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EDITORIAL

Dear Readers,

The scope of veterinary services has been extended to cover animal health, animal welfare and veterinary public health to address the challenges of animal, human and environmental health. Veterinarians play key role to ensure the availability of high-quality animal protein to ensure food security by curbing the malnutrition in our society. In addition, veterinarians play central role in ensuring food safety. Accordingly, veterinarian's role and responsibilities together with the competency has been increased manifold along with the conventional clinical services. It is, therefore, necessary to strengthen the knowledge and skill of veterinarians to address the issues of animal and human health.

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 archived in Nepjol, comprising original scientific peer-reviewed research and review articles in the field of veterinary science and allied subjects. The publication aims to share scientific knowledge and technology among members and stakeholders within home and abroad.

It is our pleasure to present our readers the 36th volume of NVJ in the eve of New Year 2020. The current issue of NVJ includes articles on various aspects of animal husbandry including breeding, nutrition, health and management of livestock, pets and wildlife. It also includes scientific articles on the effect of climate change on animal husbandry; epidemiology, prevention and control of parasitic and infectious animal disease including zoonoses; food safety, laboratory techniques in veterinary medical science, animal welfare, antimicrobial resistance pattern, and drug efficacy trial. We believe that the information will be useful to the concerned stakeholders.

NVJ welcomes original scientific research and review articles preferably focusing on the issues faced by farmers in the field. The guidelines for authors can be accessed through: <https://www.nepjol.info/index.php/nvj/about>.

On behalf of Editorial Board, I would like to express my sincere thanks to the respected authors and reviewers for their hard work and very valuable volunteer contribution to the veterinary profession. I would like to express my very sincere thanks to all the members of Editorial Board and especially to Dr. Surendra Karki, for their valuable contribution. I would also like to acknowledge Mrs. Pramina Shrestha for her secretarial and computer services. I am also indebted to executive committee of NVA for their support and encouragement.

I apologize for errors, if any, despite of our effort to correct the technical and grammatical mistakes as far as possible. We respect and welcome the constructive suggestions from our readers.

Editor-in-Chief

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Immunohistochemistry of Lymphocytes Subsets in Subclinical Experimental Paratuberculosis in Goats

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ABSTRACT

The present experiment was carried out to find out the lymphocytes subsets reactions in experimentally induced subclinical paratuberculosis in goats. Twelve goats of 8-12 weeks age were infected with 4.23×10^9 Mycobacterium avium paratuberculosis on 8 occasions. Seven goats were kept as in-contact controls and 4 as uninfected controls. Immunohistochemistry for detection of cellular reaction of CD2⁺, CD4⁺, CD8⁺, CD25⁺, MHC I and MHCII in the lymphocytes present in the intestine and lymph node revealed more reactive cells in the infected goats as compared with the in-contact and infected control goats.

Keywords: Paratuberculosis, Immunohistochemistry, Lymphocyte subsets MHC I and MHCII

INTRODUCTION

Johne's disease is one of the economically important chronic diseases affecting all domestic and wild ruminants and is caused by *Mycobacterium avium* subsp. *paratuberculosis*. The disease is characterized by chronic granulomatous enterocolitis, lymphangitis and lymphadenitis. Clinical manifestations of the disease include incurable diarrhoea, progressive weight loss and decline in productivity of the animals (Williams *et al.*, 1979; Chiodini *et al.*, 1984; Benedictus *et al.*, 1987; Buergelt and Ginn, 2000; Godfroid *et al.*, 2000; Kramsky *et al.*, 2000). The bacterium of Johne's disease has also been implicated with an inflammatory bowel disease in humans known as Crohn's disease (Stabel, 1998; Selby, 2000).

In India, paratuberculosis in goat was first reported by Pande in 1942 from Assam. The disease has now been reported from many parts of the country with prevalence varying from 2.5 to 18.9 % (Srivastava and More, 1987; Kumar *et al.*, 1988; Singh *et al.*, 1998; Tripathi and Parihar, 1999, Tripathi *et al.*, 2002). Various authors have described lesions in paratuberculosis, which occurs due to host's inflammatory immune response to the invading mycobacteria (Paliwal and Rajya, 1982; Rubin and Habekar, 1999; Hirsh and Zee, 1999).

Navarro *et al.* (1998) demonstrated different lymphocytes subsets by immunohistochemistry in the intestinal mucosa and mesenteric lymph nodes of three goats with natural paratuberculosis using monoclonal antibodies against CD2⁺, CD4⁺, CD8⁺, γ/δ , and MHCII. In the intestinal mucosa of goats with tuberculoid lesions, no significant changes were observed but in the cortical area of the mesenteric lymph nodes, the number of CD4⁺ T lymphocytes increased. In two goats with lepromatous lesions, there was a decrease in the CD4⁺ subpopulation and increase in the CD8⁺ T lymphocytes in the lamina propria of the intestine and in cortical area of mesenteric lymph nodes.

