significant difference between carcass characteristics due to dietary treatments containing fats.However, the use of an unsaturated fat source to reduce lipid accumulation could negatively affect carcass quality characteristics due to the excessively low melting point of the deposited fat (Hrdinkaet *al.*, 1996).It had also been previously reported that broilers fed isoenergetic diets rich in polyunsaturatedfatty acids showed lower abdominal fat deposition compared with those fed diets containing saturatedfat (Barroeta, 1989) which concords to finding of this trial. The use of fatty acids in the diet increased theaccumulation of abdominal fat and increased carcass (Hrdinkaet *al.*, 1996) which was similar to our finding.

Economics of broiler production

Table 3. Economic analysis of Cobb 500 broiler Fed with saturated and unsaturated fats alone or in combination in diet at Mahalaxmi Poultry Farm, Bhandara-09, Chitwan from 30th April 2017 to 10th June 2017.

Commodity	T0	T1	T2	T3	T4	T5
Expenditure (Rs.)						
Chick cost	30	30	30	30	30	30
Feed cost	151.75	158.16	149.59	145.65	147.15	140.17
Equipment	27	27	27	27	27	27
Labor	31	31	31	31	31	31
Medicine & Vaccination	12.77	12.77	12.77	12.77	12.77	12.77
Total cost	252.52	258.93	250.36	246.42	247.92	240.94
Income (Rs.)						
Live weight	303.28	308.45	325.65	378.53	367.29	326.71
Net profit	50.76	49.52	75.29	132.11	119.37	85.77
Benefit Cost Ratio	1.20	1.19	1.30	1.53	1.48	1.35

The total expenditure was highest in the bird fed with T1 diet (Rs. 243.52) which contained 6% mustard oil and lowest in T5 diet (Rs. 225.94) which contained 6% tallow. However, the total income was highest (Rs. 378.53) in T3 diet that contained 3% mustard oil and 3% tallow. The net benefit was highest (Rs. 147.11) in T3 which contained 3% mustard oil and 3% tallow and lowest (Rs. 64.52) observed in T1 diet containing 6% mustard oil. The benefit cost ratio was greater (1.63) in the treatment diet containing 3% mustard oil and 3% tallow and lowest (1.26) in diet containing 6% mustard oil.

Blood parameters

Table 4. Effect of using saturated and unsaturated fats alone or in combination in broiler diets on some blood metabolites and mineral concentration in Mahalaxmi Poultry Farm, Bhandara-09, Chitwan from 30th April 2017 to 10th June 2017.

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb: Glob
T0	261	123	3.5	1.2	2.3	0.52
T1	240	102	3.2	1.0	2.2	0.45
T2	228	112	3.7	1.1	2.6	0.42
T3	204	88	3.6	1.1	2.5	0.44
T4	194	106	2.4	0.9	1.8	0.50
T5	212	80	2.9	0.9	2.0	0.45

The birds consuming diet T0 had highest (123) cholesterol value that contained no added fats which decreased linearly and the lowest (80) in the birds consuming diet T5, diet containing 6% tallow. The birds consuming the diet T2 had the highest (3.7 g/dl) protein level that contained 4.5% mustard oil and 1.5% tallow and the lowest (2.4 g/dl) in T4 that contained 1.5% mustard oil and 4.5% tallow. Similarly the birds consuming the diet T0 that contained no added fats had highest (1.2 g/dl) albumin concentration in serum whereas birds consuming the diet T4 that contained 1.5% mustard oil and 4.5% tallow and T5 that contained 6% tallow had the lowest (0.9 g/dl). Similarly the bird consuming the diet T2 had highest (2.6 g/dl) globulin concentration whereas the bird consuming diet T4 that contained 1.5% mustard oil and 4.5% tallow had lowest (1.5 g/dl) globulin concentration in serum. The Albumin: Globulin ratio was highest in T0 (0.52) that contained no added fats and lowest in the bird consuming diet T2 (0.42) that contained 4.5% mustard oil and 1.5% tallow. The birds receiving diet T0 that contained no added fats had highest (261 mg/dl) serum glucose level and lowest in the bird receiving diet T4 (194mg/dl) that contained 1.5% mustard oil and 4.5% tallow.

DISCUSSION

The results showed that the dietary fat improves the feed conversion rate because of cholecystokinin hormone secretion and prolonged gastrointestinal transit time of food and thus increased the rate in activity of bile and pancreatic lipase secretion which results in digestion and

absorption. According to the Tawfeek *et al.* (2014) states that the heat stress condition significantly increase serum cholesterol, glucose and malondialdehyde and decreased protein and glutathione peroxidase. In the research using saturated and unsaturated fat alone or in combination had low serum cholesterol as compared to diet containing no any oil or fat, this indicated that fat containing diet reduced the heat stress.

Sah (2013) stated that the increased amount of the globulin suggested increased level of immunoglobulin which ultimately results the increased immunity. The albumin: globulin ratio indicated that lower the ratio, higher the globulin amount which indicated the immunity power. So, this result indicated that birds consuming diet T2 had higher immunity than others.

Donkoh (1989) reported reduced plasma protein concentration during heat stress where as in the research done using saturated and unsaturated fats the serum protein was highest in T2 as compared to others which indicated that diet T2 reduced the heat stress following T3, T0, T1, T5 and T4. According to the Khan *et al.* (2002) states that birds exposed to heat showed increased ($P < 0.01$) level of blood glucose in respect to increase temperature.

Similar finding also given by Tawfeek *et al.* (2014) who states that blood glucose level significantly increased in heat stress condition. In this research using saturated and unsaturated fat had low blood glucose level which showed that fat in diet reduced the heat stress.

CONCLUSION

The result of the study showed that during the period of heat stress, inclusion of fat and oils in the diet of birds positively affect the production performance. Inclusion of a correct ratio of unsaturated: saturates is important for optimum utilization and hence better production. It is also concluded that saturated and unsaturated fats have some immune-enhancing properties and also reduced the heat stress. Further research should be carried out in this regard in future. Hence, the poultry growers are suggested to use fats and oils in the diet of broilers when the temperature and humidity are high.

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Molecular Characterization of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) Water Buffalo in Chitwan, Nepal

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ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's disease (JD) in ruminants which is an economically important and endemic in many parts of the globe. This disease regarded as highly prevalent disease of domestic and wild animals, especially ruminants, is manifested as chronic granulomatous enteritis with decreased milk production, progressive emaciation and death. Understanding the genetic variability of MAP strains are important in diagnosis, epidemiological investigations, and formulation of strategies for prevention and control of the disease. However, MAP strains in Nepal have never been characterized. This study was conducted to understand the molecular characterization of MAP isolates of Nepal. A total of 49 MAP isolates obtained from buffalo population of three different dairy pocket areas of Chitwan, Nepal were typed using IS1311 polymerase chain reaction-restriction endonuclease analysis (PCR-REA). The extracted DNA samples ($n = 49$) were analyzed for the presence of MAP specific sequences (IS900) using PCR. DNA samples were further subjected to genotype differentiation using IS1311 PCR-REA and IS1311 L2 PCR-REA methods. All the DNA samples were positive for the entire three MAP specific sequences based PCRs. This study revealed that 'Bison type' strain is the most prevalent MAP genotype circulating within the riverine buffalo population of Nepal. IS1311 PCR-REA showed that MAP DNA samples of Nepal origin belonged to 'Bison type', whereas, IS1311 L2 PCR-REA method showed similarity with "Indian Bison type" and different restriction profiles of 'Bison type' genotype as compared to non-Indian strains. The study concludes that "Bison type" MAP strains was prevalent in all the MAP samples obtained from riverine buffaloes of Chitwan, Nepal. These results provided important insights in the epidemiological understanding of paratuberculosis in Nepal and will be helpful while designing its control and prevention strategies.

Keywords: Bison type, IS1311/PCR-REA, MAP, Molecular characterization, buffalo, Nepal

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Paratuberculosis, also known as Johne's disease (JD), which is an economically important and endemic in many parts of the world including Nepal. This disease is considered as highly prevalent disease of domestic and wild ruminants and is manifested as a chronic granulomatous enteritis with decreased milk production, progressive emaciation and death (Yue et al., 2016). MAP is a fastidious, slow growing acid-fast organism (Behr & Kapur, 2008). Clinical symptoms of paratuberculosis include slowly progressive wasting and chronic or intermittent, therapy-resistant diarrhoea (Stabel, 1997), which

are intermittent initially, become progressively more severe and remain constant. Affected animals typically die as a result of dehydration and severe cachexia (Cheng et al., 2020).

Paratuberculosis causes huge economic impact on livestock industry, because of premature culling of animals, reduced weight gain, reduced feed efficiency, high morbidity (Kaur et al., 2011), poor carcass quality, reduced milk yield, increased disease susceptibility to mastitis and reproductive disorders leading to increased calving intervals, poor fertility and additional veterinary costs (Hasonova & Pavlik, 2006). Paratuberculosis is chargeable for considerable economic losses causing disease within the dairy industry within the U.S., estimated about over \$200 million each year (Groenendaal et al., 2015), and thus draws significant attention in developing as well as well developed countries. No country claims that it is free from MAP (Yue et al., 2016). The disease is taken into account as one amongst the foremost important diseases of ruminant population (Sivakumar et al., 2005), however, MAP has also been isolated from a large range of non-ruminant including horses, pigs, rabbits, foxes, stoats, weasels (Beard et al., 2001), humans (Chiodini et al., 1984) and non-human primates (McClure et al., 1987). Paratuberculosis is similar to Crohn's disease in humans (McAloon et al., 2019), which reflects that MAP is potential etiology of Crohn's disease (Sechi & Dow, 2015), and thus, has amplified the possible zoonotic threat.

Control of MAP infection is the priority of developed countries to secure animal productivity and to reduce human exposure. Vaccination is considered as the reliable method of choice for the control of JD in animals, however, efficacy of vaccines depends on the genotype of candidate strain used (Singh et al., 2007). According to Sonawane et al. (2015), the genetic variability of various MAP strains and their impact on infection and pathogenesis has significant implication for diagnosis and control of JD. Therefore, better understanding of the genotypes infecting livestock species is necessary for designing disease control strategies. Based on IS1311 polymerase chain reaction restriction endonuclease analysis (PCR-REA) method, MAP isolates are grouped into three genetically distinct genotypes, namely, 'Cattle type', 'Sheep type' and 'Bison type' (Sevilla et al., 2005).

Several research has identified genomic variations in terms of genetic rearrangements, in-del polymorphisms and locus polymorphisms in 'Bison type' genotype of Indian origin in comparison with genotypes reported from other parts of the world, and thus, new nomenclature 'Indian Bison type' was assigned to 'native Bison type' genotype (Sohal et al., 2010). As contrast, molecular signature (sequence variations of native genotype) was deletion of two base pairs (TG) at 64th and 65th positions of IS1311 element particularly at locus 2 as compared to non-Indian isolates (Sohal et al., 2010) and with advantage of this molecular signature, a brand new IS1311 locus 2 specific PCR-REA (IS1311 L2 PCR-REA) assay was optimized for discrimination of 'Indian Bison type' from other isolates (Sohal et al., 2013).

The prevalence of MAP in dairy buffaloes of Nepal was detected to be 16.59% by faecal PCR (Singh et al., 2020). However, these MAP strains have never been molecularly characterized to understand the circulating strains in Nepal. Thus, this present study was conducted to understand the molecular characterization of MAP isolates from water buffalo in different dairy pocket areas of Chitwan, Nepal.

MATERIALS AND METHODS

Sample and sampling

Samples were collected from three major dairy pocket areas namely, Ratnanagar, Gitanagar and Mangalpur of Chitwan district, Bagmati province, Nepal. The buffaloes herds were selected on random basis and sampling were done from individual lactating cows old ≥ 2 years showing diarrhea and poor body score with suspect of the disease. The faecal samples were kept at 4°C until they were transported to the laboratory and subashrimal2051@gmail.com for further processing.

Bacterial isolates, DNA extraction and IS900 PCR

Fecal cultures of the samples were done under protocol as recommended by Ristow et al. (2006) and DNA extraction from MAP colonies was done as stated by van Soolingen et al. (1993). Later, PCR using specific IS900 commercial PCR test primer was performed done for confirmation of DNA samples and standardization. The use of IS900 in this type of PCR is sensitive to reveal very low numbers of MAP, but insufficient for accurate quantification of CFU within the sample, since it is present in many copies within the bacterial genome (Singh et al., 2018).

Molecular characterization of MAP by IS1311 PCR-REA

A total of 49 MAP isolates (harvested by fecal culture and later confirmed by IS900 gene PCR) of various locations were processed for the investigation of molecular genotyping (molecular characterization) using IS1311 PCR-REA and IS1311 L2 PCR-REA. Out of the 46 MAP isolates, 16, 15 and 15 were from Ratnanagar, Geetanagar and Mangalpur, respectively, of Bagmati Province, Central Plain region of Nepal. Similarly, to examine the specificity of IS1311 L2 PCR-REA, a set of five mycobacterial isolates aside from MAP [*M. marinum*, *M. fortuitum*, *M. kansasii*, *M. bovis*, *M. bovis* (BCG)] were used for this research. All the mycobacterial isolates were subjected to isolation of genomic DNA as per the method designed by van Soolingen et al. (1993). DNA samples recovered from isolates samples were subjected to IS900 (Singh et al., 2020) specific PCRs for the molecular identification of MAP.

IS1311 PCR-REA (Genotyping of isolates): Genotyping of every MAP DNA obtained from mycobacterial isolates was allotted by IS1311 PCR-REA method (Sevilla et al., 2005). In brief, the reaction was distributed in 30 µl volume, containing 20 µl positive IS1311 PCR product, 3 µl 10X buffer (Fermentas, USA), 2 units of every endonuclease *HinfI* and *MseI* (Fermentas). Reaction mixture was incubated at 37°C for 1.5 hrs. Band patterns were visualized after electrophoresis on four percent high resolution agarose gel stained with ethidium bromide and genotype profile analysis was done as described by Sevilla et al (2005).

IS1311 locus 2 PCR-REA (Sub-genotyping of MAP isolates): IS1311 L2 PCR-REA was used to specify all MAP isolates DNA included during this study. Initially, locus 2 of IS1311 was amplified using the distinct primers (P1: CACCAACCATGCAGAGGTAA; P2: GGAATCCGCAACTCCAAAT) and then amplicons were subjected to restriction digestion using *BsaJI*. PCR reaction mix contained primers (10 pmoles), *Taq* polymerase (1 unit), MgCl₂ (1.5 mM), dNTPs (0.2 mM), buffer 10X (2.5 µl) and template DNA (5 ng) during a final volume of

25.0 µl at thermocycler conditions: denaturation at 95 °C for five minutes followed by 40 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C at 30 sec, extension at 72 °C for 1 min. followed final extension at 72 °C for 7 min. PCR products were visualized on 1.5 percent agarose gel. Amplification products (~425 bp) were digested with *Bsa*II enzyme for two hours at 37°C and band pattern was detected on four percent agarose gel.

RESULTS

Molecular detection of MAP isolates by using PCR targeting IS900 sequence

All the samples were tested by IS900 PCR before proceeding to Polymerase Chain Reaction - Restriction Endonuclease Analysis (PCR-REA). MAP specific IS900 PCR showed presence of MAP DNA in all 49 DNA isolates (samples) as shown in Fig. 1, except those extracted from isolates of mycobacteria other than MAP. The reference MAP strains K-10 and ATCC19698 were found positive by PCR method.

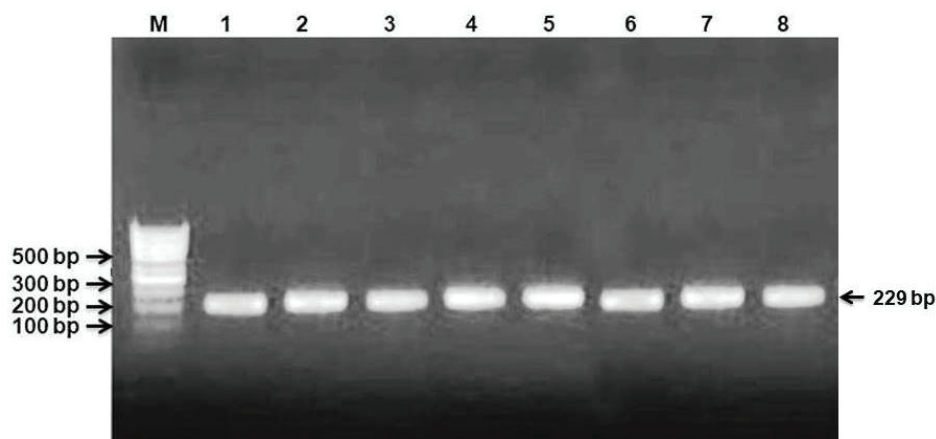


Figure 1. Molecular detection of MAP isolates by using PCR targeting IS900 sequence (specific product size 229 bp). Lane M: 100 bp marker; Lane 1: Positive Control (Indian Bison type MAP S5); Lane 2: Sample no. R09 (MAP positive); Lane 3: Sample no. R13 (MAP positive); Lane 4: Sample no. R16 (MAP positive); Lane 5: Sample no. G06 (MAP positive); Lane 6: Sample no. G10 (MAP positive); Lane 7: Sample no. M03 (MAP positive); Lane 8: Sample no. M07 (MAP positive).

Molecular typing of MAP by IS1311 PCR-REA and IS1311 L2 PCR-REA

IS1311 PCR-REA (Genotyping): All 49 MAP DNA samples showed the pattern of ‘Bison type’ genotype (Figure2). Thus, it was confirmed that all MAP DNA samples of three different regions of Nepal belonged to ‘Bison type’.

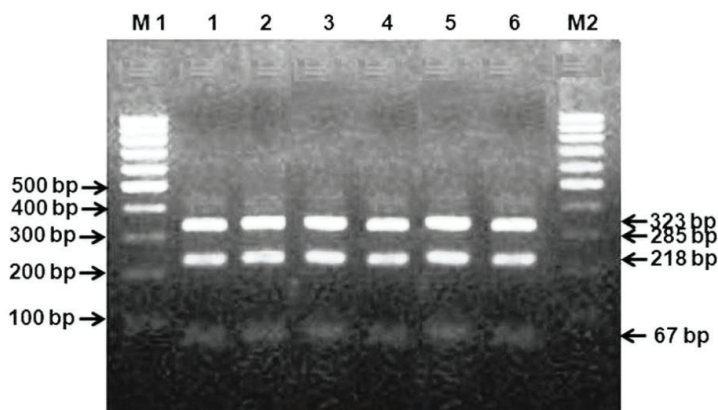


Figure 2. Genotyping of MAP isolates by using *IS1311* PCR-restriction endonuclease analysis. Lane M1: 100 bp marker; Lane 1: Isolate no. R07 (Bison type MAP positive); Lane 2: Isolate no. R11 (Bison type MAP positive); Lane 3: Isolate no. G06 (Bison type MAP positive); Lane 4: Isolate no. G09 (Bison type MAP positive); Lane 5: Isolate no. M07 (Bison type MAP positive); Lane 6: Isolate no. M11 (Bison type MAP positive); Lane M2: 100 bp DNA ladder/marker.

***IS1311* L2 PCR-REA (Sub-genotyping):** Restriction profile and band pattern for marker *IS1311* L2 PCR REA results similar with genotype of ‘Indian Bison type’ MAP from all the samples (Figure3). After restriction digestion of ~425 bp product (belonging to *IS1311* element at locus 2) with *Bsa*II enzyme, four digestion products were visualized in the 4 percent high resolution agarose gel (34, 67, 78 & 230 bp) for other MAP isolates. However, digestion of *IS1311* locus 2 amplicon of MAP DNA of Indian origin resulted into only three detectable bands in the gel (67, 78 & 262 bp).

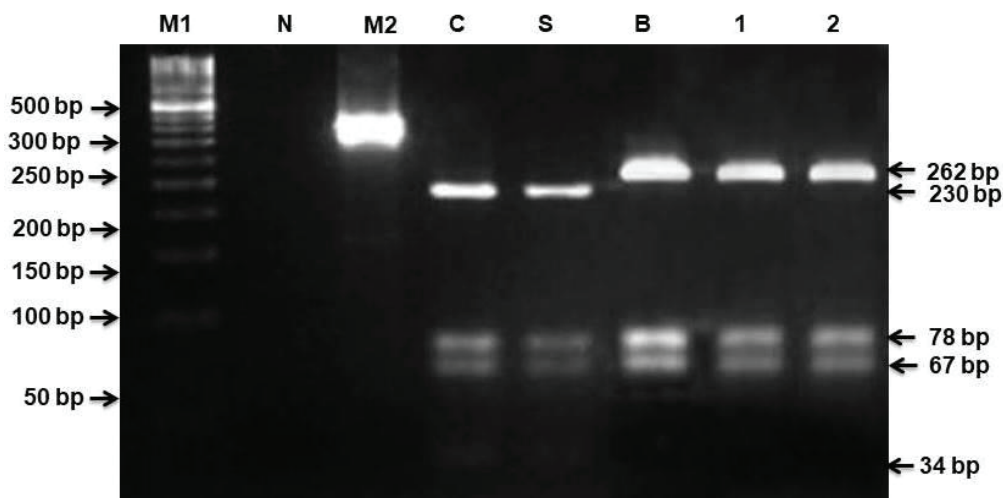


Figure 3. Discriminatory restriction pattern of typical MAP isolates using *IS1311* PCR locus 2-REA method. Lane M1: Marker (50bp); Lane N: Negative control, Lane M2: Undigested PCR product (~425bp); Lane C: Cattle type MAP positive; Lane S: Sheep type MAP positive; Lane B:

Bison type MAP positive); Lane 1: Isolates no. R16 ('Bison' type Indian origin MAP positive); Lane 2: Isolates no. M09 ('Bison' type Indian origin MAP positive). Note: MAP = *Mycobacterium avium* subspecies *paratuberculosis*; PCR = polymerase chain reaction; REA = restriction endonuclease analysis.

DISCUSSION

The molecular epidemiology of MAP has been neglected due to slow growing nature of bacteria in artificial medium. Thus, rapid strain differentiation methods are important to understand the origin of infection, disease transmission and to design disease control strategies. In the present study, IS1311 PCR-REA method was applied on MAP isolates from different dairy pocket areas of Nepal and MAP positive faecal DNA samples for determining their genotype. DNA based studies using 'Restriction Fragment Length Polymorphism (RFLP)' of genomic DNA from MAP isolates from a range of hosts confirmed the existence of two strains referred to as either sheep (S type) or cattle (C type) (Whittington & Sergeant, 2001), and one MAP strain referred to as bison (B type) (Sevilla et al., 2005). According to Thibault et al. (2012), it was found that the "Indian Bison type" strains were a sublineage of Type C strains.

All DNA samples obtained from MAP infected animals of Nepal were genotyped as 'Bison type' which is the first report to identify the strain of MAP present in Nepal. In present study, we could not detect any 'Cattle type' and 'Sheep type' genotype of MAP in animals. These results suggested that in Nepal, "Bison type" strains might be circulating among the buffaloes population, which is quite interesting, given that bison strains have been reported only in bison from USA (Sonawane et al., 2015). These findings were similar to that reported in previous epidemiological investigations conducted in north India (Singh et al., 2009). Similarly, Yadav et al., 2008 has published first report of the infection of buffaloes with MAP 'Bison type' strain. Contrary to the present finding, 'Bison type' was found to be the foremost prevalent MAP genotype whereas "Cattle type" was present in minority of cases from cattle, buffalo, and goats of India (Kaur et al., 2010). Likewise, studied done in India revealed that 'Cattle type' genotype of MAP was found as predominant genotype infecting domestic livestock, wild ruminant and non-ruminant species in other countries (Motiwala et al., 2006). 'Sheep type' strains are rarely associated with paratuberculosis in species other than sheep (Sevilla et al., 2007).

The results showed that new IS1311 L2 PCR-REA assay successfully discriminated genotype exactly similar to 'Bison type' genotype of Indian origin ('Indian Bison type') from MAP isolates of other genotypes (Cattle type, Sheep type and Bison type). The test was found to be very specific as all mycobacterial isolates (except *M. avium*) apart from MAP could not be amplified by the MAP specific primers. Due to high genetic similarity between *M. avium* and MAP, IS1311 L2 PCR amplified the DNA of both. However, the restriction profiles of IS1311 locus 2 by *Bsa*I restriction enzyme were different among both species. This study confirmed that 'TG' gap deletion at 64th and 65th position of IS1311 element at locus 2 was a stable marker and could be used in future strain typing as 'Molecular signature' and in epidemiological investigations (Singh et al., 2015).

This study confirms that MAP strains "Bison type" found in Nepal has similarity exactly with the "Indian Bison type" but different with other strains. This fact has significant epidemiological

implications and support the scientific aspects that diagnostics and vaccines prepared from foreign MAP isolates might not be very effective, except vaccine prepared from "Indian Bison type" strain.

Thus, in this study, only 46 isolates obtained from dairy buffaloes of three locations from Chitwan districts were considered, however, large numbers of isolates from different ruminant species and geographic regions have to be characterized so as to assess a national scenario about the foremost prevalent MAP genotype in Nepal.

CONCLUSION

In Nepal, MAP isolates infecting buffaloes have been first time genotyped as 'Bison type' causing Johne's disease. Based on PCR-REA patterns of the MAP isolated obtained from riverine buffaloes of various locations of Nepal, we conclude that the "Bison type MAP strain" was the sole circulating strain, which has important epidemiological implications in controlling paratuberculosis in Nepal. The current study validates that IS/311 L2 PCR-REA assay is a rapid and simple method for the differentiation of 'Bison type' MAP. MAP is an important livestock pathogen worldwide, which besides inviting trade restrictions, may be a potential human pathogen.

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Review of Veterinary Legislation for Prevention and Control of Infectious Diseases of Animals and its Situation in Nepal

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ABSTRACT

The morbidity and mortality caused by the animal infectious disease outbreaks result in significant losses to the farmers and the national economy. Besides, several animal diseases are zoonotic, thereby negatively impacting public health. It is imperative to control infectious diseases of animals effectively and promptly from both economic and public health perspective. Veterinary legislation plays an important role in the prevention and control of animal infectious diseases. The objective of this study is to review the legal provisions currently available to control animal infectious diseases in different countries and to identify the gaps in the context of Nepal. We found that several countries in the world including some countries in South Asia have already enacted the laws to control animal infectious diseases that have provided them a legal basis to effectively control infectious animal diseases. However, there is a lack of such a comprehensive legal basis for the control of animal infectious diseases in Nepal. There are some existing legal documents in Nepal such as the Animal Health and Livestock Services Act and its Regulation, Animal Slaughterhouse and Meat Inspection Act and its Regulation, and the Bird Flu Control Order. However, several provisions that are vital to successfully control animal infectious diseases effectively and promptly are missing in the existing legal documents. Further, these laws were prepared before the existence of the federal system of governance. Since the livestock and fisheries sector is rapidly commercializing in Nepal, the possibility of larger outbreaks in the animal population is increasing with intensified farming. This will also lead to the possibility of zoonotic disease spillover events in humans. It is necessary to immediately control animal diseases at their source to minimize the impacts that can be caused by infectious animal disease outbreaks. For this, it is recommended to formulate the animal infectious diseases control act and provide a legal framework for the timely control of animal infectious diseases. The new act with a clear funding mechanism should also consider the federal structure of the country and recognize the roles of stakeholders including federal, provincial, and local governments.

Keywords: Animal diseases; Animal Health Acts; Emerging and reemerging diseases; Economic losses; Regulations

INTRODUCTION

Infectious diseases are diseases that are caused by microorganisms including bacteria, viruses, fungi, and parasites (WHO, 2020). Both humans and animals are affected by a myriad of infectious microorganisms causing huge public health and socioeconomic impacts (Otte et al., 2004; Smith et al., 2019). It is estimated that nearly 60% of all infectious diseases and 75% of emerging and reemerging infectious diseases in humans are caused by pathogens of animal-origin (Weiss, 2001; Doorn, 2014). The most cost-effective way to prevent the emergence of emerging infectious diseases (EIDs) in humans is to invest in controlling diseases at the animal level and preventing disease spillover events (Ellwanger et al., 2019). There is at least two-fold advantage of controlling infectious animal diseases. First, it enhances the food security, food safety, and socio-economic status of farmers through increased production and productivity by preventing the losses caused by disease outbreaks in the animal population (Otte et al., 2004; Domenech et al., 2006). Second, it promotes public health by reducing the burden of zoonotic diseases and disease spillover events (Borremans et al., 2019; Dobson et al., 2020). The enormous loss of human lives and the economy that a single disease spillover event, if it takes the pandemic form, can cause has been well demonstrated by the ongoing COVID-19 pandemic (Pak et al., 2020; Nichola et al., 2020) and Nepal can remain no exception from the global pandemics (Dhakal and Karki, 2020). Further, many of the infectious animal diseases cause trade restrictions causing huge economic losses to the country.

In Nepal, the livestock sector is estimated to contribute about 10% to the national gross domestic product (GDP) and more than 25% to the agricultural GDP. The livestock sector is still largely subsistence-based with a large number of farmers rearing a small number of multiple livestock species which acts as an important source of cash income in the rural economy. Nepal has a huge livestock population with 7.3 million cattle, 5.1 million buffaloes, 10.9 million goats, 0.8 million sheep, 0.96 million pigs, and 68.6 million poultry (CBS, 2017). From these animals, 1.85 million metric ton milk, 0.32 million metric ton meat, 1.3 billion eggs, and 569 metric ton wool are produced annually (CBS, 2017). Despite the huge livestock population, the value of import of food and live animals has skyrocketed from 15,838.3 million rupees in 2007/08 to 130,622.7 million rupees in 2016/17 (CBS, 2017). During the same period, the export barely increased from 13,164.9 million rupees to 18,305.9 million rupees (CBS, 2017) resulting in a huge trade deficit. The Government of Nepal has been promoting the commercialization of the livestock industry to reduce the trade deficit and become self-sufficient in livestock products. In the last two decades, the poultry industry in Nepal has progressed a lot and is currently nearly self-sufficient in broiler meat and eggs. However, it relies heavily on other countries for inputs required such as parent stocks and feed ingredients. The dairy sector, goat farming, and pig industry are also rapidly commercializing in the peri-urban areas of larger cities with increasing demands for their products.

With this changing pattern of livestock husbandry, globalization, and change in the ecosystem, new challenges such as outbreaks of emerging and re-emerging infectious diseases and transboundary animal diseases are also increasing. For example, highly pathogenic avian influenza (HPAI H5N1) was detected in Nepal in Jhapa district in 2009 for the first time (Karki et al., 2014), and caused significant economic losses to the farmers (Karki et al., 2015; Karki, 2017). Other infectious diseases of animals such as Foot-and-mouth disease (FMD), Peste des petits ruminants (PPR), Classical swine fever (CSF), Hemorrhagic septicemia (HS), and a Black quarter (BQ) are

endemic in Nepal which is causing huge economic losses. Further, zoonotic infectious diseases such as Anthrax, Brucellosis, Tuberculosis, Leptospirosis, and Japanese encephalitis are also prevalent in Nepal. The Lumpy Skin Disease (LSD) has been officially confirmed in Nepal in July 2020 (OIE, 2020). There is also the possibility of the introduction of new diseases like African swine fever which is already causing havoc in the neighboring Chinese pig industry since 2018 and has been confirmed in Assam, India in May 2020 (Kabra et al., 2020; OIE, 2020; Penrith, 2020). If appropriate timely prevention and control measures are not applied, it might result in huge socioeconomic losses through outbreaks of infectious animal diseases and zoonotic diseases.

Nepal is a member of the World Organization for Animal Health (OIE) and is obliged to maintain OIE standards not only for World Trade Organization (WTO) requirements for the export market but also for local production and domestic consumption. Terrestrial Animal Health Code and other codes of OIE obliges all member countries to report disease situations in all animals including bee and fish with their new outbreaks. To meet such standard, control and diagnosis of animal diseases, surveillance of diseases, surveillance of drug residues in livestock products, need to be conducted following OIE protocols across the country. Animal quarantine is equally important to control transboundary animal diseases (TADs) in the interest of both national and global livestock production. Further, to comply with these international standards, animal welfare should also be considered during livestock production and marketing.

However, the existing laws such as Animal Health and Livestock Services Act, 1999 do not provide enough legal framework for the prevention and control of infectious diseases in animals. Currently, there is no appropriate act available to provide a legal framework for the prevention and control of infectious diseases of animals in case of an outbreak or the entry or emergence in the country. The absence of legal provisions has made it complicated to seek farmer's support in the government-led control program due to the lack of provision for providing compensation to farmers to mitigate their losses. Further, from the change of governance to the federal system with three levels of government (federal, state, and local), there is a need for new acts and policies acceptable to all levels of government with clear roles and responsibilities and a linkage mechanism. The objective of this study is to review the currently available animal infectious disease control acts in other countries and identify the gaps in the context of Nepal.

METHODOLOGY

The methodologies include a review of the national and international laws and literature relevant to the control of infectious disease in animals.

FINDINGS

Current Animal Health Structure in Nepal

Currently, Nepal has adopted three levels of government: Federal; Province, and Local. There are seven provinces and 753 local bodies (6 metropolitan cities, 11 sub-metropolitan cities, 276 municipalities, and 460 rural municipalities). In the animal health sector, the Ministry of Agriculture and Livestock Development is the apex body at the federal level, whereas the Department of Livestock Services (DLS) serves as a lead technical agency under the Ministry.

Under the new structure, there are three major divisions within DLS headed by Deputy Director Generals namely Animal Diseases Investigation and Control Division, Animal Quarantine Division, and Animal Genetic Resources and Economic Analysis Division. Under the direct supervision of the DLS, there are five laboratories: Central Veterinary Disease Investigation Laboratory (CVL), National Vaccine Production Laboratory, Veterinary Standards, and Drug Regulatory Laboratory, National Avian Disease Diagnostic Laboratory, and FMD & TADs Investigation Laboratory. Under the CVL, there are five veterinary laboratories at Biratnagar, Janakpur, Pokhara, Surkhet, and Dhangadhi.

Under the provincial government, the animal health sector is headed by the Ministry of Agriculture, Land Management, and Cooperatives. In the new structure, the previous 75 District Livestock Services Center (DLSOs) has been collapsed and replaced by 47 Veterinary Hospitals and Specialised Service Center (VHSSC) which falls under the jurisdiction of the Provincial Directorate of Livestock and Fisheries Development under the provincial Ministry. Previous livestock service and sub-service centers now fall under the jurisdiction of local government.

Existing major legal framework related to animal disease control in Nepal

Animal Health and Livestock Services Act, 1999

Under this act, there are provisions for the appointment of Veterinary inspector and quarantine officers. The veterinary inspector's jurisdiction is to inspect the quality and standard of veterinary drugs or biological products while the quarantine officer shall be responsible for the inspection of animals at the quarantine check posts. The Office In-charge or the Quarantine Officer may restrict the movement and transport of animals from the place of the Kingdom of Nepal where there is an outbreak of contagious diseases in animals to other places of the Kingdom of Nepal. Besides, there is a provision to establish temporary quarantine check-posts and restrictions on the movement of Nepal from areas of a contagious disease outbreak. There is also the provision of a permit requirement for the import and export of animals and animal products.

If there is any suspicion of contagious disease, a veterinary inspector might enter into the location to examine animals, animal products, or animal production inputs. If those inspected animals are found to be infected with diseases specified in the notification published in Nepal Gazette, the office in-charge remove or destroy such animal and animal products. There is also provision for the prevention of cruelty to animals by notification in the Nepal Gazette.

Animal Health and Livestock Services Regulation, 2000

The major provisions in this regulation include provisions to notify infectious diseases to the nearest livestock services center or sub-center or the Livestock services office and upon an investigation by the concerned technician, the information thereof has to be given immediately to the Veterinary Officer designated by the Department. In the regulation, there is provision for quarantine periods for different notifiable diseases. The procedure for disinfection has also been described in this regulation. Likewise, there is a provision that allows the office in charge in case of suspicion suspects for contagious disease, in any animal, animal products, or animal production inputs to examine such animal, animal products, or animal production inputs by entering the places

of their location. Also, procedures for physical examination of animals have been described in the regulation.

Animal Slaughterhouse and Meat Inspection Act, 1999 and its Regulation, 2001

This act and regulation were enacted to regulate the establishment of slaughterhouse and arrangements for meat inspection to safeguard the health and welfare of the people in general and to control adulteration in meat and meat products and to maintain a reasonable standard of meat by protecting the wholesomeness, quality, and adequacy of meat. The major provisions under this act and regulation include the requirement of a permit to establish a slaughterhouse, provisions related to the establishment of the slaughterhouse, the appointment of the Meat Inspector and Supervisors; ante-mortem and post-mortem inspection of animals, the prohibition of selling adulterated meat, and power of meat inspector and supervisor to enter the slaughterhouse for inspection.

Bird Flu Control Order, 2064

The Bird Flu Control Order, 2064 provides guidelines and legal framework to conduct control activities during highly pathogenic avian influenza outbreaks. Under this order, there are provisions for declaration of an infected area as well as de-notification of the outbreak area after the area becomes free from the disease. There is also the provision of culling birds to control the disease as well as compensation of killed birds under this order. However, the existence of this order is questionable as the mother law on this order was based on is replaced by a new act.

Nepal Veterinary Council Act, 2055 and Its Regulation, 2057

The Act includes a mandatory provision of registration of veterinarian (Clause 11) in order to practice veterinary profession. Nepal Veterinary Council is also mandated for policy advocacy to government for quality veterinary services including the prevention and control of animal infectious diseases.

The Criminal Code, 2074

The Criminal Code, 2074 includes few provisions in relation to animals that may have implication with the transmission, prevention and control of animal diseases. Some of those provisions include prohibition of carelessness, abandonment, bestiality, cruelty and such activities have been considered as crime and subjected to penalty in terms of fine and/or jail.

The Constitution of Nepal

The Constitution of Nepal ensures the availability of quality materials and services, food security (Clause 36), health, consumers right (Clause 44) and prosperity as the fundamental rights of citizen. The detailed description of the three-tier government for prevention and control of animal infectious diseases is described in unbundling report prepared for implementation of the Constitution. The Federal government is mandated to regulate the international trade and animal quarantine system. Provincial government is mandated for livestock development while local level

governments for management of animal health and disease control. Despite of the constitutional provision of coordination there is an urgent need to develop a mechanism of linkage between the three level of government in relation to the reporting, prevention and control mechanism of animal infectious diseases.

International legal provisions related to Animal Disease Control

Several countries across the world have legal provisions in place to govern the control of animal infectious diseases. We reviewed animal infectious diseases act from 20 countries, territories, and provinces of countries to understand the practices and provisions in other countries regarding the control of animal infectious diseases act. The list of countries, names of the act, and the promulgated year have been listed in Table 1.

Table 1. Animal Infectious Diseases Control Act in different countries

SN	Name of the Act	Year	Latest amendment	Country
1	The Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009	2009		India
2	Animal Diseases Act	1992		Sri Lanka
3	Act on Domestic Animal Infectious Diseases Control	1951	2012	Japan
4	Act on the Prevention of Contagious Animal Diseases	2002	2015	Korea
5	Decree on the prevention and control of Animal Diseases	2012		Lao PDR
6	Prevention and Control of Infectious Diseases Act 1988	1988	2006	Malaysia
7	Infectious Diseases	2003	2010	Brunei
8	Animal Diseases Act	1989	2012	Kenya
9	Animal Diseases Act, 1984	1984	1996	South Africa
10	Animal Diseases Act	2003		Tanzania
11	The Animal (Diseases and Importation) Act	1948	1982	Jamaica
12	Animal (Diseases and Importation) Act	1954	1997	Trinidad and Tobago
13	Animal Health Act, 1981	1981		UK
14	Animal Diseases Act, 2013	2013		Finland
15	Health of Animals Act	1990	2013	Canada
16	Animal Contagious Diseases Act	1962	2001	Bahamas

Below is the summary of major provisions in the animal infectious disease control acts of different countries:

Table 2. Major provisions in Animal Infectious Diseases Control Act of different countries

SN	Country	Major provisions
1	India	The Central Government has the authority to implement this act as a whole or different provision under this act in different states or areas of India. The major provisions under this act are an authority to appoint veterinary or competent officers; obligation of reporting of diseases enlisted in Schedule; authority designate controlled or free areas from certain diseases mentioned in Schedule; animal movement control; authority to vaccinate and marking; declaration or de-notification of the infected area; segregate, examine or treat animals; drawing samples from animals and practice euthanasia of infected animals when there is a possibility of zoonotic disease transmission. There are also provisions for penalties for noncompliance to this act such as a penalty for obstructing competent authority to perform their duties or for disposing of infected animals or carcass in river or failure to prevent the escape of infectious organisms for laboratories, institutions, and clinics. The act also has the provisions for the state government to delegate its power to officers or authority subordinate to it while the Central Government may omit or add the diseases listed in the Schedule as necessary.
2	Sri Lanka	The major provisions envisioned in this act are an authority to the director to seal the infected premises and area; animal movement control; immunization of animals in the infected area; seizure of infected animal and carcasses; destruction and disposal of an animal infected or come into contact with diseased animals; seizure of animal products and their destruction; disinfection of area in a prescribed manner; testing of animals for detection of specified diseases; and prohibition of sale or stock. The act also has provisions for mandatory registration of animal clinics, animal houses, or hatchery. Also, prior licenses need to be obtained under this act to manufacture the veterinary drug and veterinary biological products. Also, a license is required to maintain stud bulls and donor cows. There are provisions that require a permit and health certificate to import animal products, embryo, or semen in Sri Lanka. There is also the provision of Quarantine and the establishment of the Veterinary Drug Control Authority in this act. There are also provisions of penalties for non-compliance with the provisions in this act for a term not exceeding six months or to a fine not exceeding five thousand rupees or to both such fine and imprisonment.
3	Japan	The major provisions under this act include mandatory reporting from diagnosing veterinarian to the prefectural governor without any delay in case of diagnosis or suspicion of infectious diseases who have to them make a public notice; provision of inspection by prefectural animal health inspectors to ascertain the status and progress of outbreak; authority to order owners of animals to undergo injection, dipping or medication; issuance of a certificate of inspection; installation of equipment for disinfection; standards of hygiene rearing management; carcass

		management; movement control and restrictions of animal gathering events such as horse races; livestock shows or livestock markets. The additional provisions in the act include the provision of quarantine; permit requirements for import and export; appointment of pathogen handling officers; authority for animal quarantine officers or prefectural animal health inspectors for spot inspections; provisions of compensation; and provisions of penalties for non-compliance to provisions.
4	South Korea	Act on the Prevention of Contagious Animal Diseases was formulated in South Korea in 2002 and last amended in 2015 to develop the livestock industry and public health by preventing the outbreak or spread of contagious animal diseases. Under this act, contagious animal diseases are classified into Type I, Type II, and Type III categories. The major provisions under this act are the establishment of a system for the prevention of contagious diseases and the early detection and reporting thereof; declaration of an outbreak; establishment and operation of national integrated information system for animal disease control; establishment of Central Animal Disease Control Council; obligation of farmers and animal owner to keep their farm clean; disinfect and cooperate with local and state governments to enforce animal disease control; educating farmers and appointment of veterinary inspector and veterinary assistants to assist veterinary inspectors. Other provisions in this act include the establishment of a mobile organization for contagious animal disease control; mandatory reporting by veterinarians of dead or sick livestock they have examined; provisions for epidemiological investigation; quarantine and authority to close livestock raising facilities; movement control; order to the slaughter; prohibition of importation; compensation and provisions for penalties in case of breach of this act.
5	Lao PDR	The major provisions under this decree are disease prevention and control measures (for example risk assessment based control measures); animal disease prevention measures such as vaccination and surveillance; creating a list of notifiable diseases based on OIE lists; establishing surveillance system; authority to adopt vaccination system and declaration of an outbreak of animal diseases. Other provisions in this decree include the application of animal disease control measures and establishment of animal diseases free area; control of animals being infected or suspected of being infected; provisions for the red and yellow zone; destruction and disposal of diseased animals; compensation and notification to OIE and neighboring countries; the obligation for farmers to report; and right and duties of different levels of government.
6	Malaysia	This act mainly covers infectious disease control in humans but there are some provisions related to the controlling of contaminated animals. The major provisions under this act that cover animal infectious diseases include the appointment of authorized officers, control of contaminated articles and infected animals, entering any premises if there is suspicion

		of contaminated animals, the power to seize and dispose of contaminated animals, provisions for cost and expenses recovery, and power to make regulations.
7	Brunei	This act is primarily prepared from the human infectious disease perspective but also has provisions for control of animal infections. The major provisions related to animals in this act include quarantine measures, epidemiological surveys, disposal of animals, food, and water if they are a source of transmission of infectious diseases, animal movement control, and prohibition or regulation of importation and exportation of animals.
8	Kenya	The major provisions under this act include the appointment of veterinary inspectors, farmers, or animal owner's obligation to notify the inspectors or administrator officer; inform the neighborhood about the outbreak; provision of declaring infected area or free after the disease is controlled; movement control in the infected area; safe disposal of the carcass; prohibition of the importation of animals and provisions for the slaughtering of infected animals. There are provisions for indemnity and payment of compensation however compensation may be withheld or not paid if the person or owner of animals was found guilty for breach of any provisions of this act. The Director also has the power to search for infected animals by entering any land, farms, or vehicles. There are also provisions for penalties for breaching the provisions of this act and for obstructing any officials while executing their duties and power to seize the animals in case of breach of the act.
9	South Africa	The major provisions under this act include the appointment of authorized officers to exercise the power under this act; establishments of quarantine stations; and requirements of a permit for import and export of animals and their products. Further provisions under this act are applications of control measures for control of animal diseases and parasites; establishment of animal health schemes; obligations to farmers/owners for reporting of animal diseases; power to enter into land or animal premises to inspect; power to seize animals with suspicion of disease; compensation; requirement of a permit to conduct an investigation, experiment or research with any vaccine, serum, toxin, anti-toxin, antigen or other biological product; and objections against decisions made by the officer.
10	Tanzania	The major provisions under this act include the appointment of authorized officer to exercise the power under this act; measures for checking livestock diseases; movement control of animal and animal products from the infected area; seizing and destroying of animals and animal products; isolation of suspected animals; declaration of buffer zone between infected and uninfected zones; and obligation for reporting for owners to the nearest veterinarian or paraprofessional within 24 hours. Other provisions include setting up quarantine; authority to slaughter or destruction of affected animals; power to inspect animal facilities and disinfection; compensation; establishment of a national epidemiological

		system; antemortem and postmortem in slaughtering premises and prevention and control of bee diseases.
11	Jamaica	The major provisions under this act include control of diseases or suspected animals; declaration of infected areas; the slaughter of diseased or suspected animals; disposal of animals and provisions of compensation; requirement of a permit for the importation of animals; establishments of quarantine facilities; powers of apprehension; penalties for offenses; and power to prohibit the use of railways or public roads.
12	Trinidad and Tobago	The major provisions under this act include control of diseased, suspected, or infested animals; requirements for importation and exportation of animals; establishment of quarantine facilities; powers of apprehension; and appointment of inspectors; movement control of animals; and authority to inspect animal facilities.
13	United Kingdom	The major provisions under this act are the authority for the Minister to expend for disease eradication with the Treasury's approval; declaration of eradication or attested area; movement control; cleaning and disinfection of cleaning areas; prohibiting or regulating the holding of markets, fairs, exhibitions and sales of animals; may prohibit or regulation of the importation of animal and their products; owners should keep diseased animals in isolation during an outbreak; declaration of infected area and authority to enter any land if there is a suspicion of a disease outbreak. Other provisions in the act include authority to quarantine mammals suspected of Rabies; exclusion of strangers; seizure of diseased or suspected animals; control of Zoonoses; authority to slaughter animals with certain diseases; seizure and disposal of carcasses; compensation for the seizure; prevention of suffering; and requirement of certificate for import and export of animals.
14	Finland	The major provisions under this act include the obligation of an operator to establish procedures for preventing the spread of animal diseases; compulsory and voluntary health control measures; vaccination and other treatments; measures to be taken during the organization of animal shows; and restriction to release suspected animals into wild. Other major provisions under this act include an obligation to report animal diseases; disease surveillance plan; measures to be taken during outbreaks; investigation of the origin and spread of animal diseases; decisions to eradicate disease at an establishment; measures to be taken in connection to zoonoses; diseases in wild animals; declaration of the infected and restricted area; authority to kill animals in an infected or restricted area; implementation of emergency vaccination; establishment of compartments; regulating animals shows; animals movement control and provisions for compensation.
15	Bahamas	The major provisions in this act include authority for Governor-General to appoint officers to implement this act; authority for officers to inspect with written notice; authority to seize animals in case of infected animals or suspicion of being infected; apprehension of offenders; the obligation for notification to farmers or owner of animals or veterinarians working

		in field and penalty for negligence; compensation to farmers for animals slaughtered under this act; provisions for prohibition of importation and authority to declare area free from disease. Other provisions in this act include a requirement of a license to move animal and animal products outside the infected area; cleaning and disinfection of the infected area; provisions for quarantine; prohibition of livestock markets; fair, exhibition, or sales of an animal; expense payment as approved by the Minister through consolidated fund; and provisions for a penalty for breaching this act.
16	Canada	The major provisions under this act include obligations for owners to report disease; prohibition to keep diseases animals or bringing them to the market; requirements of a permit for import and export of animals; declaration of infected and control zones; movement control of animals from and to infected areas; authority to treat or destroy animals; power to arrest; authority to enter any premises for inspection and provisions for compensation.

Gaps to control animal infectious diseases in Nepal

Several gaps have been identified after reviewing national and international laws related to animal health particularly animal infectious disease control. The existing acts, regulations, and orders were developed before the federal system of governance was adopted in Nepal. The old acts do not recognize the federal system such as state governments and local governments. The already existing laws also need to be amended to reflect the new structure. Besides, the existing laws do not have provisions for the control of infectious diseases other than highly pathogenic avian influenza based on Bird Flu Control Order. There are some provisions of movement control and destruction of infected animals but there is no provision to compensate farmers. Further, officials from the competent authority do not have any legal right to enter farm premises if there are suspected infectious animals within the farm. The provisions to declare infected area or freedom from disease after control measures are currently lacking except for the HPAI. There is no legal provision to apply appropriate control measures such as culling, isolation, vaccination, or disinfection in case of an outbreak of animal infectious diseases. Besides, there is no legal framework to guide the management of animal carcasses which can be a source of infectious disease transmission. Finally, there is no umbrella act to provide a legal framework for the control of infectious animal diseases in Nepal.

CONCLUSIONS AND RECOMMENDATIONS

In summary, several countries in the world have enacted laws to control animal infectious disease, however, there is a lack of such law in Nepal. There are some provisions under the existing laws such as the appointment of quarantine officers and veterinary inspectors. However, these acts also need to be updated to align with the new federal structure of the governance. Several provisions that are vital to successfully control animal diseases such as power to enter the suspected premises, declaration of infected zones, and provisions of compensation are missing. The livestock and fisheries sector contributes significantly to the national economy and is commercializing rapidly. With this, there is a possibility of larger outbreaks as animal farming gets intensified. Besides,

there is also a threat of zoonotic disease transmission. To minimize the impacts that can be caused by infectious animal disease outbreaks, it is necessary to immediately control animal diseases at their source. For this, it is recommended to formulate the animal infectious diseases control act and provide a legal framework for the timely control of animal infectious diseases. The new act should address the new federal structure of the country and recognize the roles of federal, provincial, and local governments. There should also be clarity in the funding mechanism to implement the new act.

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Cysticercosis in Nepal: A Review

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ABSTRACT

Cysticercosis is the infection caused by larvae of Taenia solium, which is one of the major zoonotic diseases in Nepal. Infection of larvae in the central nervous system is neurocysticercosis and infection of adult tapeworm is taeniasis. Cysticercosis is associated with the social, cultural, and economic aspects of the country and is endemic in many developing countries like Nepal. Detection of neurocysticercosis among eight Gurkhas in Hong Kong (1987) shows its existence in Nepal since many decades ago. The occurrence of cysticercosis/neurocysticercosis is higher in males and young people are more susceptible. Lack of awareness about disease and seizure, unhygienic behaviours, free-roaming pigs, and lack of surveillance are major factors contributing to the transmission of this diseases from pigs to humans and among humans. Cysticercosis is endemic in Nepal and a large number of cases are reported in hospitals. The Social, public health and economic impact of the disease is yet to be evaluated. Increased survey and surveillance to understand the burden of cysticercosis throughout the country along with public awareness is urgently needed. The collaboration of multi-stakeholders including human and animal health, pig farmers, and policymakers using the concept of “One Health” is needed to reduce the burden of cysticercosis/neurocysticercosis in Nepal.

Keywords: Cysticercosis; Nepal; Neurocysticercosis; Taeniasis

INTRODUCTION

Cysticercosis is the infection caused by larva of *Taenia solium* (pork tapeworm) which belongs to Cestoda class of phylum Platyhelminthes (Gripper and Welburn, 2017). Neurocysticercosis; cysticercosis of central nervous system, is an ancient, commonest helminthic disease of the (Del and Sotelo, 1988). Taeniasis is the infection caused by adult tapeworm and cysticercosis is used for infection due to larval or metacestode (cysticercus) stage of *Taenia solium* (Carpio, 2002; Gripper and Welburn, 2017). Cysticercus develop as neurocysticercosis after infecting the nervous system (José et al., 2018). This disease is related to social, cultural, and economic factors of any place or country and is endemic in developing countries like Nepal (Del Brutto et al., 1988). The occurrence of Taeniasis in society is considered a biological indicator of social and economic development (Pal et al., 2000). Headache, seizure, and focal deficits are major neurological symptoms (Fogang et al., 2014), but has a different range of pleomorphic clinical manifestation depending on the number and size of larvae, stage of development, and localization within the brain (Gripper and Welburn, 2017). Clinical history, neuroimaging, immunological evidence in combination with epidemiological factors can lead to a diagnosis of the disease (Del Brutto et al.,

2001; Gripper and Welburn, 2017). Pleomorphic and heterogenicity of the clinical appearance of disease not only affects diagnosis but also makes it difficult to treat (Gripper and Welburn, 2017). Improve sanitation, education, and awareness, corralling pigs to prevent contact human feces and development of a vaccine for pigs helps in the prevention and control of the disease (Gripper and Welburn, 2017; Pawlowski, 2016).

Taenia solium is one of the major medical issues in Nepal and causes the highest burden among parasitic infections (Devleesschauwer et al., 2014). Nepal is an agricultural developing country, with majority population living in rural areas which is gradually changing. From 1992 to 2015 rural population of Nepal decreased from 97.1% to 59.51% (Rimal et al., 2017). There are limited toilet facilities in rural areas due to which people often defecates in an open area. In 2015, around 1.1 billion population across the world practiced open defecation (McMichael, 2018). In a study in the rural village of Nepal, 60 % of people had no toilet facilities (Karn et al., 2012), which are the primary barrier of fecal-oral disease transmission (McMichael, 2018). Human feces are the source of contamination of food and water with *Taenia solium* eggs (Gripper and Welburn, 2017). Infection with *Taenia solium* is not only due to uncooked pork meat but also caused by contaminated, unhygienic vegetables (Adhikari and Bagale, 2019). In Nepal, epilepsy cases are increasing up to 7.3/1000 population and around 50% of cases are due to neurocysticercoses (Joshi et al., 2007). Sanitary habits, low economic status, open pig rearing system, food preparation, and consuming habits, and open defecation are the risk factors associated with NCC (Joshi et al., 2007). Neurocysticercosis is one of the prioritized zoonotic diseases in Nepal by the Zoonotic Control Project (ZCP) of World Bank and Epidemiology and Disease Control Division, Department of Health Services, Nepal Government (Acharya et al., 2019). Although cysticercosis including is endemic and widespread in Nepal, very few studies have been made and approach of one health has not emphasized yet. The main aim of this article is to review different articles and literatures and propose present situation of porcine and human cysticercosis in Nepal and also to address prevention and control measures.

Etiology

Taenia solium belongs to phylum, Platyhelminthes, family, Taeniidae, order, Cyclophyllidea and subclass, Eucestoda (Pawlowski, 2016; Del Brutto et al., 2001). The adult tapeworm is two to four meter in length which consist of a scolex and a strobila and lives in the intestine of human beings (Carpio, 2002). Scolex consists of four suckers and a rostellum with 22-32 hooks and strobila (elongated segmented tape-like body) which consist of 700-1000 segments known as proglottids (Carpio, 2002; Pawlowski, 2016). Each proglottid consists of about 40,000 eggs and *Taenia solium* sheds up to 300,000 eggs per day (Carpio, 2002). An egg consists of an oncosphere with six hooklets and is covered by an embryophore (Pawlowski, 2016). The egg can act as a source of infection of people after shedding in feces. Mature oncosphere is a globular larva, 30µm in diameter and consist of six characteristic embryonic hooklets (hexacanth embryo) and a pair of penetration gland for migration (Carpio, 2002). Development of oncosphere into metacestode i.e. cysticercus occurs in the intermediate host (pig). The oncosphere quickly changes from a solid larva into a bladder form filled with fluid and has groups of cells that differentiate into invaginated scolex (Pawlowski, 2016). Cysticercus is an ovoid bladder stage filled with an opalescent fluid and contains an invaginated scolex. From behind of scolex of cysticercus, adult tapeworm develops (Carpio, 2002).