Little *et al.* (1996) performed immunohistochemistry in ileum of 12 normal and 18 naturally *M. avium* subspecies. *paratuberculosis* infected sheep, using monoclonal antibodies against ovine CD4⁺, CD8⁺, and γ/δ T cell receptor markers. Higher densities of lymphocytes were present in villus than in crypt areas. CD8⁺ cells were located principally around the epithelial basement membrane where as CD4⁺ cells were located towards the central villus of lamina propria.

Redcliff *et al.* (2004) used immunoperoxidase labeling for CD4⁺, CD8⁺, TCR γ/δ , WC1, CD1b, IFN γ , CD45 R, CD56 and lysozyme to investigate changes in cell mediated immune effector cell population in the intestinal Peyer's patches and mesenteric lymph node of lambs after experimental infection with *M.a.paratuberculosis*. Infected sheep had significantly more CD4⁺ cells in the mucosa, domes and interfollicular areas of the terminal ileum and in the interfollicular areas of the jejuna Peyer's patch and increase number of WC1⁺ cells in the ileal Peyer's patch.

MATERIALS AND METHODS

Experimental Design

Twenty four (24) goats were randomly divided into three groups namely the infected (n=12), the in-contact control (n=8) and uninfected control (n=4). The animals in the infected group were orally administered with 5 ml of bacterial suspension (4.23×10^9 /ml) on eight occasions at 3 days interval within a month. Similarly, the uninfected control animals received 5 ml sterile PBS. The animals of the in-contact group were administered similarly with PBS and kept along with the animals of the infected group. The experimental goats were euthanized 3, 6, 9 and 12 months post infection by intravenous injection of Thiopentone injection (Anesthal, Jagsonpal Pharmaceutical, Faridabad, India).

Fixation and Sectioning of Tissues:

Tissue sections for microscopic examination included one section of duodenum, four sections of jejunum, three sections of ileum, one section each of mesenteric lymph node, ileocaecal lymph node, caecum, colon and rectum. All tissues sections were collected in 10% neutral buffered formalin. Representative tissue samples of intestines and mesenteric lymph nodes were processed and embedded in paraffin wax after going through all the steps of dehydration by different grade of alcohol and cleaning in Xylene as per the routine protocol. rendered for tissue sections which were cut into pieces of 5 μ m thickness (Culling, 1968) and mounted on poly-L Lysine coated glass slides and dried overnight at 37°C.

Immunohistochemistry for Identification of Lymphocytes Subsets

Immunohistochemical staining were performed for delineation of different lymphocytes sub population such as T cells subset, B cells and MHC class II bearing cells by using monoclonals against various CD molecules in tissues (lymph nodes and small intestines). Using different Immunohistochemistry of goat paratuberculosis monoclonal antibodies immunohistochemistry was carried out on intestinal and lymph node tissues. (Table-1)

Table1: Monoclonal antibodies (MoAb) and their specifications

MoAb	Ig isotype	Specipicity	Target species	Source
MUC2A	IgG ₂	CD ₂ , αβ T cells and a subset of WC1 negative γδ T cells	Bovine, Ovine, Caprine	VMRD Pullman Inc. USA
GC50A	IgM	CD ₄	Bovine, Ovine, Caprine	
CACT80C	IgG ₁	CD ₈	Bovine, Ovine, Caprine	
CACT116A	IgG ₁	CD ₂₅	Bovine, Ovine, Caprine	
H58A	IgG _{2B}	MHC I	Bovine, Ovine, Caprine	
TH14B	IgG _{2B}	MHC II	Bovine, Ovine, Caprine	

Staining Procedure:

Immunohistochemistry procedure was followed as per the protocol described by Vector Lab. Inc. Burlingame CA94010, USA for demonstration of different types of surface markers of immune cells (CD2⁺, CD4⁺, CD8⁺, CD25⁺, MHC I and MHCII, VMRD Inc. Pullman, USA). The mounted tissue sections on slides were deparaffinized by treating in xylene I for 15 minutes and Xylene II for 5 minutes. Rehydrations of tissues were carried out in descending grade of alcohol I, II and III, 90% alcohol, 80% alcohol and finally 70% alcohol for 1 minute each.

The slides were rinsed in tap water for 5 minutes. Unmasking of antigen was achieved by boiling in citrate buffer (0.1mM, pH6) for 2-3 min. The sections were treated with Phosphate buffered saline (10 mM Sodium Phosphate, pH 7.5, 0.9% saline) for 10 minutes. The sections were incubated in 0.3% H₂O₂ in Methanol for 30 minutes followed by washing for 5 minutes in PBS. The sections were incubated for one hour with diluted normal blocking horse or goat serum, 1-4 drops (50-200 µl) in 10 ml buffer. Excess serum was drained off the section and incubated overnight at 4°C with optimally diluted (1:10) monoclonal antibodies. The sections were washed in buffer for 5 minutes and incubated for one hour with diluted secondary antibody ie biotinylated antimouse IgG/IgM (Vector laboratories, Burlingame, USA).