Life Cycle and Pathogenesis

The life cycle of *Taenia solium* consists of two hosts and an environment. Human beings act as the final host; consist of adult tapeworm while pigs act as intermediate hosts; which ingest egg that develop into cysticercus. Human beings acquire the infection after consumption of cysticerci in meat, which develop into an adult tapeworm in the intestine which produces eggs (CDC, 2013) (Figure 1). Human beings can also be infected by eggs via external and internal autoinfection (Gemmell and Johnstone, 1976; Lawson and Gemmell, 1983). Fecal-oral infection with *T. solium* eggs in an individual with intestinal taeniasis is external autoinfection and infection with eggs through reverse peristalsis is internal autoinfection. Internal autoinfection is unlikely to happen because eggs have to pass through a brief period of peptic digestion, which is necessary for dissolving of embryophore before they can invade human tissue (Carpio, 2002; Pawlowski, 2016). Heteroinfection occurs when eggs are ingested in contaminated food, as occurs in areas where water for drinking and irrigation carries feces or when taenia carriers handle food (Carpio, 2002). Eggs and gravid proglottids are present in human feces which is ingested by intermediate host i.e. pig. The egg develops into the oncosphere which hatches and penetrates muscles and develops into cysticercus (Figure 1). Cysticercus can affect both humans beings and pigs and develop as neurocysticercosis after infecting the nervous system (José et al., 2018).

Neglected Tropical Disease

There are 17 neglected tropical diseases (NTDs) prioritized by the World Health Organization (WHO), taeniasis/cysticercosis is one of them. It is one of the NTDs due to its public health impact on socioeconomically disadvantaged people (Ito et al., 2014). A large number of cases of taeniasis/cysticercosis are unreported because people do not realize they are infected, thus can infect the other peoples too. Identification of *T. solium* eggs without laboratories and advance molecular tests are impossible and eggs are found in feces, which may go unnoticed. So, human cysticercosis is underreported in endemic areas (Praet et al., 2009). Absence of clinical symptoms in affected pigs and poor meat inspection service in endemic regions aids in the under-recognition of porcine cysticercosis (Praet et al., 2009).

Signs and Symptoms

Clinical manifestation of infection with cysticercosis in humans is pleomorphic and heterogeneous (Gripper and Welburn, 2017). The clinical appearance of cysticercosis depends on the site of the cyst and the burden of the cyst (Hawk et al., 2005; Kraft, 2007). The cyst can settle in the brain, spinal column, skeletal muscle, subcutaneous tissue, and eye (Kraft, 2007; Rath et al., 2010). Cyst in the brain (most common site) and eye (least common site) cause the most morbidity (Hawk et al., 2005). Parenchymal neurocysticercosis is the common cause of focal and generalized seizures and heavy cyst burden in neural tissue can cause, headache, nausea and vomiting, encephalopathy with fever, altered mental status, and seizures (Kraft, 2007). Cysts when occurring in the subarachnoid or ventricular spaces can cause, meningeal signs and symptoms, obstructive hydrocephalus, or cranial nerve palsies caused by nerve entrapment (García et al., 2003). A cyst can lodge in the extraocular muscle of the eyes, and affect eye movement which mimics cranial

nerve paralysis (García et al., 2003; Mohan et al., 2005). The presence of a cyst in skeletal muscle and subcutaneous tissue can cause nodule formation and pain (García et al., 2003; Kraft, 2007).

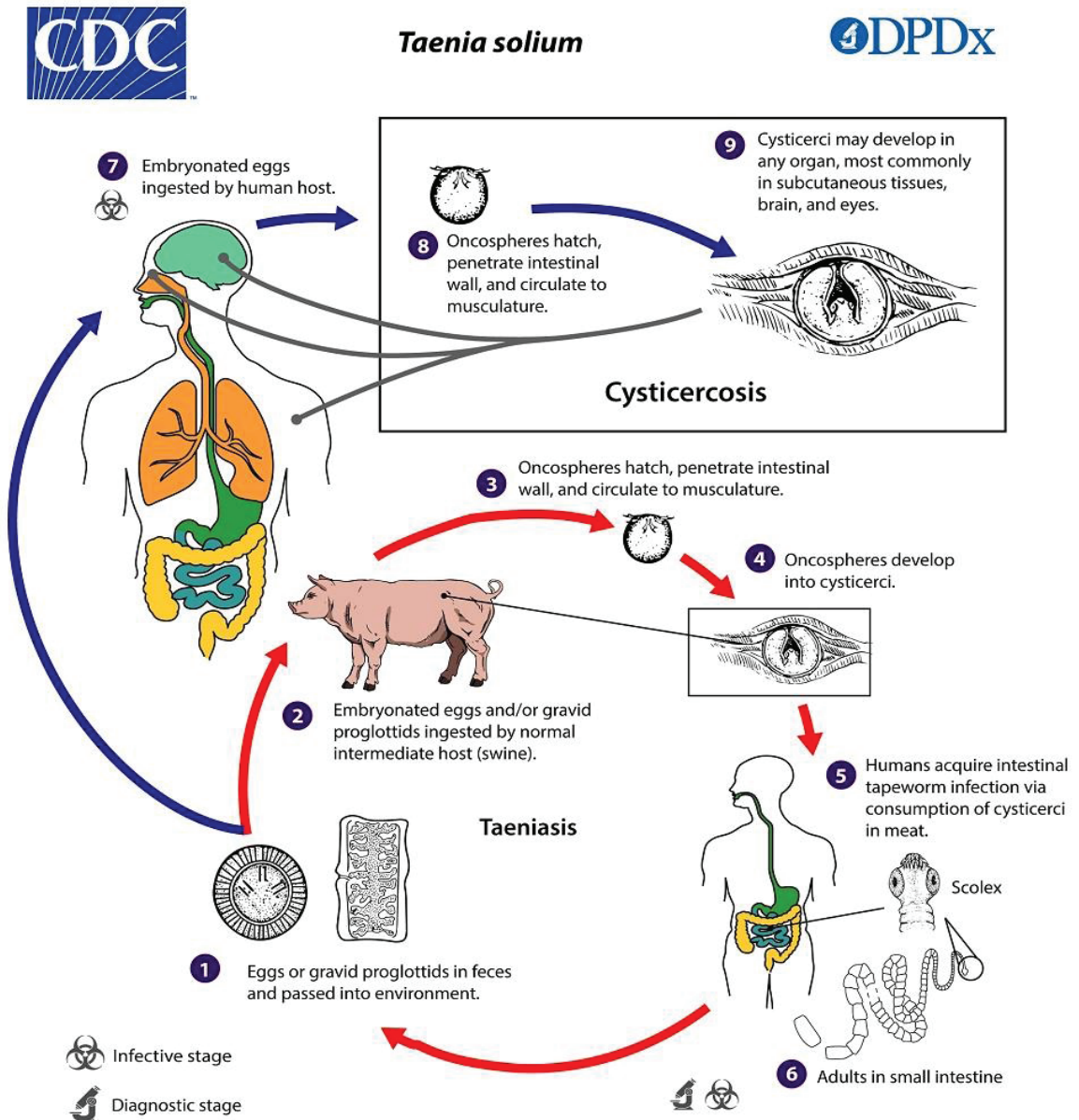


Figure 1: Life Cycle of *Taenia solium*

Diagnosis, Treatment, and Prognosis

The ultimate diagnosis of porcine cysticercosis is necropsy; detection of cysts which are usually present in striated muscle and brain (Gauci et al., 2019). Sign and symptoms of NCC in humans are not specific, neuroimaging findings may not be pathognomic, the serological test usually has low sensitivity and specificity (Carpio, 2002), because this combination of the different diagnostic tool helps in accurate diagnosis of NCC. The diagnosis of neurocysticercosis can be done accurately by clinical symptoms in combination with a history of the disease, x-ray of dependent parts, CT-scan or Magnetic Resonance Imaging (MRI), serological tests, and laboratory examination (Joshi et al., 2004). ELISA (enzyme-linked immunosorbent assay) is a serological diagnostic tool for demonstrating anticysticercal antibodies. Pathological confirmation via biopsy or autopsy is the only true reliable gold standard (Carpio et al., 1998). MRI and CT scan are major tools in the diagnosis of NCC and can be taken as gold standard (Carpio, 2002; Carpio et al., 1998). Complement Fixation Test (CFT), indirect haemagglutination, ELISA, and Enzyme-linked Immuno-electrotransfer Blot (EITB) are some immunological diagnostic tools of NCC (Carpio, 2002).

Treatment of cysticercosis and neurocysticercosis is complex and vary among different individual. Antiepileptic drugs for seizures are widely used in NCC. Mannitol or oral glycerol is used if high intracranial pressure and corticosteroids are commonly used in inflammation and edema around dying parenchymal cysts. There are controversies for the use of anthelmintic drugs like albendazole and praziquantel in the treatment of NCC, because this drug may lead to arachnoiditis, arteritis, and hydrocephalus when cysts are usually present in subarachnoid space (Carpio, 2002; Pal et al., 2000).

Cysticercosis and neurocysticercosis usually have a good prognosis in parenchymal form but the prognosis for the extra parenchymal form is not favorable when patients have arachnoiditis, arteritis, and hydrocephalus. The majority of patients had a good prognosis after treatment among different cases of different hospitals in Nepal (Basu et al., 2007; Khanal and Shrestha, 2019).

SITUATION IN NEPAL

Porcine Taeniasis and Cysticercosis

Very few studies have been made about porcine cysticercosis in Nepal. Between 1997 and 1998, 250 pigs from Kathmandu and Dharan were slaughtered, among them 13.6% were found positive for cysticercosis (Joshi et al., 2007). The study by Joshi et al. (2007) showed that 23.50% (204 pig sera) seroprevalence by ELISA and 32.50% (419 pig tongue) prevalence by lingual palpation (Joshi et al., 2007). In a study conducted by Joshi and Willingham in 2005, the prevalence of porcine cysticercosis was 10.5% (21/200) by lingual examination, seroprevalence was 22.5% (45/200) by ELISA method, and prevalence of *T. solium* cysticercus was 20.5% (41/200) in the post-mortem carcass organs examined (Joshi et al., 2007). Diagnosis with lingual palpation and post-mortem examination seems to be less accurate than ELISA, which may be due to human error and neglect. In a study in 2006, 320 pigs in Chitwan and Kathmandu Valley, the prevalence of cysticercosis was 0.63%, 0.94% for lingual and carcass examination respectively (Joshi et al., 2008). Out of the 250 pigs examined, 34 (14%) were positive for porcine cysticercosis in Kathmandu Metropolitan City and Dharan Municipality (Joshi et al., 2004). Seroprevalence of *T.*

solium cysticercosis was 13.8% among 742 pigs sampled from 2007 to 2010 from Kathmandu Valley (Devleesschauwer et al., 2013). From 2004 to 2005, the prevalence for cysticercosis was 0.99% (5 positives) of the 504 porcine carcasses inspected in Kathmandu Valley, Nepal (Sapkota, 2008). In 2014, 384 pig serum samples were analysed by Ag-ELISA test, 33 pigs were found positive for cysticercosis infection where apparent seroprevalence was 8.59% and the true seroprevalence was 7.9% (Chaulagain et al., 2017).

In Udaypur, Hirminiya and Betahani Village Development Committee (VDC) of the Banke district, 32 of the 110 animals were found positive for *T. solium* cysticerci (29%), of which 30 (27%) were found to have viable cysticerci (93% of the infected animals) (Sah et al., 2017). This is among the highest prevalence of porcine cysticercosis described for any region in the world (Sah et al., 2017). Most of the studies of porcine cysticercosis was carried out in limited districts like Kathmandu and Chitwan (Table 1), this may be due to the high numbers of veterinarians and animal health workers in these districts and due to availabilities of laboratory or easiness of conducting research. Very limited research and study are done in a remote place of the country, where the prevalence of cysticercosis might be high as in the study of Banke district. People of rural area are more susceptible to the neurocysticercosis because of social and economic status and few studies in the pig population of this area shows how vulnerable people are. Without study or research in pigs, human cysticercosis cannot be controlled, so, programs should be launched to reduce the burden of disease in pigs, which can eventually decrease the number of human cases.

Table 1. Prevalence of Procine Cysticercosis in Nepal

S. N	Year	District/Place	Detection Method	Prevalence % (Total Sample Size)	Reference
1.	1997-1998	Kathmandu Metropolitan City and Dharan Municipality	Post-mortem Carcass Evaluation	13.60% (250) 14.28% (196) Kathmandu 11.11% (54) Dharan	(Joshi et al., 2007)
2.	2001	Nepal	ELISA	23.50% (204)	(Joshi et al., 2007)
			Lingual Palpation	32.50 % (419)	
3.	2003-2004	Kathmandu Metropolitan City and Dharan Municipality	Post-mortem Carcass Evaluation	14% (250)	(Joshi et al., 2004)
4.	2004-2005	Kathmandu Valley	Post-mortem Carcass Evaluation	0.99% (504)	(Sapkota, 2008)
5.	2005	Nepal	ELISA	22.50% (200)	(Joshi et al., 2007)
			Lingual Palpation	10.51% (200)	
			Post-mortem	20.50% (200)	

			Carcass Evaluation		
6.	2006	Chitwan District and Kathmandu Valley	Lingual Palpation	0.63% (320)	(Joshi et al., 2008)
			Post-mortem Carcass Evaluation	0.94% (320)	
7.	2007-2010	Kathmandu Valley	ELISA	13.80 (742)	(Devleesschauwer et al., 2013)
8.	2014	Kathmandu Valley	ELISA	8.59% (384) Apparent 7.90% (384) True	(Chaulagain et al., 2017)
9.	2016-2017	Banke District Udayapur, Harminiya and Betahani VDC	Post-mortem Carcass Evaluation	29% (110)	(Sah et al., 2017)

Human Cysticercosis

Neurocysticercosis (NCC) is endemic in Nepal and there is a practice of visiting traditional healers before presenting to the hospital by many seizure patients (Ojha et al., 2015). There are very few published materials on cysticercosis/ neurocysticercosis and only hospital-based data are available. In the investigation in 1987, seven of eight epileptic Gurkha soldiers (Nepalese) in Hong Kong were diagnosed with Neurocysticercosis (Heap, 1990).

Cysticercosis was prevalent for many decades ago and people often used to visit traditional healers after epileptic signs and symptoms. Lack of hospitals, neurological departments, and lack of awareness and knowledge about diseases forced people to visit them (KC and Kaphle, 2019). Very few people were aware of the disease and some patients from Nepal used to pursue neurological treatment outside the country, especially in India (Rajshekhar, 2004). In 2014, there were only six neurosurgeons and six CAT (Computed Axial Tomography) scanners active in Nepal, all of which, except for one CAT scanner in Dharan, were located in Kathmandu (Joshi et al., 2004). According to the Nepalese Society of Neurosurgeons, there were 63 neurosurgeons in Nepal in 2017. At the present days, those numbers might be increased, but are limited in big cities like Kathmandu, Pokhara, Bharatpur, Butwal, Dharan, etc. After the 2000s, many people were aware of the disease, awareness programs on radio, and the establishment of health posts and clinics in the village made people visit hospitals. After then, many cases started to report in hospitals and neurological departments (Table 2).

Table 2. Cases of Human Cysticercosis in Nepal

S. N	Years	Number of Confirmed Cases	Disease Confirmation Place/Hospital	Infection and Diagnosis	Comments	Reference
1.	1987	Seven out of eight epileptic patients	Hong Kong among Gurkhas soldiers	NCC by CT Scan	Gurkhas acquire NCC in Nepal.	(Heap, 1990)
2.	1993 - 1998	62 out of 23,402 biopsies	Patan Hospital (Approximate 60 hospital and clinics of Nepal)	Cysticercosis by Histopathology	38-Female 24-Males	(Joshi et al., 2004; Rajshekhar, 2004; Amatya and Kimula, 1999)
3.	1995 - 1997	Four out of 25,033 cases	Bir Hospitals	Cysticercosis by Histopathology		(Joshi et al., 2004; Rajshekhar, 2004)
4.	1995 - 1997	11	Kanti Children's Hospital	Cysticercosis by Histopathology	Only children hospital in Nepal	(Joshi et al., 2004; Rajshekhar, 2004)
5.	2000 - 2004	124 Children	Western Nepal	NCC by CT Scan	Susceptibility is high among children	(Joshi et al., 2004; Rajshekhar, 2004; Basu et al., 2007)
6.	2003 - 2015	299 out of 1355 seizure cases	Department of Paediatrics Manipal Teaching Hospital	NCC by CT Scan	99- 6 to 10 years old 91- 11 0 15 years old	(Rao et al., 2017)
7.	2006 - 2007	15 out of 112 seizure cases among 14,118 indoor patients	Nepalgunj Medical College	NCC by CT Scan	80%- Males 20%- Females Mean age: 21 years old	(Pirayani et al., 2007)
8.	2016	54 out of 142 seizures cases	COMS-TH	NCC by CT Scan	29-Male 25-Female All patients were related with	(Khanal and Shrestha, 2019)

					agriculture and farming	
9.	2017	6.25% (5) Seroprevalence out of 80 patients	Neurological Department, Tribhuvan University, Teaching Hospital	Seroprevalence of NCC using Ag-ELISA	8.1%-Male 4.6%-Female Age group of 10-30 years	(Poudel and Maharjan, 2018)
10.	2017 - 2018	29 out of 103 seizures cases	B and C Medical College	NCC by CT Scan	21-Male 8-Females	(Thapa et al., 2020)
11.	2019	22-year old man	Kathmandu	Ocular Cysticercosis	Disseminated neurocysticercosis with bilateral papilledema	(Shrestha and Shrestha, 2019)

Sixty-two of 23,402 biopsy cases were detected as cysticercosis which was examined from July 1993 to February 1998 in Patan Hospital, which was collected from approximately 60 hospitals and clinics of Nepal (Amatya and Kimula, 1999). Among them, forty cases were from Kathmandu valley, and the rest from outside, and the mean age was 21 ± 11 years (Amatya and Kimula, 1999). Easily available pork meat in Kathmandu valley and crossing the religion barrier by young age people might contribute to a high number of cases in young people in Kathmandu. Also, young aged people are more vulnerable to eggs of *T. solium*. Between 1995 and 1997, four (0.01%) out of 25,033 pathological specimens examined at Bir Hospital, Kathmandu, were diagnosed as cysticercosis (Amatya and Kimula, 1999; Joshi et al., 2004; Rajshekhar, 2004) and 11 confirmed cases were reported in Kanti's Children Hospital in the same time (Joshi et al., 2004; Rajshekhar, 2004).

In western Nepal, 124 children cases of NCC were diagnosed from 2000 to 2004, the majority of age group affected was 10-12 years and youngest patient was of 11 months (Basu et al., 2007; Joshi et al., 2004; Rajshekhar, 2004). The partial seizure was the most common clinical manifestation and patients were treated with albendazole for 28 days along with antiedema and anticonvulsant drugs with a follow-up period of 1-3 years (Basu et al., 2007). After 1 year, 98 patients were successfully treated and 87 had complete disappearance of the lesion in CT scan. The recurrent seizure appeared in 6 patients and they have a calcified lesion in CT scan (Basu et al., 2007). Vulnerability and susceptibility of cysticercosis/neurocysticercosis are high among children and young people because of frequent exposure to soil, raw vegetables, also they play on bare feet and eat soil. Out of 131 patients admitted to the Neurology Department, Bir Hospital, Kathmandu, Nepal from April 2012 to February 2014, 21 (16%) were diagnosed with NCC (Ojha et al., 2015). Seven patients (33.33%) initially visited traditional visitors before presented to the hospital and eight patients (38.1%) were treated with antiepileptics drugs in local health posts without neurological studies (Ojha et al., 2015). This is not a very old incident, although many hospitals with the neurological department are established, people initially visit traditional healers and small health post due to unawareness of the disease and weak socioeconomic condition. Many educated people of the city area also believe in “*Phukauney*”; a way of traditional healing and

farmers in the village have conservative life and still believe and depend on “*Dhami*” and “*Jhankri*”; traditional healers (Ito et al., 2014).

1,355 cases of seizure disorders were admitted to the Department of Pediatrics, Manipal Teaching Hospital, Pokhara, Nepal from 2003 to 2015, out of which 229 (16.90%) were diagnosed as NCC (Rao et al., 2017). There were 99 (43.23%) and 91 (41.09%) patients in the age group 6 to 10 years and 11 to 15 years respectively (Rao et al., 2017). From 2006-2017, out of 14,118 indoor patients admitted in Nepalgunj Medical College, Teaching Hospital, Kohalpur, Nepal, 112 had a seizure and 15 were diagnosed with Neurocysticercosis (Piryani et al., 2007). The mean age of patients was 21 years, among them 80% were male and 20% were female (Piryani et al., 2007). Male is more prone to infection with NCC than female because of their working nature (pig farmer, butcher, field worker) and personal hygiene. Female is more likely to be hygienic than male, this may be a cause of less prevalence of NCC in most of the cases in Nepal.

For 2016, 142 patients with seizures were admitted to the College of Medical Sciences, and Teaching Hospital (COMS-TH), Nepal, 54 (38%) were CT scan confirmed Neurocysticercosis cases (Khanal and Shrestha, 2019). Among NCC cases, 29 (54%) were male, and 25 (46%) were female, and 17 (31.5%) patients were 10-20 years old. All patients were related to agriculture and farming and 10 (18.5%) patients were vegetarian (Khanal and Shrestha, 2019). Personal hygiene and sanitation of farmers is an equally important risk factor as pork consuming behavior of a person. People involved in agriculture and farming have a high chance of exposure to cyst present in fields, vegetables, water, and pig and are at high risk of infection of NCC. From September 2017 to August 2018, 103 patients were admitted to the B&C Medical College Teaching Hospital, Birtamode, Jhapa, with clinical features of seizure disorder, 29 (28%) patients were diagnosed with Neurocysticercosis, among them 21 (72.41%) were males and 8 (27.59%) were females (Thapa et al., 2020). 80 patients visited in the neurology department of Tribhuvan University Teaching hospital were screened using the Ag-ELISA method, where 6.25% seroprevalence of neurocysticercosis was found among symptomatic and non-symptomatic neurological patients (Poudel and Maharjan, 2018). Cysticercosis is one of the most encountered diseases in the neurology department of many hospitals in Nepal. Also, in 2019, ocular cysticercosis with multiple disseminated subcutaneous nodules and papilledema in both eyes was reported in a 22-year-old man in Nepal (Shrestha and Shrestha, 2019). Hospitals with the well-equipped neurological department are only located in major cities and beliefs of “*Phukauney*” (Traditional Healing) during epileptic disorder is still practiced in the country. Still, most of the epileptic patients initially visit traditional healers (“*Dhami*” and “*Jhankri*”) and then visit the hospital after unsuccessful treatment. Delay in reporting in hospital, increase the number and burden of the cyst, and make them a worse condition of the disease.

One Health practice is rudimentary in Nepal and only applied in a few diseases like Highly Pathogenic Avian Influenza and rabies. Improvement in pig rearing system, awareness about food hygiene and epilepsy, increased number of health workers and hospitals, improved communication and internet facilities and other factors are helping in the control of cysticercosis but still highly prevalent in the country. The collaborative effort of animal workers, veterinarians, public health officer, human doctors, and stakeholders related to sanitation and the environment can help in mitigation of the disease.

PREVENTION AND CONTROL

High cost and complexity in diagnosis and treatment of NCC are major demerits in developing countries like Nepal where NCC is endemic. Many people cannot afford the cost of diagnosis which drives them towards traditional healers. Prevention and control are the better way to reduce the number of cases and health care and economic burden of NCC.

1. Awareness and Education

Exposure to different risk factors of the disease can be minimized through awareness and educating the pig farmers, butcher, pig meat consumers, and communities which practice open pig rearing system. Knowledge sharing programs and training related to personal hygiene, hand washing, use of toilets, environmental sanitation, and pig rearing system should be provided to high-risk peoples. Efforts should be made to raise awareness about the seizure and its causes; to stop the flow of seizure patients toward traditional healers. Public health authorities should convince high-risk peoples that, their habits and way of living are major risk factors so that they can improve personal hygiene and sanitation. In a country like Nepal where infection with *T. solium* is endemic, awareness and education are basic yet most important tools for the prevention of disease. So, all stakeholders related to human health, pig farming, animal health, the Nepal government, and different organizations should work together to raise awareness and provide education to those people who are at risk with infection of *T. solium*.

2. Personal Hygiene and Environment Sanitation

Personal hygiene and environment are the mediators for the continuation of *T. solium* life cycle in pigs and human beings. In Nepal pig rearing communities generally have low economic status and they do not much care about their hygiene, food hygiene, and environmental sanitation. Washing hands with soap water after defecation and working in the field, wearing boots/slippers while working in field/farm, freezing and cooking meats and vegetables, and use of toilet can decrease the risk of infection with *T. solium*. Cleaning of pig barn and proper disposal of feces of pigs can help in the prevention of the disease. The establishment of pig farms in a different area than human settlement and prevention of pigs from free-roaming can decrease the risk of infection among humans.

3. Slaughter House and Meat Inspection

Proper management of slaughterhouse and meat inspection before selling can play a significant role in breaking the life cycle of the parasite and thus help in the prevention of disease. Slaughter of pigs in the open area must be prohibited and the establishment of a hygienic slaughterhouse is urgently needed.

4. Vaccination

Vaccination against the infection of *T. solium* is not immunologically and logistically appropriate because of the oculute nature of infection and minor effects during intestinal infection. Vaccination of pigs is possible and can break the life cycle of the parasite. Vaccination of pigs using a combination of recombinant antigens, TSOL16 and TSOL18 has the potential to stop transmission in pigs and infection in humans (Jayashi et al., 2012).

5. Epidemiological Investigation and Surveillance

Time to time surveillance of the disease is important to know the disease status in any region and prevention of the disease. Surveillance among pig farmers, pig meat consumers, butchers, and communities with a free pig rearing system can help in the prevention of disease and to make programs, control strategy and policies for the infection with *T. solium*. Fecal examination, analysis of hospital-based data, personal interview to know signs and symptoms are some tools for the investigation of infection with *T. solium* in humans. Serological surveillance in pigs can help in the mitigation of disease in humans. Risk-based surveillance programs in animals as well as in humans can help in the mitigation of disease (Alban et al., 2020).

CONCLUSION

The present situation of cysticercosis and neurocysticercosis is alarming in Nepal. High numbers of cases in hospitals indicate that people are unaware and lack knowledge about the disease. Awareness programs along with other control strategies are urgently needed. Efforts should be made to improve self sanitization, food hygiene, and environmental cleanliness to reduce transmission among humans and from pigs. Identification of risk areas and people and launching different programs can reduce the social and economic burden of the disease.

Conflict of Interest: None.

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Epidemiology and Pathogenesis of COVID-19, a Pandemic Novel Coronavirus Disease; Review from Global Perspectives

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) is an emerging global public health issue that was declared as a pandemic by the World Health Organization (WHO) on March 11, 2020. The disease is causing substantial mortality and morbidity after the first case emerged in the Wuhan city of China from the Huanan Seafood Market in late December 2019. This mini-review summarizes the information regarding the virology of the etiological agent, epidemiology, pathogenesis, clinical manifestation, laboratory diagnosis, and prevention by reviewing published literature as well as situation reports of WHO and other international organizations. The chronological incidence of COVID-19 uncovered a significant increasing trend ($P < 0.01$) of confirmed and death cases in all regions of WHO. As of November 22, 2020, total cases of 57,882,183 and 1,377,395 deaths have been reported globally. Among WHO regions, the regions of the Americas and West Pacific contributed to the highest and lowest confirmed cases and death cases, respectively. Minimizing person-to-person contact, travel, and gathering is an effective way to control the current pandemic situation together with increased coverage of vaccines developed as specific antiviral drugs are not available to date for treatment. To combat the underlying serious global health problem caused by COVID-19, a controlling framework in the joint effort from governments is essential following the WHO guidelines at the global and regional levels.

Keywords: COVID-19; Coronavirus; Epidemiology; Pandemic; Pathogenesis; WHO

INTRODUCTION

Taxonomically, Coronaviruses belong to the genus *Coronavirus* within the family Coronaviridae, subfamily Coronavirinae, and order Nidovirales, which comprises single-strand, positive-sense RNA viruses (Sahin, 2020). Morphologically, they are enveloped with size 120-160 nm in diameter, containing crown-shaped peplomers, from which the name ‘Corona’ was derived (Woo, Huang, Lau, & Yuen, 2010). They are remarkable among all RNA viruses due to the largest genome size i.e., ranging from 26 to 32 kb in length. Coronaviruses can rapidly replicate in a wide

range of hosts i.e., humans, pigs, turkey, guinea fowls, bats, cats, dogs, camels, whales, ducks, and avian hosts (Lu et al., 2020; Miłek & Domańska, 2018). Till now, there are seven types of Coronaviruses reported to cause human diseases i.e., 229E, NL63, OC43, HKU1, MERS-CoV, SARS-CoV, and COVID-19. Former four human Coronaviruses are low pathogenic, confined to upper respiratory tract infections, and cause cold-like respiratory illness, meanwhile, the latter three are highly pathogenic human Coronaviruses that predominantly infect the lower respiratory tract and cause fatal pneumonia (Channappanavar & Perlman, 2017). As the antigenic structure of Coronaviruses infecting animals, avian species, and humans are similar; there is a possibility of a shift between the hosts (Sahin, 2020). Previous epidemiological incidences caused by Coronaviruses i.e. SARS (Severe Acute Respiratory Syndrome), and MERS (Middle East Respiratory Syndrome) uncovered that derived strains are more pathogenic and could cause severe infection in humans. Recently, a new strain of Coronavirus first isolated from Wuhan, China in late December 2019 is causing massive infections and millions of deaths worldwide. This new strain of Coronavirus is also believed to have originated in bats but the intermediary animal host form which it jumped into a human is yet to be determined.

EPIDEMIOLOGY OF COVID-19

Incidences of pneumonia cases of unknown etiology were officially reported in December 2019. Later, it was disclosed that the outbreak is associated with Huanan Seafood Wholesale Market, Wuhan, China. The etiological agent of the disease i.e., a new type of Coronavirus was first isolated on January 7, 2020 (*WHO, Coronavirus disease 2019, Situation Report-01*, 2020). On January 13, 2020, the first incidence of the disease outside mainland China was confirmed in Thailand, which was the imported case from Wuhan, China. Further, imported cases were reported in two neighboring countries of China i.e., Japan and the Republic of Korea on 15 January 2020 and 20 January 2020, respectively (*WHO, Coronavirus disease 2019, Situation Report-01*, 2020). After 7736 confirmed cases and 170 deaths in China (Figure. 1), with very high risk in China and high risk at the regional and global scale, WHO officially declared a “public health emergency of international concern” on January 30, 2020 (*WHO, Coronavirus disease 2019, Situation Report-10*, 2020). On February 11, 2020, an official name was purposed by the World Health Organization for the disease i.e., COVID-19, which is an acronym that stands for coronavirus disease 2019 (*WHO, Coronavirus disease 2019, Situation Report-22*, 2020).

With 118,319 confirmed cases from 114 countries and 4,292 deaths though mostly from epicenter China, the regional and global level

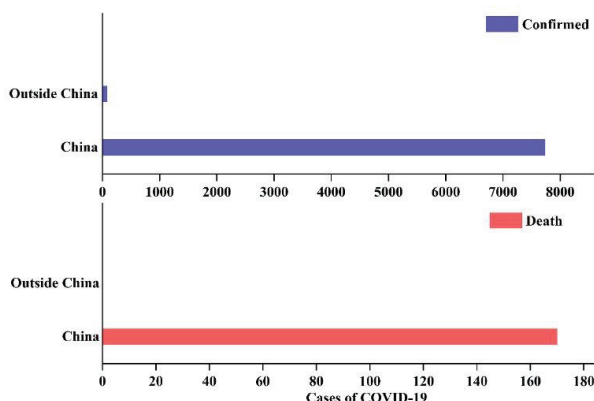


Figure 1. Incidence of COVID-19 infections and death cases when WHO declared a public health emergency of international concern (January 30, 2020)

risk was very high. Considering the rapidly spreading coronavirus outbreak, WHO declared it a pandemic on March 11, 2020 (*WHO, Coronavirus disease 2019, Situation Report-51*, 2020). Based on the chronological order of case data published by WHO, confirmed and death cases of COVID-19 were higher in China till March 15, 2020, meanwhile, both confirmed cases and death cases peaked outside China from March 16 onwards (Figure. 2).

Currently, both confirmed and death cases have been increasing in all regions of WHO i.e. African Region, Region of the Americas, South-East Asia Region, European Region, Eastern Mediterranean Region, and Western Pacific Region. Chronological incidence of COVID-19 infections (Figure. 3) with the highest-burden in the Americas and European Region. As of November 22, 2020, total cases of 57,882,183 and 1,377,395 deaths have been reported globally. Region of the Americas and West Pacific contributed to the highest and lowest confirmed cases and death cases, respectively. Region of the Americas alone contributed for 42% and 51% of total cases and deaths due to COVID-19 infection, respectively (*WHO, COVID-19 Weekly Epidemiological Update- November 24, 2020*). Based on trend analysis using Kendall's tau-b test (Adhikari et al., 2019), the monthly confirmed and death cases showed a significant increasing trend in all regions of WHO ($P < 0.01$). The case fatality rate (CFR) i.e., deaths in COVID-19 patients divided by the total number of COVID-19 cases is different in different

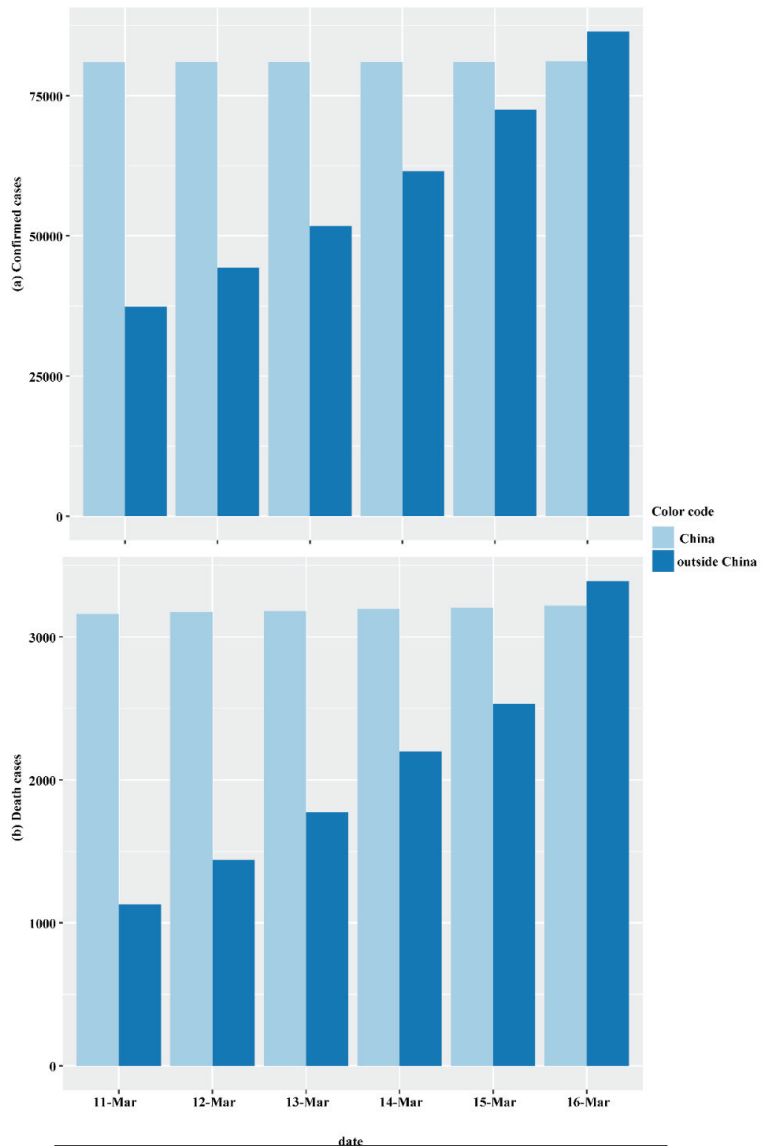


Figure 2 Chronological order (March 11-16) of confirmed (a) and death (b) cases of COVID-19 infections in China and outside China after the declaration of a pandemic by WHO. Data were extracted from the situation reports of WHO

countries, which may be due to the variation in the population of elderly people, population density as well as available health facilities in the selected region. The highest case fatality rate (CFR) was reported in Italy (Iype & Gulati, 2020), probably due to a larger proportion of elderly people.

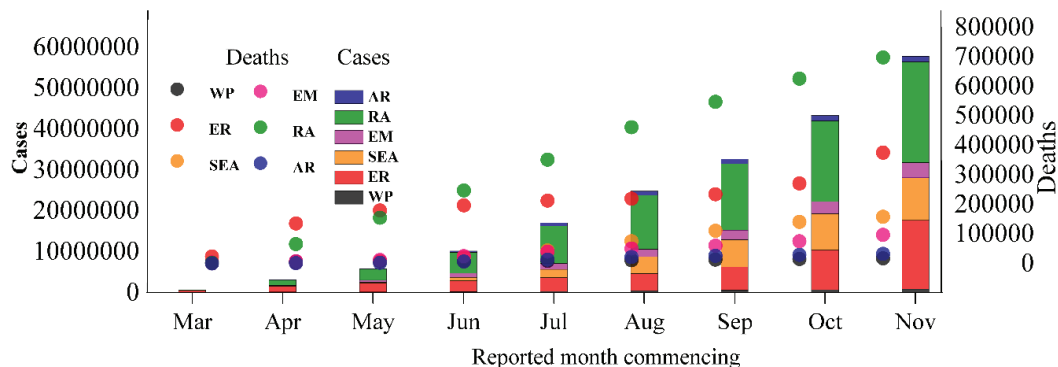


Figure 3 Chronological incidence of COVID-19 infections in different regions of WHO. Data were extracted on monthly basis from the situation reports of WHO. AR: African Region, RA: Region of Americas, EMR: Eastern Mediterranean Region, SEA: South-East Asia, ER: European Region, WPR: Western Pacific Region.

PATHOGENESIS OF COVID-19

Virus transmission occurs during close contact through respiratory droplets released from coughing or sneezing of infected individuals (Rothan & Byrareddy, 2020) or by fomites (WHO, *Coronavirus disease 2019 Situation Report-66*, 2020). Viral particles reach alveolar epithelial cells (the suspected primary target cells) by either of the ways. Envelope, the structural protein of the virus plays an important role in the pathogenesis of this virus. The entry of Coronavirus into host cells is mediated by spikes on their envelope, which then fuses into host cell membranes to release viral nucleocapsid into the host cell cytoplasm (Figure. 4). Viral spikes comprise S1 and S2 subunits. The S2- subunit of spike protein is the receptor-binding domain of the virus, which docks with the angiotensin-converting enzyme 2 (ACE2) in the host receptor (Rothan & Byrareddy, 2020; Wan, Shang, Graham, Baric, & Li, 2020). ACE-2 is a membrane-bound aminopeptidase expressed in the respiratory system, gut, heart, kidney as well as arterial and venous endothelial cells (Albini, Di Guardo, Noonan, & Lombardo, 2020). The expression of ACE-2 is comparatively higher in the lungs and heart (Zheng, Ma, Zhang, & Xie, 2020).

As soon as the viral nucleocapsids are released in the host cell cytosol, they use their own RNA Dependent RNA Polymerase (RdRP) and encode various structural and non-structural polypeptides that facilitate the adaptation of the virus into the human host. Matured virions are released from the infected cells via a process called exocytosis and form the main sign and symptoms. Virions disseminate to other body parts i.e., liver, kidney, intestines as well as T-lymphocytes (Sahin, 2020) (Figure. 4). Proliferated viruses activate immune cells and induce the secretion of pro-inflammatory and inflammatory cytokines as well as chemokines (Rothan & Byrareddy, 2020).

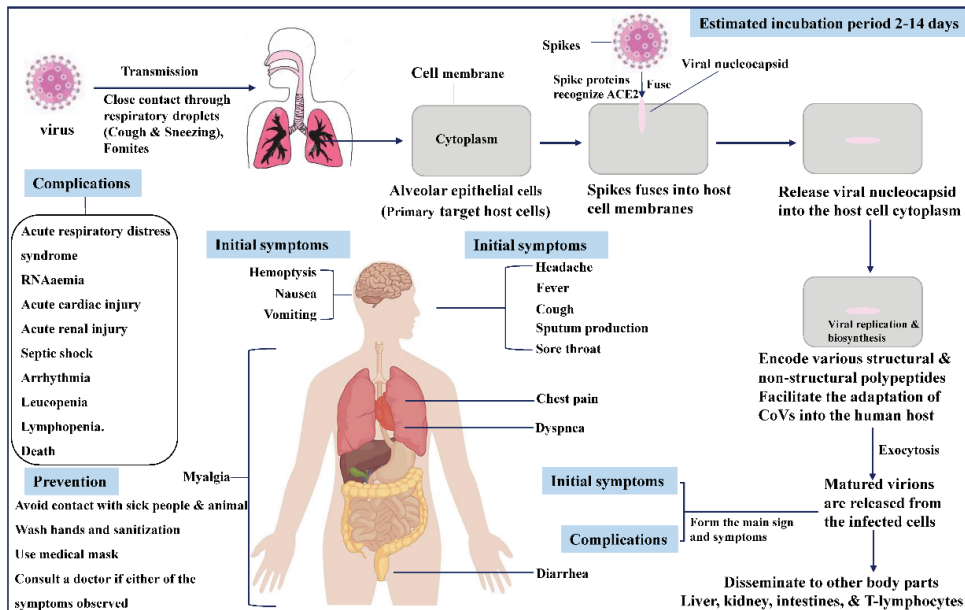


Figure. 4 General diagrammatic sketches showing the transmission, pathogenesis, and clinical manifestations of COVID-19 infection

CLINICAL MANIFESTATIONS

Based on the study including 425 COVID-19 subjects in Wuhan, the mean incubation period was 5.2 days, with the 95th percentile of the distribution at 12.5 days (Q. Li et al., 2020). A similar study on 88 subjects revealed an average incubation period of 6.4 days (Backer, Klinkenberg, & Wallinga, 2020). Though an unusual case showed 19 days incubation period, experts proposed 14 days quarantine period, considering a maximum incubation period of 14 days (Rockx et al., 2020). Clinical signs and symptoms are the consequences of viral replication in human cells. Initial symptoms reported in hospitalized COVID-19 patients are fever, cough, myalgia, dyspnea, headache, sore throat, chest pain, hemoptysis, sputum production, diarrhea, nausea, and vomiting (Albini et al., 2020; Jiang et al., 2020; *WHO, Coronavirus disease 2019 Situation Report-66*, 2020; Zheng et al., 2020). Complications of initial symptoms lead to acute respiratory distress syndrome, RNAemia, acute cardiac injury, acute renal injury, septic shock, Arrhythmia, and death (Jiang et al., 2020) (Figure. 4). The progression of viruses in multiple organs leads to leucopenia and lymphopenia. Host immune cells also respond to the viral infection by producing various cytokines and chemokines i.e., IL1B, IL1RA, IL7, IL8, IL9, IL10, basic FGF, GCSF, GMCSF, IFN γ , IP10, MCP1, MIP1A, MIP1B, PDGF, TNF α (Huang et al., 2020).

DIAGNOSIS OF COVID-19

The primary diagnosis of COVID-19 is based on epidemiological data i.e., history of exposure to the infected area or infected individuals, signs and symptoms, as well as ancillary examinations like molecular tests, radiographic imaging, and serological tests, were conducted in pioneer treatments (Albini et al., 2020; Chen et al., 2020; Fengxiang et al., 2020; Jiang et al., 2020; X. Li, Geng, Peng, Meng, & Lu, 2020; Zheng et al., 2020). Throat swabs, bronchoalveolar lavage, sputum, blood, and feces can be potential specimens for laboratory diagnosis. The nucleic acid test (RT-PCR) is considered the gold standard for the authorized identification of viruses (X. Li et al., 2020). However, due to the huge gap between the number of contracted individuals and laboratory capacities to perform RT-PCR promptly, antigen-based rapid diagnostic tests (RDT) are also for COVID-19 diagnosis (Porte et al., 2020). Chest CT of a hospitalized patient in Wuhan revealed ground-glass opacity lesions were observed in the peripheral and posterior lungs. In addition to molecular and radiographic tools, histological tests are applicable in the diagnosis of COVID-19 as hospitalized patients had leucopenia and lymphopenia. Development and commercial production of more sensitive immunological detection kits targeting viral antigens or antibodies are being high demand in the recent scenario.

PREVENTION AND CONTROL

The first and foremost way to prevent this disease is vaccination. Till now, Pfizer-BioNTech COVID-19 vaccine and Moderna's COVID-19 vaccine are authorized and recommended to use for preventing Covid-19 by the Centers for Disease Control and Prevention (CDC) (<https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines.html>). Also, several candidate vaccines are in clinical and preclinical evaluations. The vaccines use inactivated virus particle, DNA plasmid, RNA, protein subunit, replicating, and non-replicating viral vectors as a platform (*Draft landscape of COVID-19 candidate vaccines*, 2020). However, the best way to get rid of illness is to be aware of activities that may lead to exposure to the virus. CDC has recommended some preventive measures to minimize exposure to the causative agent. They are self-isolation to avoid person-to-person transmission, minimizing unnecessary travels, washing hands with soap-water or hand sanitizer, cleaning, and disinfecting frequently touched surfaces. Contact isolation wearing masks, gowns, and gloves is essential. Similarly, eye protection is also recommended to avoid possible ocular transmission.

Some broad-spectrum antiviral drugs have been experimented with and found to be successful for the treatment of COVID-19 infection. Some of the drugs used during the early outbreak of the disease in China, Japan, and South Korea are IFN- α , lopinavir/ritonavir, and ribavirin, Arbidol, Chloroquine phosphate, Favipiravir (Dong, Hu, & Gao, 2020). A study revealed that the combination of hydroxychloroquine and azithromycin resulted in viral load reduction/disappearance in COVID-19 patients (Gautret et al., 2020). Though, there is a great effort of scientists and experts for finding a suitable drug specific to COVID-19, neither of the drugs has been officially confirmed for usage yet.

CONCLUSION AND FUTURE PERSPECTIVES

There is a history of recurrent outbreaks of Coronavirus-associated diseases, and most of them are zoonotic in origin. The emergence of such diseases leads to the death of a large number of people and also causes great economic loss. COVID-19 cases and deaths are increasing globally, implying a serious global health problem. Thus, in this unprecedented situation, everyone should strictly follow the guidelines and preventive measures published by the WHO CDC, and national public health authorities. Reduction of person-to-person contact, and prohibition of traveling, and unnecessary gathering would be crucial to control the current pandemic. Special precautions and care should be applied in the susceptible population to prevent transmission. Intergovernmental and inter-continental cooperation is essential in the current situation. Detailed information on the epidemiological outbreak at global, regional, and country levels should be displayed out to develop strategies for future management. A controlling framework in the joint effort following one health approach is necessary following the WHO guideline in global, regional, and local communities to prevent future outbreaks. This might assist in structuring the specific and comprehensive public health measures.

COMPETING INTEREST

The authors declare that no competing interest exists.

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Emergency Animal Health Management during Natural Calamities and Disasters in Nepal

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INTRODUCTION

Ecologically, Nepal is divided into three regions namely; the Terai (23%), the Hills (42%) and the Mountains (35%) (CBS, 2013). Agriculture sector contributes 26.5% in GDP, livestock and fishery contribute 29% of agricultural GDP and it is about 11% of total GDP (CBS, 2019). Livestock forms an integral part of agriculture system in Nepal. Large indigenous population of cattle, buffaloes and goats are there with less production per animal. There are 7.3 million cattle, 5.2 million buffalo, 12.28 million goat 0.8 million sheep, 1.43 million pigs, 69,588 yaks, 59,822 horses and 75.7 million fowls in Nepal. There is production of 21, 68,434 MT of milk, 386 thousand MT meat and 1,760 million eggs in Nepal (MoALD, 2019).

In poultry meat, Nepal is self-reliant. The prices of chicken meat however remains fluctuant which decreased significantly during Covid-19 pandemic because of reduced consumption triggered by fear among the consumers. Chicken contributes to about 20% of meat consumption in Nepal. Compared to meat, egg market remained as usual which fetched satisfactory prices even during the prolonged lockdown period in 2020 March to August. Nevertheless, movement of chicks, birds and live animals suffered great hurdles especially during the initial periods of lockdown. There was lower impact in goat and buffalo production but consumption was decreased due to COVID-19 related economic impacts. Nepal has 12.5 million goat population and 3.3 million goats are slaughtered every year in Nepal to meet public demand for goat meat. The goat production is doing well even in adverse situation. Nepalese people prefer goat meat and it contributes to nearly 20% of meat consumption in Nepal. Buffalo meat contributes over 50% of meat consumption in Nepal. Pork contributes about 10% of meat consumption in Nepal (MoALD, 2019). There is 95,000 MT of fish production in Nepal and around 9,000 MT are imported from India and abroad. There was some difficulty in transportation and marketing of fish during Covid-19 lockdown period. There is restriction of rearing, importing and transportation of live cat fish in Nepal. Farmers have problem of getting animal feed on time and selling of their products on time.

In term of butter storage there should be 2,000 MT storage facility near by city areas to cope with increase production. Ware house for Skim Milk Powder (SMP) shall be in place which will cater up to 3,000 MT. There is lack of poultry, pig, goat and buffalo slaughter house facility in the country. The cold storage facility for meat storage shall be in place which will be up to 10,000 MT capacity. The facility largely caters the demand of frozen chickens. Covid-19 pandemic effects largely on production of meat, milk, eggs and their products. Marketing and distribution chain become defunct. At present Nepal becomes self-reliant in milk and basic

milk products. Formal sector carries 25% stake in marketing of milk in Nepal. 30% milk distribution is from informal sector, where as 45% milk is consumed in household in rural areas. Pasteurization of milk, product diversification can be carried out in formal sector. In the lock down period collection of milk in formal sector increases dramatically even though it is dry period of milk production in Nepal. It leads to pile up of skimmed milk powder and butter in dairy industries. Nepal is able to export butter to Japan in the period.

The Government of Nepal has announced the 15th five years plan 2020-2025. The major objective of this 15th 5-years plan is to reduce poverty from 18.7% to 9.5% by the end of the plan year. This plan targets to increase the production and productivity of agriculture to ensure food security. Also, the plan targets to reduce the burden of infectious diseases to create a healthy society. Likewise, the United Nations Development Assistance Framework (UNDAF) 2018-2022 has four priority areas. The DLS animal health programmed contributes to the third priority area: Resilience, Disaster Risk Reduction (DRR) and Climate Change Adaptation by decreasing the threat of zoonotic pathogens. The “Health Policy 2019” also aims to reduce the burden of zoonotic diseases and Antimicrobial Resistance through the One Health approach. The Government of Nepal has endorsed the “One Health Strategy 2019” and have outlined 5 strategies including multi-sectoral collaboration, integrated disease surveillance, and capacity building of stakeholders.

Nepal is the member of World Organization for Animal Health (OIE) and regularly provides disease information to it. There is immediate notifications to OIE if there is outbreak of emerging and notifiable diseases. There are 753 local municipalities where there is livestock services section and they would send epidemiological information to the DLS. Similarly, from 77 districts Veterinary Hospital and Livestock Expert Centre (VHLEC) will report to epidemiology section of DLS. Ministry of Home Affairs (MoHA) is the focal ministry in Nepal to deal with disaster and natural calamities.

These are the major laws and regulation which are under livestock sector.

- Animal health and livestock services act, 1998 and Regulation, 1999
- Slaughterhouse and meat inspection act, 1998 and Regulation 2000
- Nepal veterinary council act, 1998 and Regulations, 2000
- Feed act 1976 and Regulations 1984

DISASTER SCENARIO IN THE COUNTRY

In a global comparison, Nepal ranks 4th in terms of climate risk according to the Global Climate Risk Index which assesses the impacts of meteorological events in relation to economic losses and human fatalities (Eckstein, et al., 2019). The country ranks 11th in terms of global risk for earthquake occurrence and impact (Maplecroft 2011). The country is in top 20 of all the multi-hazard countries in the world. Rugged and fragile geophysical structure, very high relief, high angle of slopes, complex geology, variable climatic conditions, active tectonic processes, unplanned settlement, dense and increasing population, poor economic condition and low literacy rate have made Nepal vulnerable to various types of natural disasters. Most part of the country are seismically active. Hence, the geomorphology is very

fragile. The constant tectonic action of different degree along with varied intensity of weather condition has adverse effect on stability of earth surface and river course.

The Himalayan region of Nepal can be considered as one of the severe flood hazard zones of the world. Besides, Heavy precipitation, high wetness and steepness of watersheds and river channels contribute to flood magnitudes. Mainly, the middle Hills are prone to landslides and the Terai to flood and forest fire. Thus, flood, landslide and fire are the most frequent natural disasters in Nepal. About 80 percent of the population of Nepal is risk form natural hazards (MoHA, 2017). FAO states that agriculture sector absorbs 23 percent of the total damage and losses in developing countries. Further, flood, drought and other climatologically disasters along with disease pest have 37%, 19% and 44 % respective contributions to the loss on agriculture and livestock production.

MAJOR NATURAL DISASTER IN AGRICULTURE AND LIVESTOCK SECTOR

Drought

Change in magnitude and frequency of droughts will have severe impacts on agriculture, especially crop production, cropping system and livestock (Karl et al., 2009). According to MoHA (2018) every year drought impacts agriculture, Livestock, environment and ecosystem negatively in the Terai and Western hills of Nepal and it has been identified as the most hazardous for agriculture. The droughts happened in 1972 and 1979 were the most seriously damaging and harmful to the people, livestock and crops and that of 1994 was the worst drought in its history that affected 35 districts of western hilly and Terai regions (NDRRP, 2019).

Flood

Flood Monsoon carries more than 70 percent of South Asia annual precipitation in a brief of four-month period. A good monsoon brings strong harvests and financial security, but a poorly timed monsoon, can result in human suffering and economic and animal loss due to either flooding or drought (WB, 2012). Nepal is also extremely vulnerable to water-related hazards. Nepal's hydrology is highly variable, with the monsoon bringing 80 percent of Nepal's rainfall in less than three months during the summer (WB, 2012). The flood water has wiped out 79.8 million USD worth of crops and livestock, as the massive floods inundated huge tracts of land in 31 districts of Nepal (MoALD, 2018). According to MoHA (2018), 42,995 hectares of agriculture land had been affected by heavy rainfall, landslides and flood in various years. Furthermore, this has damaged the irrigation system and rural roads and other physical infrastructure.

Earthquake

Although the frequency of earthquakes, in Nepal is low, it has affected the maximum number of people and has destroyed the country's economy among all disasters that occurred between 1990 and 2015. Earthquake has a significant effect on the agriculture production system. As per the Post Disaster Need Assessment Team (PDNA), the devastating 2015 earthquake in Nepal destroyed stockpile of stored grains and devastate the livestock sector. The loss of over 17,000 cattle and about 40,000 smaller domesticated animals has resulted in the downward revision of the projected growth in agriculture from 2.2 to 1.8 percent. The total effect of the disaster in agriculture and livestock was NPR 28,366 million.

Landslides

Landslide is one of the very common natural hazards in the hilly region of Nepal. Natural and human factors such as steep slopes, fragile geology, high intensity of rainfall, deforestation, unplanned human settlements are the major causes of landslide. In between 1971 to 2015, Nepal had to bear more than 2000 landslides, and this has great impact on livestock loss, destroyed irrigation system, roads, bridges and other infrastructure, causing great economic loss.

Hailstorm and Windstorm

Hailstorm is another most critical factor causing crop damage. Thunderstorms usually occur during the monsoon season; and hailstorms at the beginning and end of the monsoon season, causing potential damage to property and crops and increasing vulnerability to food insecurity, for instance (MOHA & DPNepal, 2015).

EXISTING POLICIES AND INSTITUTIONAL FRAMEWORK FOR DISASTER RISK MANAGEMENT IN NEPAL

- Government of Nepal has promulgated various acts, regulation, policies, standard, action framework and codes related to the management of natural disaster.
- For the first time the Constitution of Nepal (2015) has spelled out in its Directive Principles, Policies and Obligations of the State (Clause 51) about disaster management as to make advance warning, preparedness, rescue, relief and rehabilitation in order to mitigate risks from natural disasters. It has identified disaster management as one of the key priorities of 3 tiers of government in the list of concurrent powers of federal, provincial and local levels.
- In Nepal, disaster management is focused mainly on early preparedness, rescue, relief and rehabilitation. The Local Government Operation Act (LGOA) 2017, empowers local bodies to govern themselves. It recognizes that local people and local bodies as the most appropriate points of entry to meet the disaster management needs at the local level.

National policy

1. Legislative provisions for disaster risk management

Major legal arrangements dealing with the disaster risk management in Nepal.

- Soil and Watershed Conservation Act 1982;
- Water Resource Act 1992;
- The Forest Act 1993;
- National Building Code 1994;
- National Building code act 2004;
- Prime Minister Relief Fund Regulation 2007;
- Local Government Operation Act 2017;
- Disaster Risk Reduction and Management Act 2017
- Environment Protection Act 2019

Institutional set up for the disaster management in agriculture and livestock sector

- Federal Ministry of Agriculture and Livestock Development (MoALD) is the leading organization to deal with the disaster management in agriculture and Livestock sector.
- Among the 11 clusters identified by the Disaster Preparedness Response Plan (2019), MoALD is the leading organization for the food security cluster.
- Agriculture loan, Insurance and Disaster Management section under the Agriculture and Livestock Business Promotion Division is the focal section to coordinate, plan and prepare the disaster related programs and policies.
- In the provincial level, agriculture biodiversity and climate change section has been assigned to look after this subject.
- At local level, the agriculture section has the sole responsibility to deal with the entire agriculture and livestock related tasks including disaster.

Strategies for the disaster risk reduction and management in agriculture and livestock sector:

- Planned land use with crop rotation, rainwater harvesting, drought monitoring, using recycle water, developing irrigation system, water rationing are some of the strategies which help to minimize impact of drought.
- Nepal has limited crop and relatively low livestock insurance coverage. According to ADB (2019) the impact of natural hazards on farmers in Nepal would be significantly mitigated using insurance scheme. This calls for the crop and livestock insurance scheme to be extended and made ease access to all farmers.
- In Nepal, federalism is still in fledgling stage. The capacity and experience of the sub national government to manage disaster is poor. The institutional and organizational setup according the NDRRM act 2017 yet to be completed.
- There are skills and capacity gaps in the newly formed municipal and weak incentives for local governments to include Disaster Risk Reduction in development activities. So, the capacity of the local and provincial government in dealing with the natural disaster needs to be enhanced.
- Many municipalities do not have systematic process to integrate disaster risk management into their planning and programming despite the high level of risk. Some local governments have considered such components on an ad-hoc basis without any systematic procedure (WB, 2019). So, integrating the disaster risk management in annual budget from the 3 tiers of government is becoming fundamental to secure the livelihood of majority of the people

CONCLUSIONS AND RECOMMENDATIONS

Revision of existing laws and regulations pertaining to Disaster Relief Regulations (DRR) is envisaged to address the overlap, conflict or gaps in the legal provisions, and the process shall be prioritized. However, collecting, analyzing and managing disaster and climate related data remains inadequate, and it is not accessible to many of the local level planners, non-governmental organization and the private sector.

To support the process of prioritizing investments, and to further the understanding of sectoral impacts of climate change and disasters to the vulnerable sectors, collection and analysis of data should be reinforced at all levels of government through technical support and capacity building. A need remains to collect local level risk information based on levels of risk for all urban and rural municipalities. The vulnerable sectors of which more data is required to determine risks included. As a member of World Organization of Animal Health (OIE) Nepal needs to send regular epidemiological reports, disease surveillance and disease status to OIE in stipulated time frame. There is contingency plan for control of highly pathogenic avian influenza and PPR. The HPAI control decree shall be updated and harmonized with federal structure of the country. The research mandate is given to the Nepal Agriculture Research Council (NARC) of Nepal. There is limited research on disaster relief and animal diseases in NARC. More focus on the area is needed. Contingency plan of the infectious animal diseases shall be made, and more focuses shall be given to the zoonotic diseases. The importance of One Health approaches shall be kept in mind of policy makers.

It is very necessary to establish a Regional Information Center to share the information to SAARC region which will help to cope with the natural disasters. Moreover, there should be regional planning and joint effort to solve the disaster problems particularly in agriculture and livestock sector. Natural disaster has significant negative impact on economic sector. The impact is more visible on agriculture, livestock and fishery. Annually a huge amount of loss is being observed in those sectors. Despite the several efforts made by national and international to rebuild the agriculture and livestock resilient disaster management, the profound effect is yet to be seen.

Strengthening the institutional set up in the three tiers of government, establishment of information flow system and early warning system along with incorporation of disaster risk management in annual plan, programs and budget are essential to prevent and address the impact of disasters in agriculture and livestock sector of Nepal. Field epidemiological study, laboratory strengthening shall be done in very transparent ways. Capacity building of veterinarians and paraprofessionals are important tool to strengthen the national veterinary services of the country. Priority diseases of the livestock in Nepal includes FMD, HS, PPR, HPAI, IBD, ND and rabies.

Though covid-19 does not affect livestock directly, but there is direct effect on sale of meat, milk, eggs and break of their distribution chain. There is outbreak of lumpy skin disease (LSD) in eastern Terai of Nepal during COVID 19 pandemic. Central Veterinary Laboratory (CVL) procures RT-PCR kits and LSD identifies for the first time in Nepal. It is immediately notified to OIE. There is formation of task force to deal with Covid-19 problems in agriculture and livestock sector. MoALD takes lead on the team. There is continuous engagement of Chief Veterinary Officer (CVO) with concerned agencies to ease lock down problem case by case basis. The mobile number of CVO was given for emergency communication point. It makes soothing effect in livestock related problems including transportation of feed and livestock products. Movement of animals were restricted due to LSD problem. But chickens, goats and fish transportation were made easy by following Covid 19 protocol. There is difficulty in transportation and marketing of fish in Covid-19 lock down period. Egg production is as usual, and eggs have got satisfactory prices even in prolong lock down period. Movement of chicks, birds and live animals have great hurdles in lock down period. Even though it was hard time, but Nepal becomes self-reliant on livestock products for the first time in history. It is basically due to strict quarantine measures aftermath of

COVID-19 pandemic. Farmers have problem of getting animal feed on time and selling of their products on time. Therefore, there is good animal husbandry practices and value chain mapping for each municipalities and supply chain shall be maintained even in pandemic situation with the help of 3 tier government in Nepal. There will be poultry, pig, goat, and buffalo slaughter house in and around urban area is essential to cope with emerging demand. The cold storage facility for meat storage shall be in place which will be up to 10,000 MT capacity.

Nepal is natural calamities prone country and contingency plan for disease surveillance, animal feeding and marketing of livestock products in the time of disasters shall be done by three tiers of government in coordinated approach. The approach shall be sustainable and farmers friendly. Because majority of farmers are of small holder in Nepal. The livestock insurance is doing well in Nepal and 95% of insurances shall be done by livestock and fishery sector. Implementation of animal slaughter house and meat inspection act, 2055 is an urgent need of the country. The role of the local municipality is great and it should be harmonized with our federal systems. Formulation of the infectious animal disease act is very much essential.

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Recent Dynamics of Poultry Production in Nepal: Food Security to Sustainable Production

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ABSTRACT

Poultry sector in Nepal is growing rapidly and contributes a significant share in the agricultural GDP. In this context, we present major push factors and challenges and interventions required to sustain the poultry farming in Nepal. Poultry sector has observed a huge growth in last two decades (2000-2020). During the same period, government policies and strategies including Bird flu control order 2064 were also formulated to develop the poultry sector. However, transient layoff was observed in the past decade (2010-2020) due to several crisis such as outbreaks of Bird flu, Earthquake, unofficial blockade from India and lately COVID-19 pandemic. Moreover, ineffective government relief packages in the crisis further exacerbated the situation. Nevertheless, some initiatives such as livestock Insurance were taken to mitigate potential losses. This review identifies reliance to foreign countries for feed ingredients, lack of integration of native breeds of poultry in mainstream market, fragile pricing of poultry products, and lack of stringent governmental rules/regulation/standards/acts to develop the poultry sector as major challenges for the poultry industry in Nepal. These gaps need to be fulfilled to develop the poultry sector further in Nepal.

Keywords: Challenges of poultry industry, Growth, Nepal, Poultry

INTRODUCTION

The poultry sector in Nepal has sustained through the ages and has shared a significant portion of the Nepalese economy. Nepalese indigenous breeds of poultry like Sakini and Ghantikhile have been associated with Nepalese households for past centuries and continue to the present day. Poultry sector alone contributes 4% (CBS, 2012) to gross domestic product and 8% to agriculture gross domestic product, with a total investment of over NRs 80 billion and direct employment for 1.5 million people (CBS, 2018). The introduction of improved breeds by American Citizen Aid in 1959 has been a benchmark for the poultry sector in Nepal (Mishra and Spradbrow, 1991). Almost half a century after the introduction of improved breeds, Nepalese poultry has grown exponentially with the integration of modern farming practices (FAO, 2014; Mishra & Spradbrow, 1991).

Nepal ranks 92nd in the worldwide egg production and 112th in poultry meat production (FAO, 2014) which justifies the fact that the poultry sector is an emerging agribusiness in Nepal and can be a source of the cheapest animal protein. Apparently, Nepalese poultry sector has seen vibrant growth, however, the backyard poultry sector still seems to have a significant share in total poultry

population. This article aims to present recent progress and changes in the poultry sector of Nepal and correlate these changes with the premise behind them in a rational way.

MATERIALS AND METHODS

Literature review were conducted using various sources; scientific publications, newspaper, reports, governmental publications, non-governmental publications (FAO, Heifer International, and Hellen Kellen International), magazines, and proceedings from conferences and symposiums.

RESULTS AND DISCUSSIONS



Fig 1: Word cloud with keywords used in article

Stages of the poultry sector in Nepal: The introduction of improved breeds from New Jersey, USA for cross-breeding purpose (200 cocks and 1500 hens) was the first step toward commercialization of poultry industry in Nepal. (Mishra & Spradbrow, 1991). Since then many policies, programs, and strategies have been formulated to uplift the poultry industry in Nepal. (FAO, 2014)

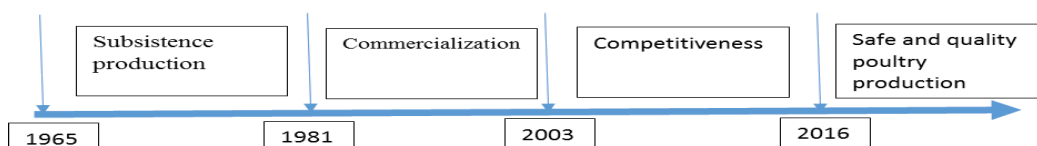


Fig 2: Chart showing Stages of Poultry Production in Nepal.(FAO, 2014; Rijal, 2018)

Growth of commercial poultry farms over the period of 1974-2015 in Nepal

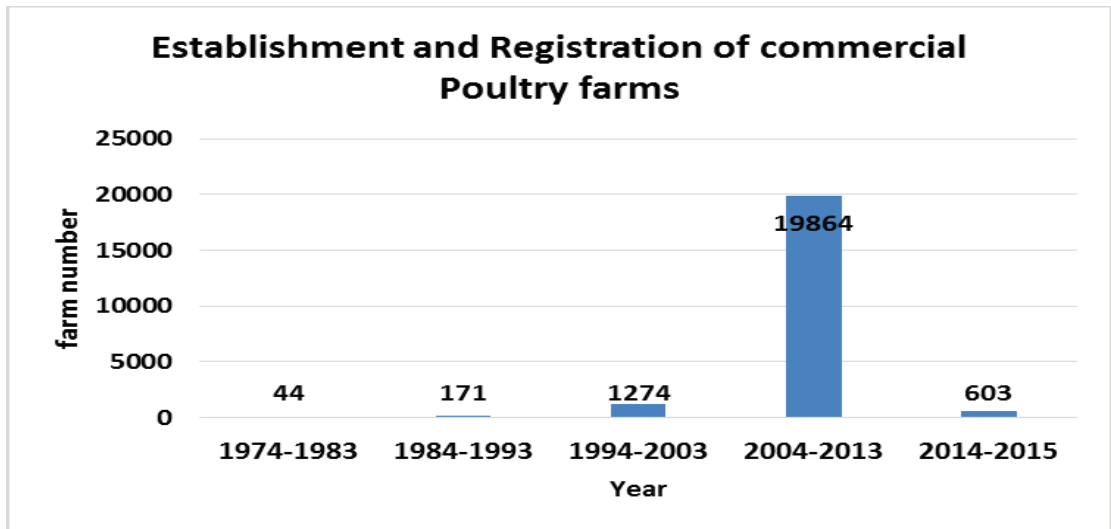


Fig 3: Graph showing establishment and registration of commercial poultry farms (CBS, 2018)

Nepal sees exponential growth of poultry sector in the decade 2004-2013 (CBS, 2018; FAO, 2014). The burgeoning poultry sector can be attributed to factors like political stability after the decade-long Maoist conflict (1993-2003), change of attitude among Nepalese consumers to consume poultry meat, increase in tourism, increased demand for poultry meat and eggs, and acceptance and integration of modern technology supported by the governmental and non-governmental sectors.

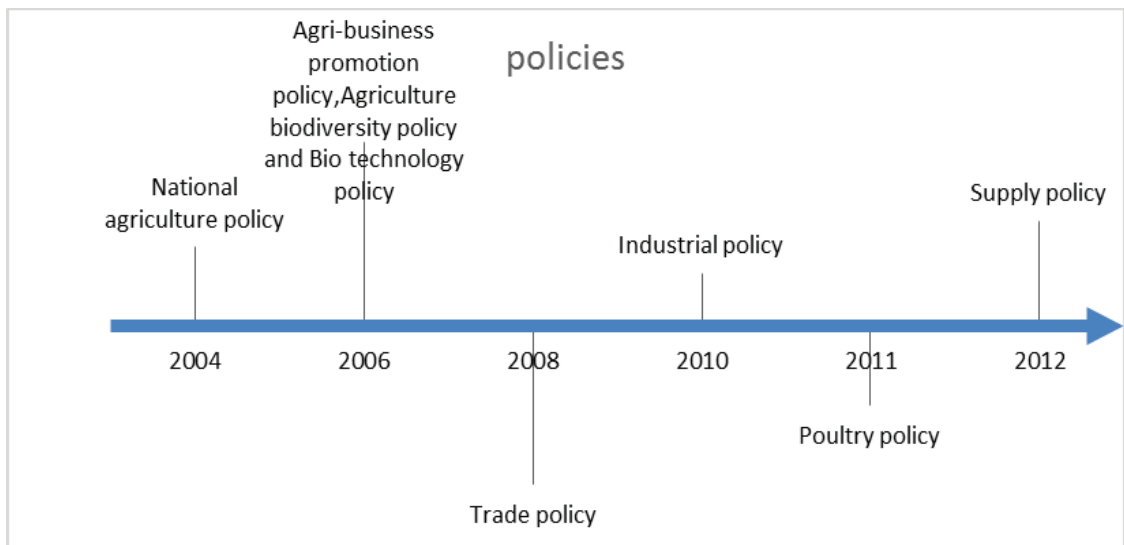


Fig. 4: Chart representation of the governmental policies over a period of time which are directly or indirectly related to the Poultry Sector.

Governmental policies to uplift Nepalese poultry industry

The then HMG (His Majesty's Government) of Nepal had formulated the different policies to uplift the poultry industry in Nepal. The pace of policy formulation and implementation has continued since then. The policies drafted and implemented had some impact on the poultry sector of Nepal but the achievements did not align with goals set.

Major events hampering the poultry sector in a decades (2010-2020)

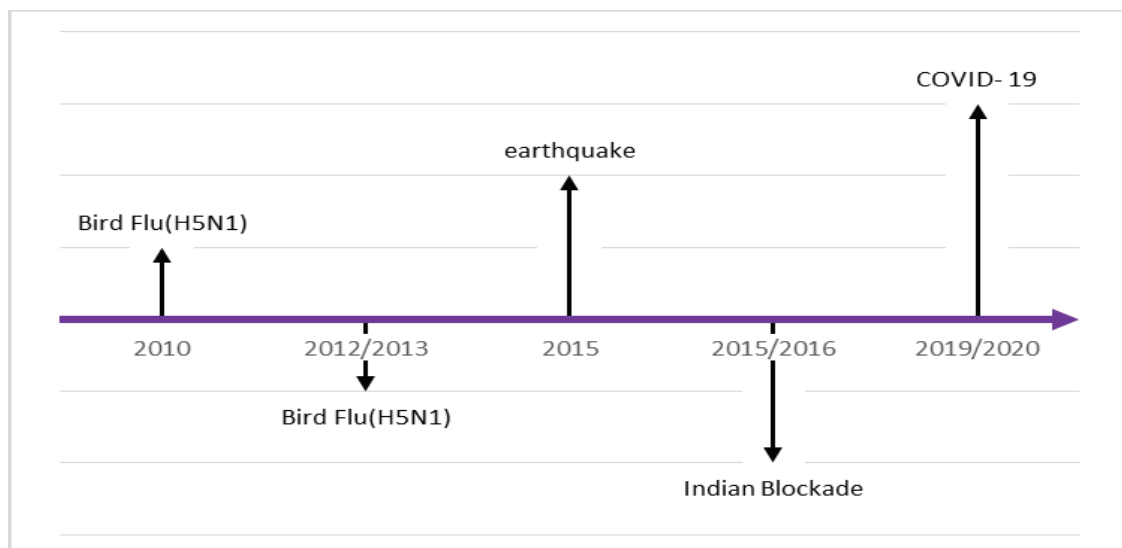


Fig 5: Major events hampering the Poultry sector in decade (2010-2020)

Bird flu (H5N1): The first case of bird flu appeared in 2009 in Jhapa district of Nepal (Karki et al., 2014) and has been impacting in the socioeconomy, especially in the microeconomy level (Karki, 2017). The highest number of outbreaks were recorded during 2012/2013 during which major poultry producing districts were affected. In these outbreaks, almost 1,84,776 birds were killed and 1.75 million chickens, 3.05 million eggs, 1, 72,181 kg of feed and 1, 55,550 kg of the meat were destroyed to prevent spread of virus. (“Farmers Receive Rs 1b for Compensation for Losses,” 2014). Low pathogenic avian influenza H9N2 has been endemic in Nepal since 2016 and has caused great loss in commercial meat and egg production.

Earthquake: Major earthquake hit Nepal on April 25, 2015. This had a devastating effect on Nepalese poultry sector like many other sectors. Earthquake killed hundreds of thousands of birds and tons of feeds were destroyed. In addition, 100 thousand chicks were destroyed daily from the hatcheries due to the lack of proper care and lack of employees in poultry farms and hatcheries (Mahato, 2015; “Nepal Needs ‘\$20 m’ to Help Quake-Hit Farmers,” 2015). Thousands of commercial broilers and layers farmers lost their poultry shed along with live birds in affected districts. Reestablishment of poultry farms got delayed (“Nepal Needs ‘\$20 m’ to Help Quake-Hit Farmers,” 2015) due to sluggish governmental actions and unofficial trade blockade from India which occurred after the earthquake. Lack of demand for poultry meat from restaurants, schools,

and banquets has led to transient decreases in poultry meat prices. After a month of post-earthquake, the supply of poultry was shrunk by 30%-40% due to which there was an increase in poultry meat (Rs 290/kg) and egg prices (Rs 280/crate) (“Chicken Prices Soar on Drop in Output,” 2015).

Unofficial Trade Blockade from India: Nepal claims to be self-reliant in poultry meat and eggs but the majority of poultry inputs (more than 60% of corn and 90% of soy cake) comes from India. The unofficial Indian Trade Blockade in December/January 2015/2016 badly affected the poultry industry. The lack of fuel, feeding raw materials (corn and soy cake) led to the destruction of one million newly hatched chicks and further delayed breeding of new chicks (Bashyal, 2015). During the blockade time (four and half months), some of the poultry farmers imported feed from Dhangadhi and Biratnagar border points informally paying four times higher than the actual cost (Yadav, 2016).

COVID-19 Pandemic: Amid of COVID-19 pandemic, Government of Nepal initiated a nationwide lockdown on 24th March 2020 which was partially loosened on 14th June 2020 except for schools, hotels, seminar halls, tourist spots, and banquets. The dearth of demand of chickens and eggs due closure of restaurants and school, public fear to consume animal source food, people busy buying daily consumable goods, and misinformation circulated by online media—corona virus spread through the chicken—had led to sharp decrease in the price of chicken meat (Rs 70-80/kg) (Prasain, 2020b). Many farmers buried their birds due to the lack of market and feed supplies, some even sold birds on piece basis (Rs 25/ piece) during the first 11 days of lockdown period (Prasain, 2020a). In the first 2-3 weeks of lockdown, the poultry sector faced a loss of Rs 220 million daily (Kafle, 2020). After a month and a half after embarking on a nationwide lockdown, there was an extreme shortage of chickens in the market due to low production of chicks in the hatchery, decreased feed production and many farmers did not keep the next batch of chicks. These conditions favored a skyrocketing of price of finished marketed chicken meat i.e. Rs. 450-500/kg which is 1.6 times the poultry meat price at the beginning of lockdown days (“Chicken Price Skyrockets to Rs 460 per Kg in the Valley,” 2020; Prasain, 2020c).

Some government and non-governmental intervention to uplift the poultry sector and management of the crisis

Avian Influenza: After a massive outbreak of avian influenza in 2012/2013, the Government of Nepal declared a bird flu emergency and banned all imports of poultry-related items including live chicks, meat, eggs, and even feed. The government provided Rs. 170 per bird as compensation for the loss (“Farmers Receive Rs one billion as Compensation for Losses,” 2014). Risk based surveillance system is implemented in Nepal, and the country is divided into 3 risk categories—high, medium and low. The massive screening program conducted by the Nepal government helped to contain the further Avian Influenza outbreak in the country. As of the recent outbreak in Kathmandu and Bhaktapur (2019), the government provided 75% value of destroyed chicken marketed amount which equals to NRs 81,086,550.50 (Bird flu compensation report, 2019).

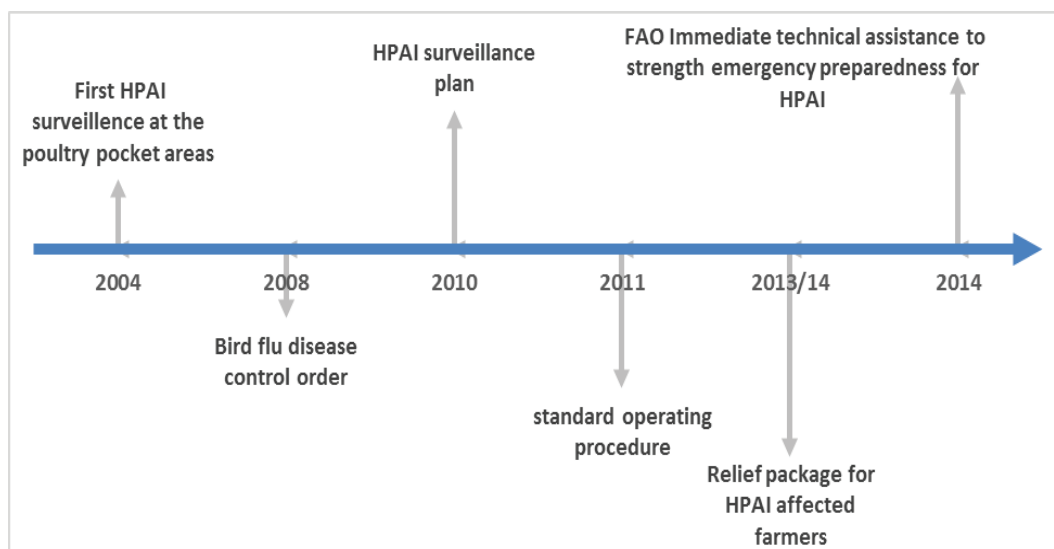


Fig 6: Bird flu control measures

New Castle disease/Ranikhet disease control: This disease has shown its effect on the native birds. The death and low production of eggs are correlated to the ND in rural and urban settings. Various works have been done by governmental and non-governmental agencies to prevent this disease outbreak and vaccinate backyard poultry in Nepal. The first ever vaccination of the ND in Nepal was made in the then CARD (central animal disease research division and Pakhribas Agricultural Research Center. Likewise, ND (lasota) was made in 1998 and ND (heat stable I2) was made in 2008 in the National Vaccine Production Laboratory. Commercial flocks are widely vaccinated as per the vaccination guidelines, however, the vaccination at Backyard Poultry seems challenging. Backyard Poultry being a source of sustainable protein supplementation and reared by marginal farmers as a cash crop is of huge importance in rural settings. The major threat to backyard poultry is ND disease, and to mitigate this problem, Nepal's government launches ND vaccination program. The SUA AHARA project by USAID has vaccinated 3 lakh backyard poultry in 3 districts--3 times with 10 lakh doses, as a result of which there is a reduction in mortality rate from 80% to 30%. With the success of this program, an extension of vaccinations was done in 26 districts (R. K. Neupane, 2018).

Earthquake and Informal Trade Blockade from India: The situation in Nepalese poultry industry is worse in times of earthquake and further exacerbated by the Indian blockade. The government has passed a relief package for disaster hit farmers. However, the relief package was further delayed by irresponsible bureaucracy and Indian Blockade.

Insurance for Poultry Farmers: With growing poultry farmers, and increased frequency of outbreak of disease (bird flu), the Nepal government has started an Insurance policy for livestock farmers with great incentives. For the broiler farmers they have to only 1.5% of total insurance premium amount and for layers and parent farmers they have to pay 5% of the total insurance amount. Insurance should be renewed with a new batch of flocks on the farm ("Plant and livestock insurance," 2019).

Rural Community Targeted Programs: Governmental and non-governmental organizations have focused on rural poultry enhancement for food security and nutrition balance. Different programs - cooperative poultry farming programs, mini hatchery establishment programs, backyard poultry and dual-purpose poultry promotion programs - and small farmers targeted with poultry farming programs have been run by government and non-government agencies (Livestock Statistics of Nepal 2016/17, 2018).

Major challenges for poultry industry in Nepal

Native / Indigenous Breed : Nepal holds three major indigenous breeds namely: Sakini, Ghanti Khuile (Naked Neck), and Pwankh Ulte (Frizzled Feather), which are known as major native poultry genome stocks. Among the three, SAKANI holds the major share with 50% of total birds. These breeds have the pros like well adopted for various altitude and temperature ranges—Temperate Hills to Tropical Terai have disease resistance, good scavenging capability and can live without regular feeding. However, they possess some cons like low dressing percentage, low hatchability, low egg production, and high chick mortality. These birds are not found in the mainstream poultry market, it is a huge challenge to bring the native birds into the commercial market and make them competitive with the exotic breed of chicken.

Feed Ingredients: Nepal imports the majority of poultry feed ingredients from the neighboring country (India).

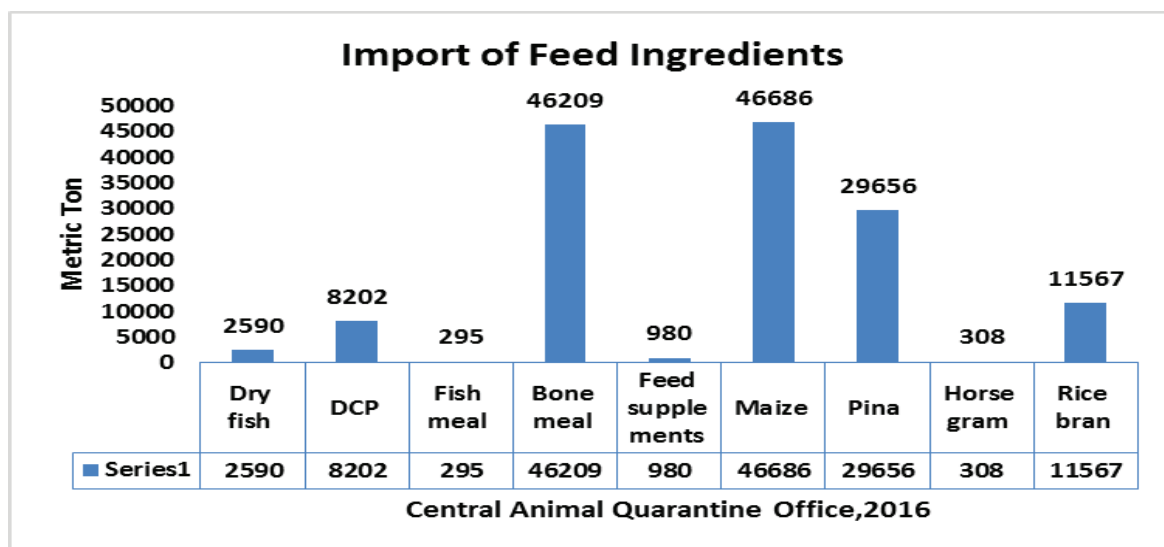


Fig 7: Import of feed Ingredients

Almost 60% of feed ingredients are imported in Nepal, with maize being the highest to be imported at 46686 metric tons. Importing feed ingredients has a direct effect on poultry products like meat

and egg. (Rijal, 2018). As per NFIA (Nepalese feed Industries Association, 2017) NRs 1.8 bilion worth of feed supplements are imported by the feed industry (Singh, 2018).

Fragile Pricing: Nepalese Chicken meat and egg is fluctuating and many variables determine the price. Likewise, the presence of a plethora of middlemen in the poultry product value chain has also affected the pricing of poultry products (Prasain, 2020c; Shrestha et al., 1998). In addition to this, a major factor for this fragile pricing is lack of proper intervention by the government and consumer association.

Veterinary Services and Diagnostic facilities: Veterinary labs and diagnostic facilities were found to be the same as those that were present in the then HMG Nepal, but only little or no change has been made to increase the facilities. Nepal still struggles to implement the federal system of administration, which has also aided poor governmental services. The dearth of skilled manpower in diagnostic and surveillance facilities along with technical equipment's (molecular level) had crippled the veterinary service sector. Only one Avian Designated laboratory NADIL (Nepal Avian Disease Investigation Laboratory) which was established in 2004, can't handle all the surveillance and diagnostic cases. Thus, the burgeoning poultry sector is not in line with the growth of surveillance and diagnostic facilities

Veterinary Acts, Standards and Regulations: Nepal has drafted numerous acts and regulations directly tied to animal heath, meat hygiene and animal feed. Some of these important acts and standards are listed below:

Table 1. Different acts and standards related to Poultry sector in Nepal (Rijal, 2018)

Acts
1. Animal health and livestock services act, 1998 and regulation, 1999 2. Nepal veterinary council act, 1998 and regulations, 2000 3. Nepal Agriculture Research Council Act, 1991 4. Feed act, 1976 and regulations, 1984 5. Slaughterhouse and meat inspection act, 1998 and regulation, 2000 6. Drug act, 1978
Standards
1. Standard for poultry breeding farms: 2005 2. Hatchery standard, 2005 3. Quarantine standard for hatching eggs, 2005 4. Quarantine standard for importation of parent and commercial chicks, 2005 5. Quarantine standard for importation of table eggs, 2005 6. Quarantine standard for importation of hatching eggs, 2005 7. Veterinary standards for biological products, 2006 8. Standard for import and export of meat and meat products, 2007 9. Standards for Broiler Poultry Management, 2007 10. Animal transportation standard, 2007 11. National microbial standards for meat, milk, eggs and MRL of veterinary drugs, 2012

12. Standards for Import Risk Analysis of Live Animals, Animal Products and Veterinary Biology, 2016
13. Protocol for import export recommendation, livestock related industry establishment and sales distribution permission, 2016

Each and every act and standard related to the poultry sector has its own importance and cannot be neglected. However, the effective implementation of these acts and standards seems challenging. Many of the acts and standards like MRL (minimum residue limit), drug act, feed act, are others are being violated due to lack effective implementation and good governance. In addition, many researchers have found violations of public health-related burning issues (antibiotic residue and antibiotic resistance) associated with chicken meat (R. Neupane & Kaphle, 2019).

Prospects and necessary future interventions

The recent scenario of poultry industry in Nepal vividly represents its vibrant growth. Some of the reasons, like preference for white meat over red meat by Nepalese consumers, cheap sources of protein, and tourism boosts have favored the growth of the poultry industry and continued the build up momentum. Despite providing a significant share of GDP and employment for more than 1.5 million people, Nepalese poultry sector faces many constraints as discussed. Some future interventions that can serve a useful purpose are given below:

Feeding materials/ingredients: The majority share of the cost of poultry farming goes to feed and the use of indigenous and local feed ingredients is a major conflict with the establishment of self-sustained poultry industries in Nepal. To produce enough (maize and soya) different approaches like contract farming, cooperative farming, could be carried out. Poultry feed industries can collaborate with local cooperatives and communities to produce the feed ingredients, which in turn benefits both the farmers and industries. Probiotech (Nimbus feed industries), had initiated the contract farming (maize, rice) with the local cooperatives and farmers with the support of International finance corporation (a world bank group), and GAFSP, till 2015 it had trained about 4000 maize farmers with 70% of them adopting new technology with an increase in 20% of local maize productivity (*IFC INCLUSIVE BUSINESS CASE STUDY and Probiotech*, n.d.). Recently, a research work on integration of black soldier fly larvae as a protein source in poultry feed was carried out at Agriculture and Forestry University, which can serve as a good alternative to imported protein feed, however, a thorough cost benefit analysis cannot be discounted.

Native breed integration in commercial poultry farming: Indigenous breeds of poultry (Sakini, Ghantikhulae, and Pwankhulte) can be an alternative to imported breeds of poultry. Incorporating these breeds into the mainstream market from the backyard can be a sustainable alternative to dependence towards foreign breeds. Different types of genetic intervention like selective mating and breed hybridization can be done in native breeds for sustainability. Currently in Nepal there is only one grandparent farm established by Cobb, but only one grandparent farm cannot hold the demand of hatcheries, thus there is urgent need of establishment of grandparent farm to sustain chicks demand.

Antibiotic resistance and residue surveillance: Many researchers have pointed out that antibiotic residue in poultry meat is higher than the minimum residue level set by WHO. 24.66% of marketed broiler meat in the Kathmandu metropolitan city has shown the presence of antibiotic residue, in a study conducted in 2019 (Maharjan et al., 2020). However, more surveillance activities are needed to understand the situation better. In order to ensure safe and quality production of poultry products, periodical screening of poultry products for residue and resistance is inevitable. There is also an urgent need to establish a separate veterinary drug enforcement agency.

Vaccine and Drug Manufacturing: Nepal imports huge amounts of poultry vaccines from India and other countries. The National Vaccine Production Laboratory produces vaccines for various poultry diseases like New Castle/IBD, however, many hatcheries and large scale poultry farms are using imported vaccines. Biovac Nepal, a private company, is also producing some poultry vaccines. Veterinary and poultry drugs (antibiotics, water acidifiers and liver tonics) are imported from India and other countries though there are few Nepalese drug manufacturers. The import of large quantities of vaccines and veterinary drugs suggest that either Nepalese manufacturers are not competent enough (quality and reliability) or they cannot supply the demand by Nepalese market.

Shift to environmentally controlled housing: The dynamics and contemporary scenario of poultry market in Nepalese context suggest that in order to sustain the farmer capability for the long run, transformation from the traditional housing system to modern environmental housing is inevitable. Now, well-established business tycoons: Chaudhary Group, Golcha Group, along with other business enterprises from FDI (Foreign Direct Investment) are keeping an eye on the fortuitous poultry business, as this type of investment can provide consumers with safe and quality meat products but can hamper small-scale poultry farmers as they are obliged to change their business or shift to costly modern farming practices.

CONCLUSION

To sum up, the Nepalese poultry market has huge potential for growth and expansion with flexibility for product diversification. However, things like fragile pricing, import of high amounts of feed ingredients, diagnostic and surveillance facilities, and native breed integration should be solved in order to sustain the Nepalese poultry farmers in the long run. The drafting and launching of relief packages for crisis hit farmers (COVID-19) should be carried out in an immediate manner to save the farmers from bankruptcy. In addition to this, there should be regular monitoring and evaluation of the governmental policies/acts/standards/specifications to ensure safe and quality poultry products.

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A Case Report of Tyzzer's Disease in Two Kittens in Japan

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ABSTRACT

Clostridium piliforme is an obligatory intracellular bacterium that causes Tyzzer's disease in a wide range of hosts. This is a report of Tyzzer's disease in kittens. Two kittens with no remarkable clinical anamnesis were sent for a necropsy. Gross necropsy findings were not promising except for the signs of diarrhea, roundworms, and foreign body. Histopathology of paraffin-embedded tissue revealed focal necrosis in the liver and diffuse degeneration of intestinal mucosa. Giemsa and Warthin-Starry stains revealed the presence of intracellular filamentous organisms arranged in parallel or crisscross bundles around necrotic areas of the liver and intestine. These findings were supported by previous reports to be Tyzzer's disease caused by *Clostridium piliforme*.

Keywords: Cat; *Clostridium piliforme*. Tyzzer's disease

INTRODUCTION

Tyzzer's disease is an acute, spontaneous infection of young animals caused by spore-forming gram-negative obligatory intracellular bacteria, *Clostridium piliforme*. Tyzzer's disease has been reported in a wide range of hosts which include laboratory animals such as a mouse, rabbit, guinea pig, hamster, gerbil, rat, and a variety of wildlife including muskrat, cottontail rabbit, snow leopard, coyote, gray fox, lesser panda, raccoon, marsupial and white-tailed deer. It is a highly fatal disease of young foals but rare in other domestic animals like dogs, cats, and calves (Ikegami et al., 1999). One case of human infection has been reported in HIV-1 infected patient (Smith et al., 1996). The disease is also reported in 2 psittacine birds and a weaver bird (Mete et al., 2011). In short, these organisms can infect a wide range of mammalian species and also the avian species.

Naturally occurring Tyzzer's disease has been reported in many instances in cats. All cats presented hepatic lesions and lesions in the small intestines with a few cases presenting colitis. Most of the cases reported included kittens of ages 2 months or less and concurrent immunosuppressive factors like viral infections and administration of corticosteroid drugs. The clinical signs and symptoms are peracute and acute as in most of the cases the animals die within three days in laboratory animals though it is noticed that lethargy, depression, anorexia, and rapid abdominal discomfort and distension are constant in dogs and cats. The actual pathogenesis of this disease is not well understood but is presumed to be transferred by faeco-oral route through ingestion of spores. *Clostridium piliforme* adheres to the intestinal epithelial cells and there is local proliferation after infection. The mechanism by which the bacteria attaches to and enters host cells

is unknown (Jones et al., 2012). When a host is immune suppressed, the organism spread through the portal circulation to the hepatic cells and causes multifocal periportal necrotic lesions. The organism has an affinity to the cells of the intestine, liver, and heart.

DESCRIPTION OF THE CASE

Two kittens of the same litter, approximately 2-3 months of age were sent for necropsy at the veterinary pathology laboratory, The University of Tokyo. Clinical anamnesis was not remarkable but one cat had bloody diarrhea. Specimens of the stomach, small intestine, large intestine, liver, kidney, heart, lungs, pancreas, tongue, thyroid, spleen, adrenal gland, hypothalamus, urinary bladder, bone marrow, bronchus, and trachea were fixed in 10% buffered formalin and paraffin-embedded blocks were prepared. The paraffin-embedded tissues sectioned at approximately 4µm were examined using routine hematoxylin and eosin (HE) stain. Liver and intestines with lesions were stained with Giemsa and Warthin-Starry stains.

Gross necropsy findings were unremarkable but both cases had dark purple colored spleen while the stomach and duodenum of both kittens revealed multiple roundworms. A plastic tube of 1.5 cm length was found in the jejunum and petechial bleeding in the mucosal surface of the pyloric part of the stomach with thinning of the intestinal wall was seen in one kitten. The intestinal content of both kittens had scant whitish liquid providing evidence of diarrhea.

Histopathological findings were restricted to the liver, small intestine, and large intestine. In the liver, multiple focal necrosis was seen in both kittens (Fig 1). The necrotic area consisted of eosinophilic materials, necrotic cellular debris, erythrocytes, and mononuclear cells. Hepatic cells bordering the necrotic area showed bundles of basophilic, filamentous structures in the cytoplasm which was faintly visible with HE stain. With Giemsa stain, the basophilic structures were visible as purple to blue-stained structures (Fig 2). The Warthin-Starry stain revealed the organisms as a brown to black colored filaments (Fig 2, inset). In both cases, small and large intestinal mucosa showed diffuse necrosis with a moderate number of lymphocytes, neutrophils, plasma cells and cell debris (Fig 3). Like in hepatic cells, the intestinal mucosal cells also had the presence of basophilic filamentous structures arranged in bundles which were vivid as blue-purple with Giemsa stain (Fig 4) and brown-black with Warthin-Starry stain (Fig 4 inset).

In this study, hepatic and intestinal involvements were seen with the presence of the filamentous, basophilic structures which resembles *Clostridium piliforme*, the causative organism of Tyzzer's disease in both cases. The lesions in the liver and intestines were similar to those reported in other species of animals with Tyzzer's disease. A majority of infected feline cases had naturally or experimentally induced immunosuppressive diseases such as feline leukemia, feline panleukopenia, and feline infectious peritonitis. Unlike previous case reports by Neto *et al.* (2015), Bennett *et al.* (1977), and Ikegami *et al.* (1999), neither of the cases had evidence of any concurrent diseases nor immunosuppressive factors. But these cases resemble with few case reports that also did not find the involvement of the immunosuppressive factors or other concurrent diseases (Wilkie *et al.*, 1985).

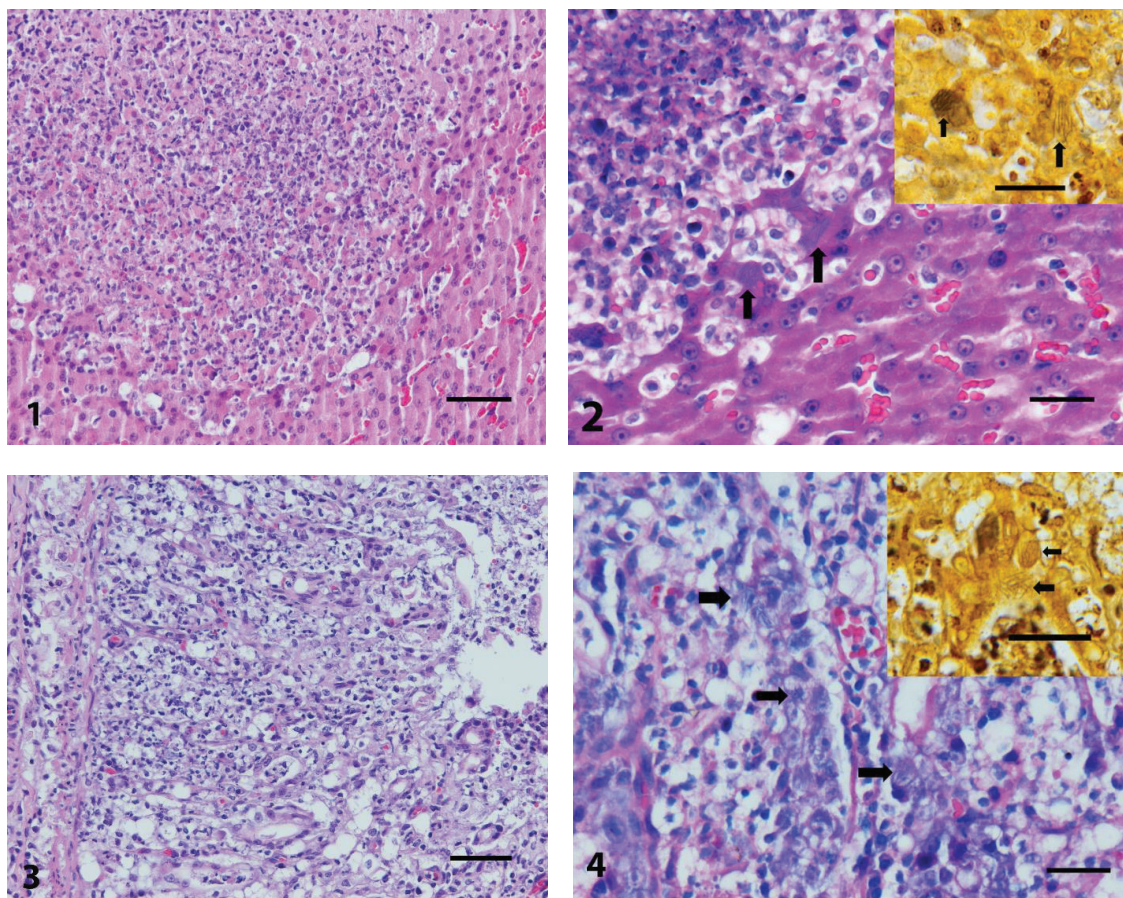


Figure 1: Focal necrosis in liver with cell debris. Hematoxylin and Eosin stain (HE). Scale: Bar= 50 μ m

Figure 2: Blue stained bundles of long filamentous organisms (arrows) in giemsa stain. *Inset*: black stained filamentous organisms (arrows) in Warthin-Starry stain. Scale: Bar = 25 μ m

Figure 3: Cell debris and inflammatory cells in intestine. HE Scale: Bar = 50 μ m

Figure 4: Blue stained bundles of intracellular long organisms (arrows) in mucosal cells of intestine seen through Giemsa stain. *Inset*: brown to black bundles of organisms in mucosal cells as seen in Warthin-Starry stain. Scale: Bar = 25 μ m

Stress as a result of weaning, overcrowding, poor sanitation, shipping, irradiation, or corticosteroid administration often predisposes Tyzzer's disease (Ganaway et al., 1971). Both kittens were 2-3 months of age that is about the age of weaning. During weaning, the change in diet and environment might have contributed for the infection of the kittens with *Clostridium piliforme* from the environment. Experimental disease has been difficult in healthy dogs and cats and most feline cases have occurred in cats with rodent contact in the lab (Jones et al., 2012). The presence of roundworms in the stomach in both cases and the plastic tube in one case were insufficient to contribute to the death of the kittens. But necrotic enteritis and focal hepatic necrosis with the presence of *Clostridium piliforme* can be contributive for the death of both the kittens. According

to Gelberg H.B. (2017), the presence of causative bacillus around the necrotic hepatic foci is the definitive diagnosis for Tyzzer's disease which supports these are the cases of Tyzzer's disease.

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Factors affecting Somatic Cell Count in Milk

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ABSTRACT

Mastitis, an inflammation of mammary gland, causes huge economic losses in dairy industry. Mastitis is indicated by the abrupt increment of somatic cell counts (SCC) in the milk. Somatic cells are the immune response cells acting against any pathogens invading mammary tissues consisting primarily the macrophages, lymphocytes and polymorphonuclear cells. The normal SCC level in milk was considered to be 200,000 cells per ml in milk and SCC count above this threshold indicate intra-mammary infections. Apart from infections, different other animal and management related conditions affect the level of somatic cells. Some of the factors contributing the fluctuations of milk somatic cells were stress, milking frequency, age, breed, stage of lactations, parity, shed management conditions and body condition scores.

Keywords: Infections, Mastitis, Somatic Cells.

INTRODUCTION

Mastitis is commonly known as the inflammation of mammary tissues with significant changes in udder parenchyma caused due to the alteration in the interaction among host, pathogen and environmental factors (Ali et al., 2008). The major contagious pathogens that causes mastitis are *Staphylococcus aureus* and *Streptococcus agalactiae* while environmental pathogens includes *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Escherichia coli* (Waller et al., 2009). Among these pathogens, *Staphylococcus aureus* was the most prevalent (Barkema et al., 2006). Mastitis has the potential to cause huge economic losses due to reduced milk production, veterinary cost, premature culling, increased labour, discarded milk and decreased milk quality (Fagiolo et al., 2005). The disease occurred in two forms, clinical and subclinical, classified on basis of clinical signs and symptoms (Dhakal, 2006). Clinical mastitis is symptomatic and characterized by fever, swollen and painful quarters, dehydration, remarkable change in milk yields and composition alterations while the sub-clinical mastitis is asymptomatic and is more hazardous as farmers are not aware of its development (Singh et al., 2018). The economic burden of mastitis in Nepal was estimated to be 63 USD/buffalo/lactation with additional costs for veterinary services, extra labour, and medicines (Ng et al., 2010). Similarly, the loss due to mammary gland infection in United States was recorded approximately 2 billion USD and similar trend was seen in European dairy industries (Donovan et al., 2005). The total annual loss of 35 billion USD worldwide was estimated only due to mastitis (Raza et al., 2011). The quality and hygiene of milk could be assured through the mastitis control strategies implemented in the farm that is possible through the regular monitoring of somatic cell counts (SCC), an indicator for udder health (Jadhav et al., 2016).

Somatic Cell Counts

The defense cells released against any intra-mammary infections (IMI) that have capacity to repair damaged cells in udder are somatic cells (Flere et al., 2016). Somatic cells are the immune response cells, part of natural defense and golden standard to evaluate IMI (Hamann, 2002). The somatic cells comprised of pathogen warning signals, epithelial secretions and leucocytes where macrophages (60%), lymphocytes (30%) and polymorphonuclear cells (10%) are present (Alhussien & Dang, 2018; Brahma et al., 2017). The leucocytes in blood stream gets massively influxed in milk when resident somatic cells activated due to IMI and showed the increment of SCC when measured as a regular monitoring (Schukken et al., 2003).

The overall milk quality and its suitability for further processing is determined by the monitoring of milk SCC and if above threshold level of 200,000 cells/ml is recorded, discarding of milk is recommended (Bytyqi et al., 2010; Dhakal, 2006). The main reason of reduced quality of milk-based products with higher SCC is because of lipolytic and proteolytic enzymes in somatic cells degrading quality of milk fat and proteins (Talukder & Ahmed, 2017). The legal limit of SCC varies in different countries for example 300,000 cells/ml in China; 400,000 cells/ml in Australia and 500,000 cells/ml in Brazil (Alhussien & Dang, 2018) whereas a researcher suggested the normal level of SCC to be up to 200,000 cells/ml in milk (Siatka et al., 2019).

The variation in normal level of SCC differs by species as well. The normal level of milk SCC was found to be 200,000 cells/ml in buffaloes (Dhakal, 2006); 1,000,000 cells/ml in goat and 750,000 cells/ml in sheep in US (Paape et al., 2001). The SCC is estimated by utilizing several methods such as Newman-Lampert staining technique (Dhakal, 2006), flow cytometry (Li et al., 2014) and electrical cell counters with fluorescent dye (Alhussien & Dang, 2018). The implementation of udder health programs in farms and estimating SCC will help to prevent new IMI (Schukken et al., 2003). The dry cow management therapy, adequate vitamins and minerals in feed/diet, using vaccines against pathogens, supplementing antioxidants, post milking dipping and shed hygiene are common preventive measures applied to control of IMI and SCC elevation (Paape et al., 2001; Raza et al., 2011; Skrzypek et al., 2004).

Factors affecting SCC

The release of SCC in milk is a natural process but its increasing number is considered the indication for IMI or mastitis (Pyorala, 2003). Major factors affecting the release of SCC are discussed below.

a. Stress

In general, stress has negative influence in the SCC level. Dhakal (2006) found positive correlation between temperature and rainfall and outbreaks of mastitis. Confinement in harsh condition with higher humidity and ambient temperature increases susceptibility to mammary pathogens and leads to increase in SCC. Likewise, injections of ACTH contribute to significant increase in SCC in milk. Usually, stress leads to 10-20% decline in milk in dairy animals (Brahma et al., 2017). During stress, free radicals are produced that are unstable and react quickly causing injury in the tissues (Sharma et al., 2011). Vaccination and estrogen administration for estrus in dairy cows resulted in significant increase in SCC independent of the change in milk yield (Haenlein, 2002).

Transportation and storage methods in different preservatives such as potassium dichromate, azidol and bronopol demonstrate to affect the SCC by increasing the cells per ml (Sharma et al., 2011).

b. Milking Systems and frequency

Milk removal from mammary glands can be done by calf, hand or machine. Nowadays, with an increase in milk yielding capacity of animals, milking machines have become integral part of larger dairy farms. If proper hygiene of the machine is not maintained, there is a increased risk of transferring infectious agents and may increase cases of sub-clinical mastitis (Brahma et al., 2017). Machine milking requires proper cleaning, functioning and maintenance for acquiring quality milk in terms of SCC (Alhussien & Dang, 2018). Even shifting the milking frequency from twice a day to thrice will decrease bulk milk SCC in cows of higher SCC but if milking intervals is shorter than 4 hours or less, it will raise the milk SCC (Pyorala, 2003; Sharif & Muhammad, 2008). SCC level is higher during stripping and lower before milking and after 4 hours of milking. Day to day variations are also observed up to 30% without any relevant changes in conditions (Sharma et al., 2011). Skrzypek et al. (2004) had well illustrated the association of lower SCC due to the manual massaging of udder before milking that may happen because of greater stimulus for oxytocin release.

c. Breed

Variations have been observed in SCC levels in different breeds and species. Higher milk producing cattle like Brown Swiss and Holstein shows cell counts ranging 300,000-400,000 per ml (Sharma et al., 2011). Udder and teat morphology also get affected due to breed type where well attached udder reveal lower SCC than pendulous ones but shorter teats with greater diameter have higher milk SCC (Alhussien & Dang, 2018). The prevalence of clinical mastitis is highly affected by the breed type and is present in decreasing order of Karan Swiss, Karan Fries, Sahiwal, Tharparker and Murrah buffaloes. This indicates buffaloes possess superiority in mastitis resistance. The higher resistivity may be due to tightly closed teat orifice and circular muscles thick squamous keratinized epithelium of streak canals (Jingar et al., 2014). However, the teat-end lesions have no significance unless there is a milk leakage. On quarter SCC, reduction in teat-end to floor distance has positive correlation to IMI (Bhutto et al., 2010). Significant difference in SCC level was not found by breed of cattle as compared to goats breeds (Paape et al., 2007). The study conducted in Slovenia showed no considerable differences in SCC between the breeds, either crossbreed or pure lines among cattle (Flere et al., 2016).

d. Age and Parity

Advancing age will influence towards increment of the SCC level due to higher possibility of exposure to pathogenic agents in cows (Flere et al., 2016; Sharma et al., 2011). The incidence of clinical mastitis may also increase with the increased parity in both the cattle and buffaloes (Jingar et al., 2014). The increased prevalence of mastitis was observed in older buffaloes where age group 9-11 years was found at higher risk compared to 3-5 and 6-8 years old buffaloes (Ottalwar et al., 2018). It might be due to the breakdown of streak canal barriers and udder tissues.

The mammary gland immunity of primiparous cows is higher as compared to multiparous throughout the lactation period. Cattle more than 4th parity shows increased diurnal variations in SCC levels (Alhussien & Dang, 2018). Multiple births and short duration of lactation is also associated with the elevation of milk SCC (Paape et al., 2007).

e. Shed Managements

Farm practices such as post milking feeding, teat dipping, flooring and milking procedures reveal lower SCC whereas unhygienic condition of farm and utensils increases the cells count (Savić et al., 2017). Proper interventions for good managerial practices in shed can have positive effects to lower the SCC count (Bytyqi et al., 2010). Somatic cells were found lowest (129,500 cells/ml) in clean and comfortable environment while it was found to be much higher (1,013,400 cells/ml) in moist and dirty environment (Dhakal, 2006).

SCC levels are lower during winter and highest in the summer season as the number of environmental pathogens increases during warmer temperature (Sharma et al., 2011). In countries where non-seasonal calving pattern exists and more influenced by temperatures and humidity, the possibility of IMI is higher and SCC levels are elevated (Alhussien & Dang, 2018). The probable reason for increased IMI and SCC in summer besides warmer temperature and higher humidity may be due to irritation and spreading of pathogens in udders by flies (Skrzypek et al., 2004).

f. Stages of lactation

Stages of lactation may be classified as early, mid and late lactation. There is a positive correlation between SCC and stages of lactation whereby scores is higher in early phase, gradually decrease in mid and then again increase in late stage (Alhussien & Dang, 2018; Pyorala, 2003). The high yielding cow experiences negative energy balance during early stage of lactation due to which non-esterified fatty acids (NEFA) and β -hydroxy butyric acids (BHB) are increased which impairs phagocytic activity of mammary gland thereby leading to increased susceptibility to mastitis (Ottalwar et al., 2018). Whether the cow is infected or not, late lactation phases have shown elevated SCC which is linked with preparation for calving and enhancing of mammary defense mechanisms (Sharma et al., 2011). The uninfected quarter demonstrate SCC of 83,000 per ml in 35 days post-partum but raised to 160,000 by day 285 (Sharif & Muhammad, 2008).

g. Body condition score (BCS) and body weight

BCS is a method of evaluating fatness or thinness of dairy animals referring to the well-defined scale. Lower SCC is associated with increased BCS and weight loss during early stage of lactation (Alhussien & Dang, 2018). Similarly, BCS and amount of body weight change from 60 to 120 days in milk is significantly associated with the higher SCC across lactation but the probability of clinical mastitis cannot be justified (Berry et al., 2007). BCS, practical indicator of feeding adequacy is highly associated with the impairment of udder health where even slight deviations will lead to significant effects (Skrzypek et al., 2004).

CONCLUSION

Udder health is one of the major issues in modern dairy industry for production of quality milk and milk products. The milk somatic cell counts can be an indicator for monitoring the dairy udder health for easy and early identification of intra-mammary infections. Factors affecting the fluctuations of SCC need to be considered and necessary actions should be adopted to maintain milk SCC level.

Conflict of Interest

Authors declare that there is no conflict of interest.

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Investigating the presence of *Oesophagostomum* species in slaughtered goats in the central Nepal

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ABSTRACT

Oesophagostomum infects various hosts like caprines, ovines, porcines, rodents, primates, and humans. The aim of the current study was to identify different species of *Oesophagostomum* in the intestines of sacrificed goats during festival seasons in the central part of Nepal. From ten slaughtered male goats, *O. asperum* and *O. venulosum* were identified by light microscopy and line drawing methods accompanied by the taxonomic keys. These methods were used to completely describe the presence of two *Oesophagostomum* species for the first time in Nepalese goats and are crucial in the laboratory with the limited facilities.

Keywords: Copulatory bursa, *Oesophagostomum asperum*, *Oesophagostomum venulosum*, taxonomy.

INTRODUCTION

The genus *Oesophagostomum* (nodular worm) (Strongylida: Chabertiidae) represents a dominant nematode parasite of suids, ruminants, primates, African rodents and humans (Stewart & Gasbarre, 1989; Thornton, 1924). Few species of *Oesophagostomum* like three species like *O. bifurcum*, *O. stephanostomum*, and *O. aculeatum* can infect humans (Polderman and Blotkamp 1995; Chabaud and Lariviere 1958; Skrjabin et al. 1961; Blotkamp et al. 1993). The freshly laid eggs of *Oesophagostomum* are passed into the feces, hatch into L1 and then into L3 in the environment in one week. When hosts ingest L3 along with soil or grasses, they develop into adult worms (<https://web.stanford.edu/group/parasites/ParaSites2002/oesophagostomiasis/epi.html>, Retrieved February 12, 2021). This parasite is the underlying cause of clinical and veterinary oesophagostomiasis characterized by weight loss, persistent diarrhea, and granuloma formation, and caseous lesions or abscesses in the intestines of variety of hosts including man and animals (Goldberg, 1952; Stewart & Gasbarre, 1989). Because the parasite can be transmitted between vertebrates and humans, it is critically important as veterinary and public health all over the world.

In Nepal, coprologic studies have been conducted to find out the prevalence rates of strongyles, however, it is difficult to identify the species of *Oesophagostomum* from other species of strongyles. It is very difficult to identify the species by microscopy only because eggs are morphologically similar to false hookworm (*Ternidens deminutus*), hookworms and other strongyles like *Trichostrongylus*, *Bunostomum*. Few laboratories use coproculture of L3 larvae or histopathology of gastrointestinal tract to isolate adult worms. In these situations, morphological

keys will be supportive in complete identification of these species when molecular studies like polymerase chain reaction (PCR) and Scanning Electron Microscopy (SEM) are lacking (Gaddam et al., 2017a). These techniques are both expensive and time consuming for developing countries like Nepal. Therefore, we aimed to identify *Oesophagostomum* species up to species level in goats using morphological keys. Although *O. columbianum*, the small ruminant nodular worm (*O. asperum*), and the large bowel worm (*O. venulosum*) are predominantly reported in goats and sheep (Goldberg, 1952; Yu et al., 2012; Zhao et al., 2013), we have recorded only two species, (*O. asperum* and *O. venulosum*) in goats in the central part of Nepal.

MATERIALS AND METHODS

Sample collection, processing, and examination

During the festive seasons of Hindus, 10 sacrificed goats were purposively sampled in the Chitwan area that lies in the central southern part of the country. The stomach and intestines of those goats were carefully collected, and they were dissected longitudinally. The worms embedded in mucosa were separated and collected in the saline solution containing 0.9% sodium chloride. Some of the worms were put in 70% ethanol. Then, they were brought to the laboratory in Kathmandu.

The nematodes (milky colored with bent hook-like structure anteriorly and posteriorly) were washed several times with saline solution (0.9%). Then, they were placed in watch glass containing lactophenol (HIMEDIA, India) for 5 minutes. The nematodes were put in a slide containing glycerine. Canada balsam (Fizmerk, India) was used to tighten the coverslip. In another context, the nematodes preserved at 70% alcohol were washed in normal saline (0.9%). Nematodes were also stained by Giemsa's stain (Fisher Scientific) and Lugol's Iodine (Fisher Scientific) differently and slides were prepared. Finally, all the specimens were observed under light microscope (Optika Microscopes Italy, B-383PLi) at a total magnification of X40, X100, and X400 and images (1920 pixels x 1020 pixels) were taken using SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright (C) 2013-2014. The live images were drawn at the tracing paper over the screen of a laptop. Then, *O. venulosum* and *O. asperum* were confirmed by the keys published by various authors (Gaddam, Murthy, & Kommu, 2017a; Khanmohammadi, Halajian, & Ganji, 2013; Yadav & Tandon, 1992). These keys were mainly based on the presence of the mouth cone, the number and the shape of the elements of corona radiata, the pattern of the cervical vesicle, and position of vagina and anus in the female, and the structure of spicules on the male.

RESULTS AND DISCUSSION

In this study, out of 10 goats, only two were found to be infected with *Oesophagostomum* spp. Both goats had multiple infestations of *O. asperum* and *O. venulosum*. Morphometric measurements of different structures of males and females *O. asperum* and *O. venulosum* were recorded (Table 1).

Table 1: Characteristic features and measurements (mm) of female and male *Oesophagostomum aspersum* and *Oesophagostomum venulosum*. The measurements are expressed in minimum to maximum and average values are put in the parenthesis.

Characteristic features	<i>O. asperum</i>		<i>O. venulosum</i>	
	Female measurements (mm) (N=2)	Male measurements (mm) (N=4)	Female measurements (mm) (N=3)	Male measurements (mm) (N=3)
Body length	8.4-8.7 (8.55)	8.49-9.12 (8.79)	12.48-17.78 (14.88)	11.35-13.1 (12.23)
Max width body	0.38-0.40 (0.39)	0.42-0.45 (0.43)	0.42-0.6 (0.50)	0.40-0.49 (0.45)
Esophagus length	0.74-0.78 (0.76)	0.76-0.88 (0.81)	0.83-0.94 (0.87)	0.80-0.90 (0.85)
Max esophagus width	0.12-0.12 (0.12)	0.13-0.15 (0.14)	0.10-0.13 (0.12)	0.11-0.12 (0.12)
Width at end of esophagus	0.31-0.32 (0.31)	0.24-0.28 (0.26)	0.31-0.42 (0.36)	0.24-0.27 (0.26)
Width at start of esophagus	0.22-0.23 (0.22)	0.15-0.16 (0.16)	0.06-0.17 (0.12)	0.10-0.15 (0.13)
Tail length	0.08-0.09 (0.09)	-	0.30-0.43 (0.35)	-
Tail tip to vulval part	0.13-0.16 (0.15)	-	0.70-0.80 (0.76)	-
Vulva width	-	-	0.02-0.03 (0.03)	-
Vagina length	-	-	0.05-0.06 (0.05)	-
Rectal length	-	-	0.20-0.48 (0.29)	0.15-0.20 (0.18)
Length of spicules	-	1.21-1.35 (1.29)	-	0.60-1.2 (0.88)
Average length of rays	-	0.51-0.56 (0.54)	-	0.18-0.30 (0.23)

The anterior end of both male and female *O. asperum* was characterized by the presence of a salient mouth collar or mouth capsule (MC) formed by the cuticle layer (Figure 1-3). The microscopic study showed that the MC was characterized by external corona radiata (ECR) with a total of 12 elements although we could draw only six of them on one surface (Figure 2). In spite of 24 internal corona radiata (ICR), we observed 12 of them on one side of the capsule. There was a cervical groove (CG), a ventrally prominent structure, extended behind the lateral line. Cuticle upper to the CG was widening to form the cephalic vesicle (CV) which was well developed, highly swollen, and was ridged producing three to four tiers (Gaddam et al., 2017a; Yadav & Tandon, 1992). However, these ridges changed into fine annular striations or lateral alae posteriorly, even after esophagus (Figure 1). In male *O. asperum*, bursa was well developed with rays. There were 12 bursal rays of various sizes located inside the flap-like bursal pouch. However, their endings were provided with sharp hook-like structures. There were paired long, tubular, alate, and equal thread-like spicules. The spicules were curved at their tips and were provided with a small rounded knob (Figure 4-5). In female *O. asperum*, the vulva and vagina were located at a short distance from the posterior end of the body. Microscopically, anus and vulva lie quite close to one another as revealed by using the scanning electron microscope (SEM) by others (Yadav & Tandon, 1992).

The reproductive pores consisted of an upper labium and a lower labium that had some longitudinal stripes (Yang, 1996). Tail tapered sharply (Figure 6-7).

In summary, a total of 12 ECR, the highly developed cervical vesicles, the paired, tubular, alate, and equal thread-like spicules with a rounded knob at the tips, the vulva and the long vagina located closely from the posterior part of the body, and the closely placed anus and vulva are the characteristic features of *O. asperum*. In contrast, 18 ECR, less developed CVs, a large bell-shaped bursa with muscular bursal rays, prebursal papillae anterior to the bursal lobe, the slender and tubular spicules, and the short, transverse, and kidney-shaped female openings are the main taxonomic keys of *O. venulosum*.

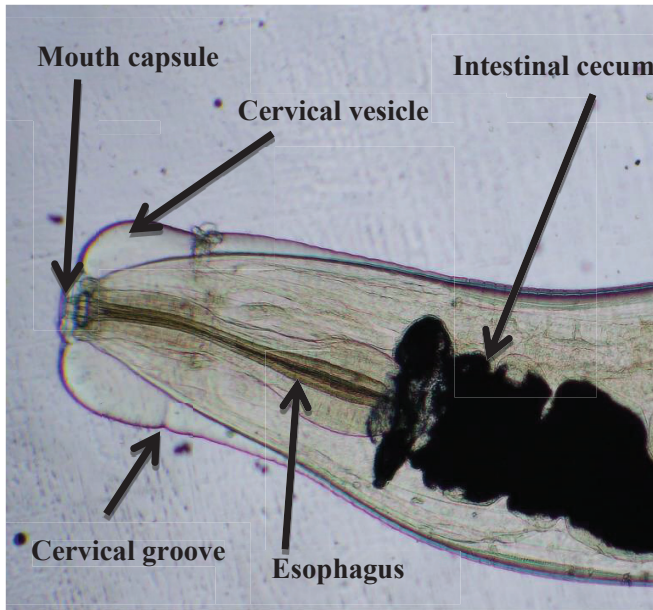


Figure 1: Photomicrograph showing the anterior end of *O. asperum* (X40).

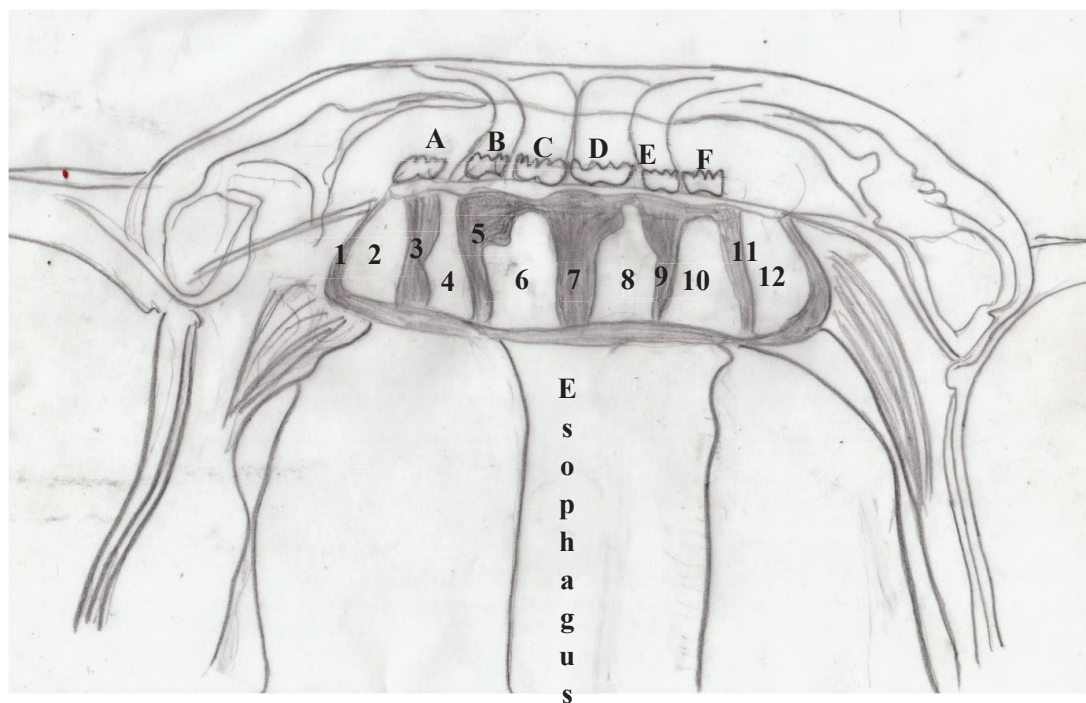


Figure 2: Anterior end of *O. asperum* (X400). 1 to 12 represents internal corona radiata on ventral surface. A to F represents external corona radiata.

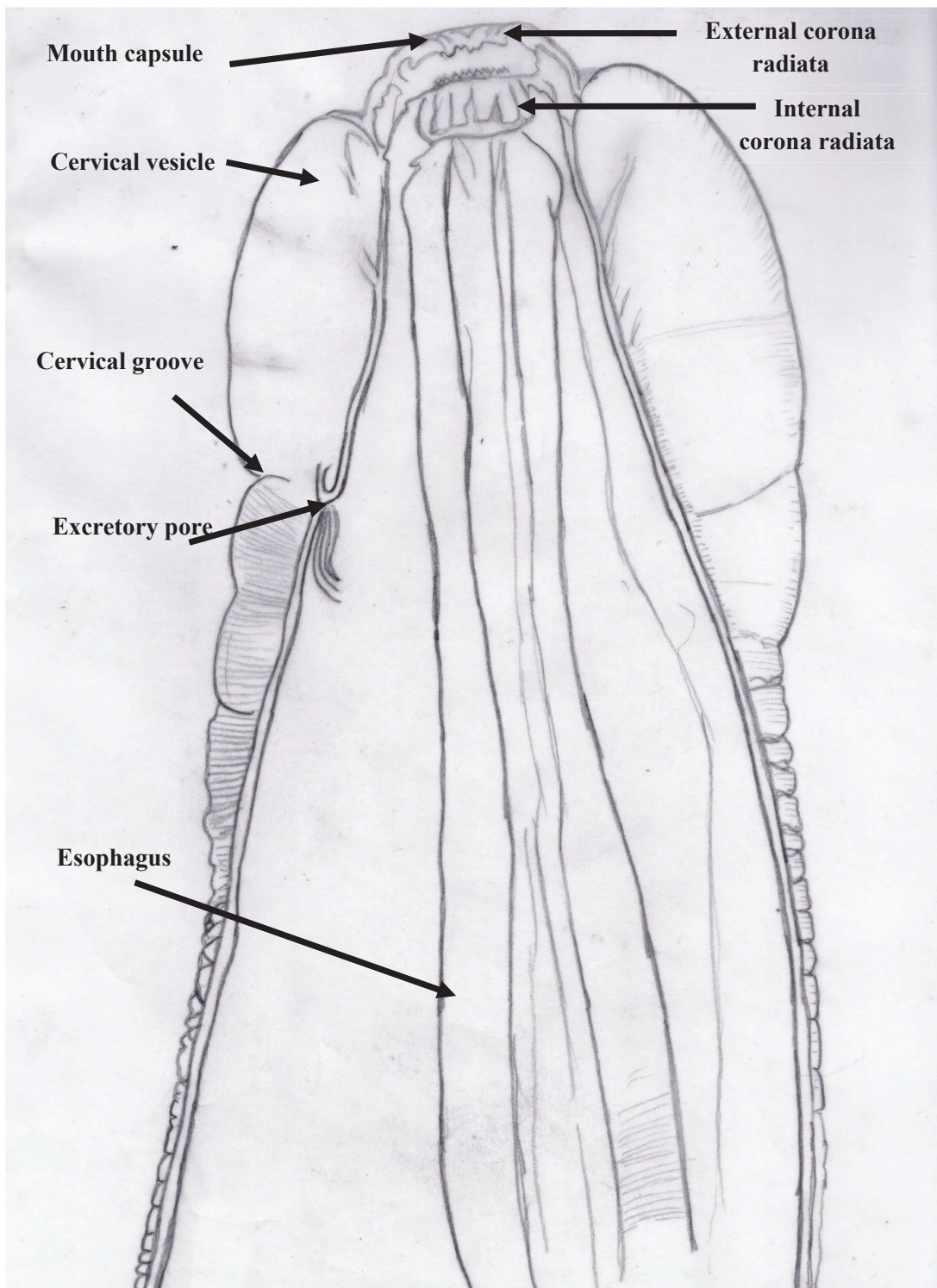


Figure 3: Anterior end of *O. asperum* (X100)

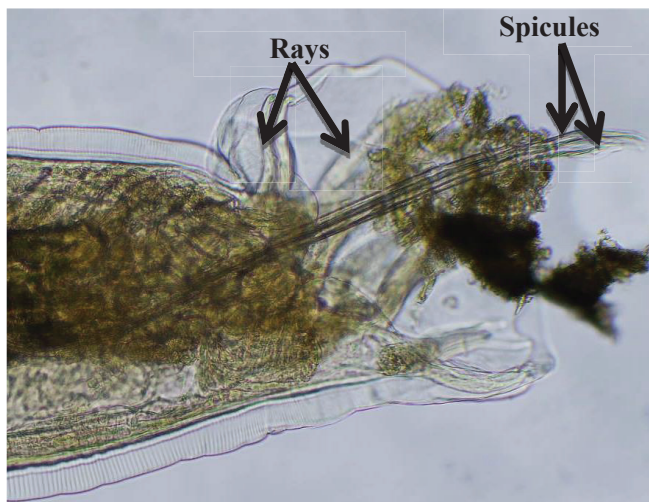


Figure 4: Photomicrograph showing the posterior end of male *O. asperum* (X100)

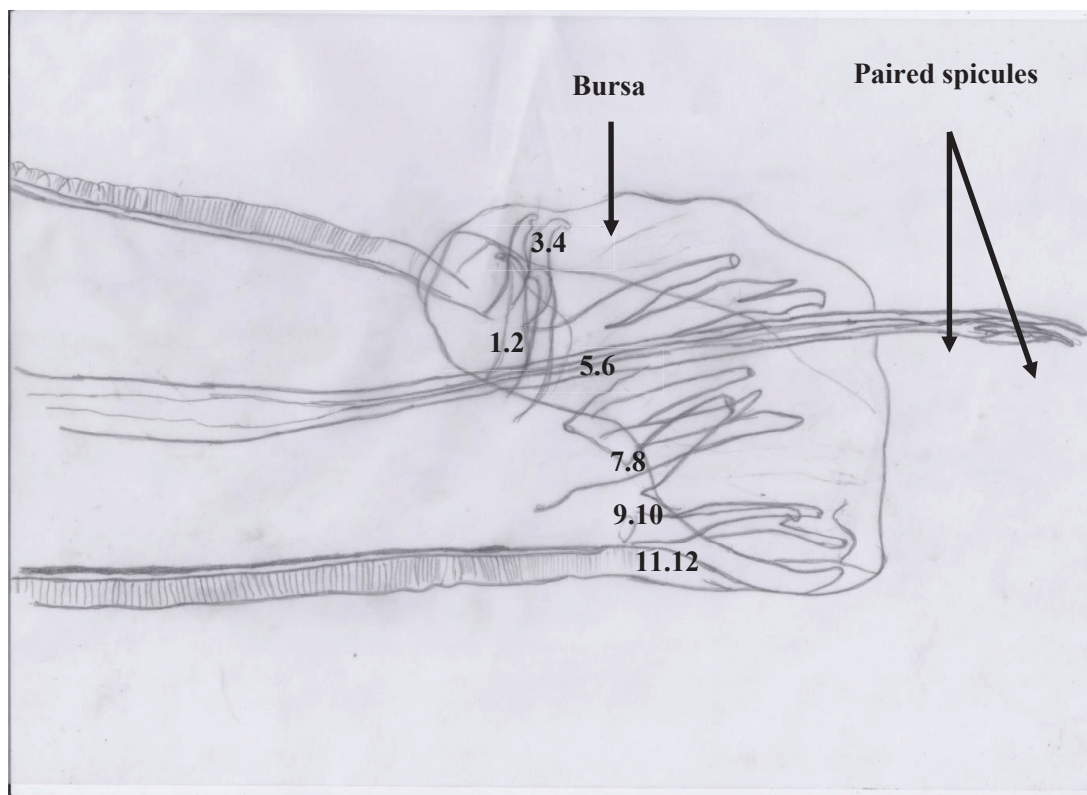


Figure 5: Posterior end of male *O. asperum* (X100). This part includes bursa copulatrix with a paired spicule and 12 rays. Each pair has been represented by point in between two rays

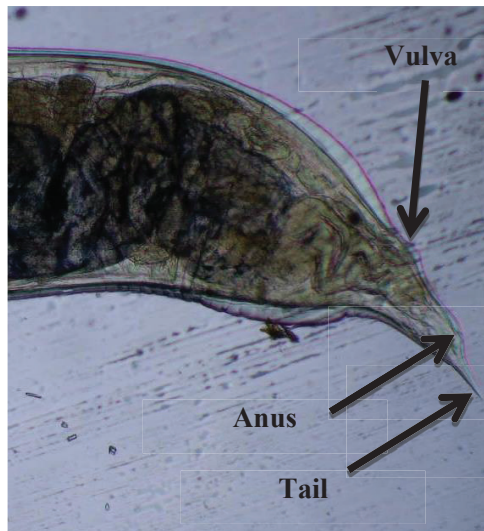


Figure 6: Photomicrograph showing the posterior end of female *O. asperum* (X40).

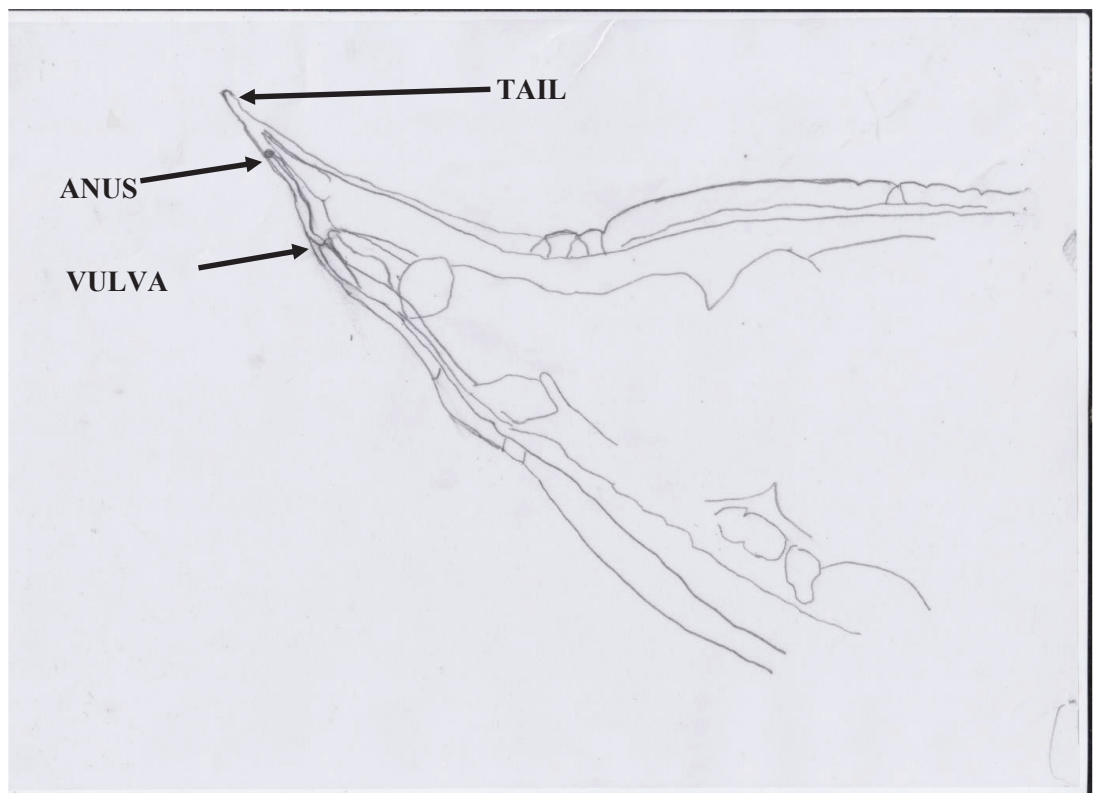


Figure 7: Posterior end of female *O. asperum* (X100). This part includes vulva with long flaps, anus, and tail

Both male and female *O. venulosum* had a well-developed mouth collar at the anterior end. The MC seemed to be a cone-like structure isolated from the body. MC contained 18 elements within the ECR although we observed only nine elements, whereas 36 elements within the ICR although only 18 of them on one surface of the collar. Cervical vesicle ranged from behind the mouth collar to CG; however, it was less developed compared to that in *O. asperum* (Gaddam et al., 2017a; Gaddam, Murthy, & Kommu, 2017b; Khanmohammadi et al., 2013). Lateral cervical alae were lacking as similar to other studies (Khanmohammadi et al., 2013) (Figure 8, 9). Male *O. venulosum* showed a large bell-shaped structure of bursa with two lateral lobes with bursal rays as described previously (Gaddam et al., 2017a). The bursal rays are muscular. Prebursal papillae were observed anterior to the bursal lobes. Spicules were slender and tubular (Figure 10, 11). In female, the vagina was very short, transverse, and kidney-shaped as described previously (Khanmohammadi et al., 2013). Its anus looks like a fissure. The tail is a finely pointed (Gaddam et al., 2017a, 2017b) (Figure 12, 13).

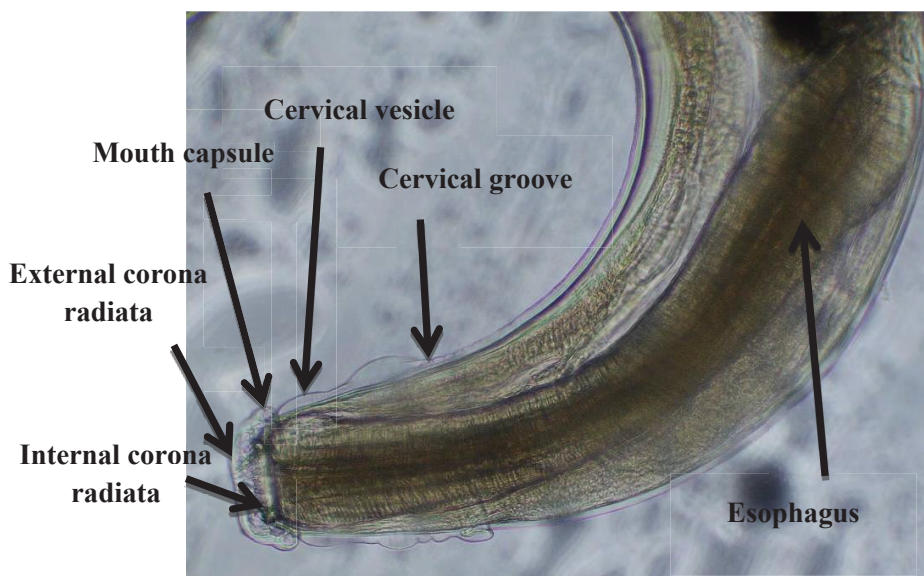


Figure 8: Photomicrograph showing the anterior end of *O. venulosum* (X100)

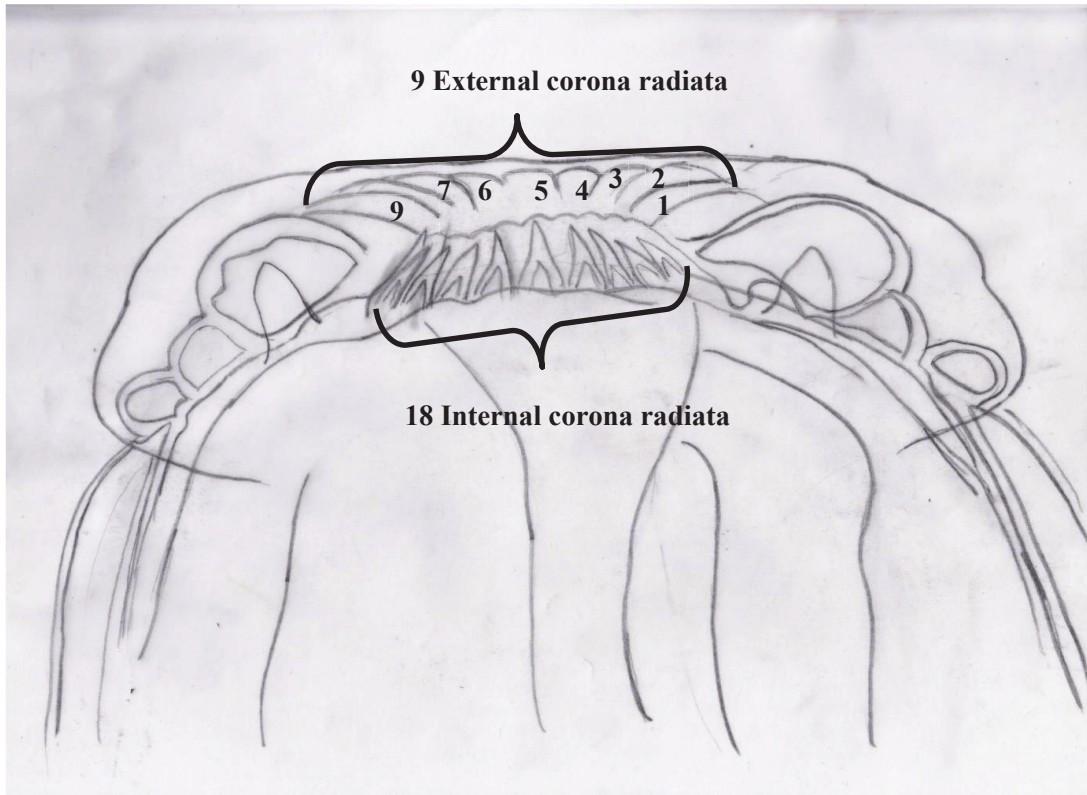


Figure 9: Anterior end of *O. venulosum* (X400). One surface shows a total of 18 internal corona radiata and nine external corona radiata.

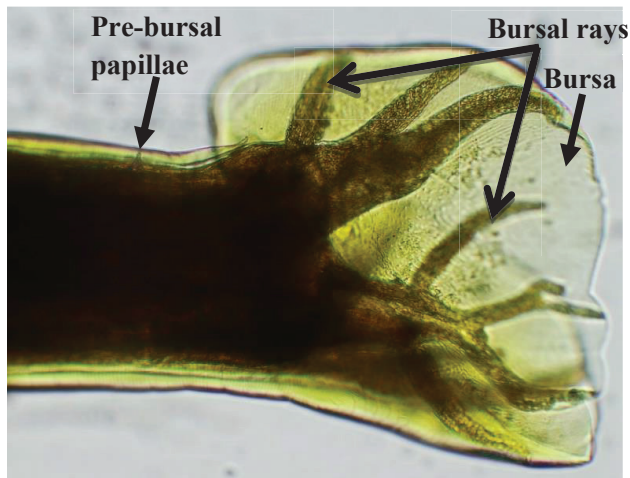


Figure 10: Photomicrograph showing the posterior end of male *O. venulosum* after processing in Lugol's Iodine (X100)

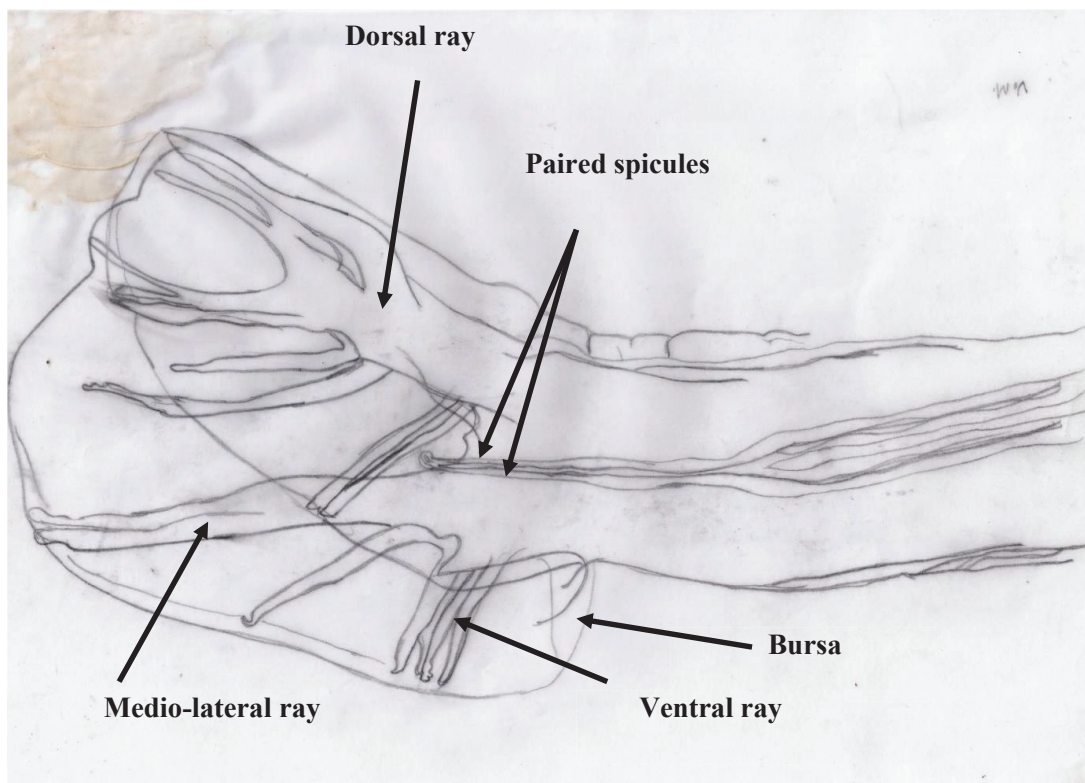


Figure 11: Posterior end of male *Oesophagostomum venulosum* (X100). This part includes bursa provided with bursal rays and spicules.

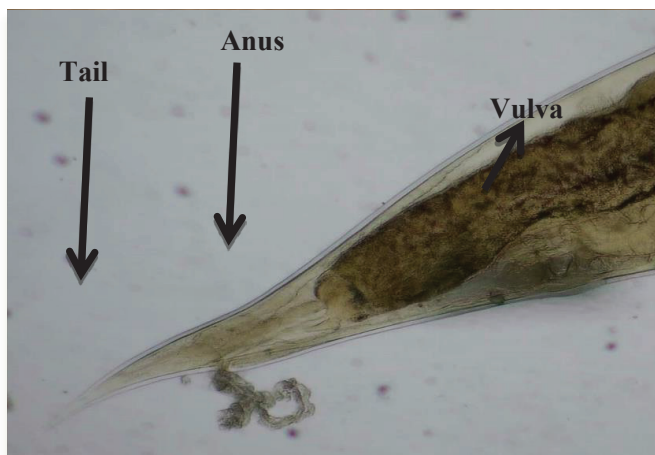


Figure 12: Photomicrograph showing the posterior end of female *O. venulosum* (X40).

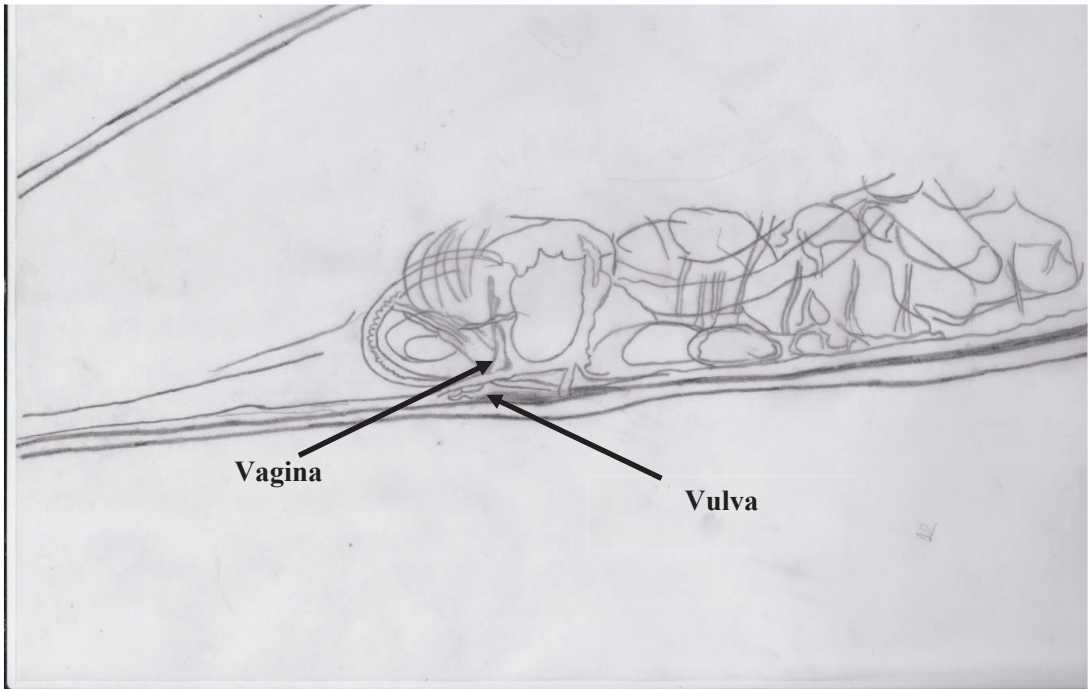


Figure 13: Posterior end of female *O. venulosum* (X100). This part includes vulva, vagina, ovary, and uterus.

CONCLUSION

In conclusions, this is the first study using light microscopes, line-drawings of camera lucida-like systems, and morphologic keys for identifying *O. asperum* and *O. venulosum* in the goats of Nepal. These materials and methodologies are crucial in taxonomy of nematodes in the low resource settings. However, the SEM data and molecular methods like PCR would be more confirmatory compared to these techniques. Therefore, further studies related to nematodes should be conducted using more advanced tools and techniques to confirm them in goats and other domestic animals.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ANIMAL RIGHTS

The authors declare that the study was conducted on naturally-infected goats which had been sacrificed for religious purposes in the central part of Nepal. No experimental infection was established during this research work.

Abbreviations: CG: Cervical Groove; CV: Cephalic Vesicle; DR: Dorsal Ray; ECR: External Corona Radiata; ICR: Internal Corona Radiata; MC: Mouth Capsule; SEM: Scanning Electron Microscope

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Major poultry diseases and their temporal pattern detected through rapid tests at the Veterinary Laboratory, Pokhara, Nepal

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ABSTRACT

Poultry industry in Kaski and surrounding districts are affected by various diseases. Veterinary Laboratory, Pokhara is one of the major diagnostic center for animal diseases in the region. A retrospective investigation was carried out for one year of duration (April 2018-March 2019) for the cases recorded at the Veterinary Laboratory (VL), Pokhara, Gandaki province. The method of rapid test used were plate agglutination test (PAT) for bacterial diseases (Salmonellosis & Mycoplasmosis), rapid diagnostic test (RDT) for viral diseases (Infectious Bursal Disease; IBD, Newcastle Disease; ND & Avian Influenza; AI), and microscopic examination for parasitic disease (Coccidiosis). Antibodies against Salmonella (47.15%) and Mycoplasma (58.63%) were found sero-positive from the sample of morbid chickens. Similarly, ND (42.11%), IBD (62.45%) and AI (9.97%) were detected on the RDT from the suspected chicken carcass. Out of 2053 caecal scrapings taken from the chickens older than two weeks of age, 28.30% were found positive for caecal Coccidiosis on microscopic examination. Rapid tests were found very easy, quick, less laborious, and non-expensive for the detection and differentiation of various diseases. Salmonellosis, Mycoplasmosis, IBD, ND and caecal Coccidiosis were found endemic diseases in the poultry sector of this region. The incidences of AI, the highly pathogenic strain with a potential to cause pandemic should be taken seriously. Our preliminary investigation indicated that the majority of diseases are correlated with poor management and biosecurity measures in farms. Therefore, farmers need to be made aware about seasonal occurrence of diseases, their timely vaccination, good farm management and overall biosecurity measures.

Keywords: Occurrence, Poultry diseases, Rapid test, Temporal

INTRODUCTION

Poultry sub-sector is one of the major components of the livestock sector which contributes 3-4% in total gross domestic product (GDP) and 8 % in agricultural gross domestic product (FAO, 2014; Kattel, 2016). It is developing as a resilient industry with huge capital investment which is directly employing and engaging large number of people across the country. Likewise, it has created multiplier effect to other industries (Winrock International, 2008; FAO, 2014). There is an estimated investment of NRs 80 billion in this sector which has provided direct employment of 1.5 lakh people (Singh, 2018). The growth rate of commercial sector is around 17-18% annually and its contribution to overall GDP is also encouraging and increasing (FAO, 2014).

Farmers from different districts (Kaski, Tanahun, Syangja, Parbat, Baglung, Myagdi, Palpa, Gorkha, Lamjung, Nawalpur) of western Nepal bring dead or morbid chicken for disease diagnosis in this laboratory (Regmi *et al.*, 2020). Most of the cases presented here are of broilers (80%), layers (12%) and remaining 8% local, giriraj, fighter chicken and turkey. Different diseases are increasing and causing huge economic losses with the advancement of poultry sector, some of which are of high zoonotic potential e.g. Avian Influenza. Diseases are major constraints in the development of poultry sector causing a huge economic losses to farmers. Many diseases have seasonal occurrence (Regmi *et al.*, 2017; Regmi *et al.*, 2020). Therefore, this study was aimed to find out the clinical prevalence of major poultry diseases and their temporal occurrence.

MATERIALS AND METHODS

All cases brought to the Veterinary Laboratory (VL), Pokhara from April 2018-March 2019, suspected during post-mortem examination (PME) were diagnosed using rapid test (microscopic examination, PAT, RDT for antigen detection) for the parasitic, bacterial and viral diseases respectively. Coccidian parasites were confirmed through caecal scrapings and their microscopic examination by viewing the oocytes. Clinical sero-prevalence of some bacterial diseases like Salmonellosis and Mycoplasmosis were performed for the morbid chicken by rapid plate agglutination test (PAT) using commercial *Salmonella* and *Mycoplasma gallisepticum* (MG) antigens to determine the presence of *Salmonella* and MG specific antibodies. Some viral diseases like Newcastle Disease (ND), Infectious Bursal Disease (IBD) and Avian Influenza (AI) were diagnosed on the basis of RDT. Then, the various pattern of diseases were analyzed using Microsoft Excel-2013 and the results are expressed in the chart forms.

RESULTS AND DISCUSSION

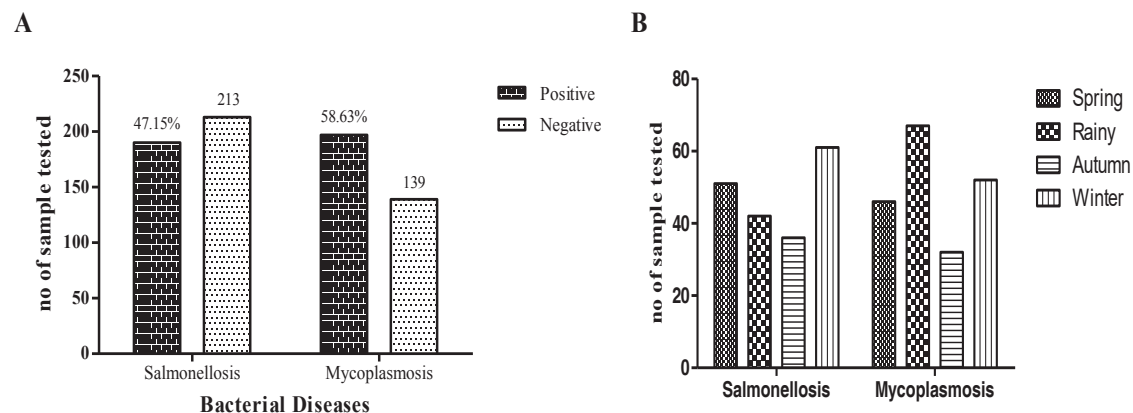


Figure 1 Graph showing cases of two important bacterial diseases, A: Sero-prevalence of Salmonellosis & Mycoplasmosis based on rapid PAT; showing the prevalence of *Salmonella* and *Mycoplasma* infection 47.15% and 58.63% respectively, B: Seasonal occurrence of Salmonellosis & Mycoplasmosis; showing the prevalence of *Salmonella* infection highest in the winter season 61 (32.10%) and *Mycoplasma* in rainy season 67 (34.01%).

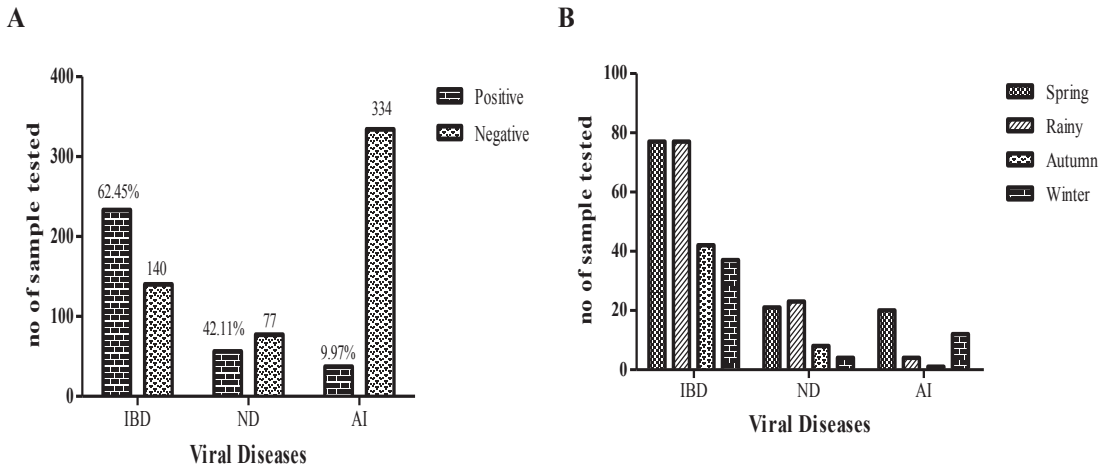


Figure 2 Graph showing, A: Different viral diseases positive on RDT. The prevalence of IBD, ND and AI was found 62.97%, 42.02% and 9.5% respectively on the RDT, B: Seasonal variation of IBD, ND and AIV diseases showing the clear higher cases of IBD and ND in spring and rainy season

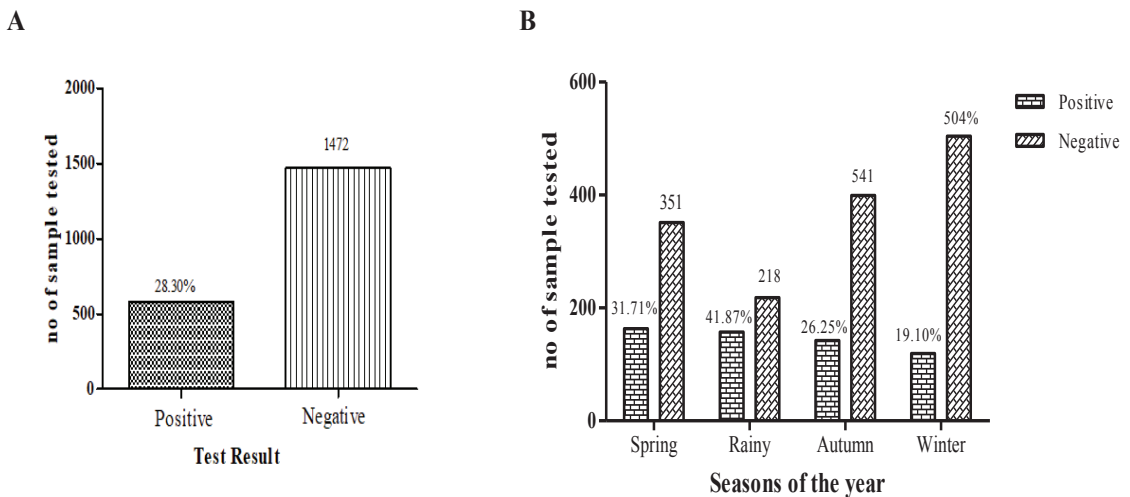


Figure 3 Graph showing cases of caecal Coccidiosis, A: Out of the 2053 caecal scrapings taken from the chicken of more than two weeks of age and tasted for caecal coccidian oocyst, 583 (28.8%) were found positive for caecal Coccidiosis, B: Seasonal variation of caecal Coccidiosis on microscopic examination. The prevalence of Coccidiosis was found more in humid spring season (28.3%), followed by rainy (26.9%), autumn (24.4%) and winter (20.4%)

The clinical sero-prevalence of *Salmonella* and *Mycoplasma* infection (47.15% and 58.63% respectively) on PAT as shown in Figure 1A, is higher than the findings of Sikder *et al.* (2005), who had recorded Salmonellosis (23.46%) and Mycoplasmosis (46.88%). This higher value indicates the poor management and biosecurity measures of farming system. The Salmonellosis

was found more prevalent in the winter season 61 (32.10%) whereas Mycoplasmosis in the rainy season 67 (34.01%) as shown in Figure 1B.

The prevalence of IBD (62.45%) on RDT as shown in Figure 2A, is similar to the finding of Lawal *et al.* (2014) who have reported the prevalence of 63.5%. This high prevalence may be due to the sample taken from only suspected chickens found on PME. Similarly, the prevalence of 48.02% was found for ND on RDT from the suspected chicken carcass which is higher than the findings of Rahman *et al.* (2012), who reported about one third sample positive. A high prevalence of IBD and ND was obtained during the rainy season as compared with in the dry winter season (figure 2B). This is in consistent with the report by Lawal *et al.* (2014), who also reported high prevalence of IBD 84.4% (rainy season) and 40.3% (dry season). The prevalence of AI was found 9.46%, in which high prevalence was in spring (43.33%) and winter season (40%) which may be due to the high susceptibility of virus in cool and moist climatic conditions.

The overall prevalence of caecal Coccidiosis (28.30%) is lower than 39.58% (figure 3A) as reported by Ebrahimi-Nik *et al.* (2018) and 31.8% reported by Lawal *et al.* (2016). The main parasitic disease, Coccidiosis is due to poor management practices in the deep litter system encouraging *Eimeria* oocysts to build-up. Coccidiosis was found more prevalent in hot and humid seasons (figure 3B).

CONCLUSION

Rapid tests are very easy, quick, less laborious, and non-expensive for the detection and differentiation of various poultry diseases. Salmonellosis, Mycoplasmosis, IBD, ND and caecal Coccidiosis were found endemic diseases in the poultry sector of this region. The incidences of AI is also increasing. These diseases are likely to be correlated with the poor management and biosecurity measures of farm which needs further investigation. We suggest farmers to be conscious about seasonal occurrence of common poultry diseases, their timely vaccination, good farm management and overall biosecurity measures.

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Alteration in Blood Parameters in Dairy Cows Affected with Acute /Chronic Mastitis: A Short Communication

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ABSTRACT

Mastitis denotes the inflammation of parenchyma of mammary gland and can be observed in acute, gangrenous, sub clinical and chronic form. Change in hemato-biochemical parameters play an important role to rule out physiological and pathological state of mastitis in affected animal. Various research studies were reviewed to understand the changes in blood parameter in dairy cattle affected by mastitis. Increase in leukocyte, neutrophil count and decrease in lymphocyte and macrophages were reported in several research. Likewise, there is no significant changes in Packed Cell Volume (PCV) and Haemoglobin level. Most of the research finding shows increase in Aspartate aminotransferase (AST) level and total protein in blood in infected cattle. There is fluctuation in blood mineral level values during mastitis and may either increase, decrease or do not change

Keywords: Blood parameter, Change; Inflammation of udder,

INTRODUCTION

Mastitis denotes the inflammation of parenchyma of mammary gland with physical, chemical and microbiological changes in the milk along with pathological change in the glandular tissue (Radiostits, 2010). Mastitis in bovine can be observed in acute, gangrenous, sub clinical and chronic form (Krishnappa, 2016). Bovine mastitis is one of economically challenging disease to the farmers all across the world. About 21% of the milk yield and is reduced and 25% of butter fat is reduced in the affected cattle (Chakrabarti, 2014). Older animals and a higher lactation has a higher chance of infection (Radiostits, 2010). Various changes are found in blood parameters in cattle affected with mastitis. Change in hematobiochemical parameters play an important role to rule out physiological and pathological state of mastitis in affected animal (Sarvesha, 2017). This short communication aims to understand the changes and findings occurred during the disease conditions conducted by different researchers.

METHODOLOGY

We conducted literature review to understand the change in blood parameters. We searched in PubMed, Sci-Hub, and Google Scholar. The keyword used to search the literature includes mastitis, and change in blood parameters during mastitis.

RESULTS

Alterations in white blood cells

Increase in leukocyte and neutrophil count in cattle with mastitis [Krishnappa, 2016; Singh, 2014; Tripathy, 2018; Atroshi, 1996; Sarvesha, 2017; Sadek, 2017; Alhussien, 2015]. Contrary to this finding by several researchers, a research conducted to know alteration in blood parameters based on causative agent shows decrease in white blood cells in gram negative mastitis (Smith, 2001). Decrease in lymphocytes and macrophages in the infected cattle (Krishnappa, 2016; Alhussien, 2015; Sarvesha, 2017).

Alteration in Hemoglobin and PCV level

Significant decrease in Hemoglobin level and PCV level were observed in affected cows (Tripathy, 2018). However, in another study it was observed there was an increased Hemoglobin concentration and hematocrit level in gram negative mastitis (Smith, 2001). In another study no significant changes in Hemoglobin and PCV level were reported (Krishnappa, 2016; Atroshi, 1996; Sarvesha, 2017).

Alteration in protein and liver enzyme

Increase in AST level and increase in albumin globulin and fibrinogen level in mastitis affected cows (Singh, 2014; Tripathy, 2018; Singh, 2019; Sarvesha, 2017). Contrary to this another research showed low total protein and albumin level in a cow with mastitis (Sadek, 2017).

Alteration in blood mineral level

In mastitis affected cow among minerals Ca, Mg, Na level were found to be increased (Krishnappa, 2016). In another study, increase in calcium level with non-significant changes in P, Mg, Na and K level was reported (Singh R., 2014). Likewise increase in Na, K and decrease in Ca and P level was reported by Tripathy (2018). Increase in Na and K level and non-significant changes in Mg, P, Zn and Fe was reported by Singh (2019). Low calcium level in blood serum with clinical mastitis with non-significant changes in Zn, Mg and Fe was found by YILDIZ (2005). Likewise, Low serum calcium level regardless of type of pathogens was observed by Smith (2001), high average value of Ca, P, Na, Cl and K by Sarvesha (2017) and non-significant changes in Na and K level by Atroshi (1996).

CONCLUSION

Increase in leukocyte, neutrophil count and decrease in lymphocyte and macrophages are found in most of the researches. Likewise, there is no significant changes in PCV and Haemoglobin level in most of the studies except few ones. Most of the research findings support increase in AST level and total protein in mastitis infected cattle. There is uncertainty in change in blood mineral level values during mastitis and may either increase, decrease or remain the same. Alteration in blood parameters cannot only confirm mastitis due to which other diagnostic tools should be used for confirmatory diagnosis. However, these changes show the physiological and pathological condition of an individual with mastitis.

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