The sections were washed in buffer for 5 minute and again incubated for 45 minutes with Vectastain Elite ABC reagent (Vector laboratories, Burlingame, USA) prepared as per instructions. The sections were washed in buffered saline for 6 minutes and incubated (2-10 min) Iperoxidase substrate solution until the desired stain intensity was developed by using Diaminobenzidine (Sigma, USA). The sections were then rinsed in tap water and counterstained with the Hematoxyline for 3-4 seconds. Following brief rinsing in tap water the sections were dehydrated in 3 changes of absolute alcohol for 1 min each. These were left for 1 min in alcohol:

Xylene mixture followed by xylene I and xylene II for 1 min each and finally sections were mounted in DPX. Sections were examined at high power objective 400X to assess the reactivity of the cells in the sections of small intestines and mesenteric lymph nodes, For assessment of the reactivity of the labeled cells were scored a + (1-10 labelled cells), ++ (11-50 labelled cells), +++ (more than 50 labelled cells). Scoring was made according to the protocol followed by Reddacliff *et al.* (2004) with slight modification.

RESULTS

The intensity of reaction of different lymphocyte subsets identified by various monoclonal antibodies are presented in Table 2.

Table 2: Reactivity of monoclonal antibodies (CD2, CD4, CD8, CD25, MHC I and MHC II) in intestine and lymph nodes of experimental goats

Goat No.		CD2		CD4		CD8		CD25
		MHC I		MHC II				
		Int.	MLN	Int.	MLN	Int.	MLN	Int.
		Int.	MLN	Int.	MLN			
		MLN						
Infected	23	++	++	++	++	++	++	++
	++	++	++	++	++			
	28	++	++	+++	++	+++	+++	++
	++	++	++	+++	++			
	4	+++	++	++	++	++	++	++
		++	++	+++	++	++	++	
	1	++	++	++	++	+++	+++	++
	+++	+++	++	+++	++			
	3	+++	++	++	++	++	++	++
	+++	+++	++	+++	++			
In-contact	35	+	+	+	+	+	+	+
	+	+	+	+	+			
	33	+	+	+	+	+	+	+
	+	+	+	+	+			
Control	39	+	+	+	+	+	+	+
	+	+	+	+	+			
	40	+	+	+	+	+	+	+
	+	+	+	+	+			

Note : + 1 10 labelled cells, ++ 11- 50 labelled cells, +++ > 50 labelled cells

CD2⁺ labeled cells were present in the lamina propria of the villi and crypt regions of intestine. In mesenteric lymph nodes, more reactivity was seen in the paracortex and corticomedullary junction. CD4⁺ cells were mostly distributed in the villus region of lamina propria of the intestine. labeled cells were seen in crypt region. In the mesenteric lymph nodes CD4⁺ cells were more in the cortical region.

CD8⁺ cells were found mostly in the base of epithelium of intestine and paracortical region of the mesenteric lymph nodes. Crypt region also showed more reactivity. Labelled cells were also visualized in the cryptal region. CD25⁺ cells were distributed in the lamina propria of the intestine. In the mesenteric lymph nodes cells were concentrated in the paracortex and medullary region

MHC I cells were found in the lamina propria and under the basement membrane of the epithelium, cortex and paracortical region of the mesenteric lymph nodes. MHC II cells expressing MHC II antigens were found in the villi and crypts of small intestine and paracortex and medullary regions of mesenteric lymph node.

DISCUSSION

There were increased reactivity of CD2⁺, CD4⁺, CD8⁺, CD25⁺, MHC I and MHCII labeled cells in the villi and crypts of the small intestine and cortex and medulla of the mesenteric lymph nodes in comparison to the in contact and control goats. These results were in line with those reported in naturally and experimentally infected in goat (Navarro *et.al.*, 1998; Gonzlez *et.al.*, 2001). The increase in CD2⁺ labeled cells reflected the increased labeling of CD4⁺ and CD8⁺ cells (Mackay *et.al.*, 1988) and the importance of CD8⁺ lymphocytes in paratuberculosis infection has been highlighted in sheep and goats (Navarro *et.al.*, 1998; Reddacliff *et.al.*, 2004). A decrease in CD4⁺ lymphocytes and an increase in CD8⁺ T lymphocytes have been observed in goats showing borderline lepromatous type lesions suggesting that deficit in macrophage activation mediated by CD8⁺ T lymphocytes plays a role in progression of paratuberculosis lesions (Navarro *et.al.*, 1998). Navarro *et.al.*, (1998) also described an increase in CD8⁺ T lymphocytes and decrease in CD4⁺ lymphocytes in mesenteric lymph nodes and suggested that the progression of paratuberculosis lesions in the goat could be due to an ineffective host immune response, which was attributed to CD8⁺ T lymphocytes subset that down regulates the activity of CD4⁺ lymphocytes required for macrophage activation. In the present study an increase in the number of CD8⁺ lymphocytes suggested strong CMI response in the animals, which could be responsible for asymptomatic paratuberculosis with mild lesions in goats. Koets *et.al.*, (2002) in a study of progressive bovine paratuberculosis indicated that when the asymptomatic stage was compared with the clinical stage of Johne's disease, there was a decrease in the number of CD4⁺ T lymphocytes where as CD8⁺ T lymphocytes remain at comparable frequencies. The pathogenesis may be mediated predominantly by the loss of protective CD4⁺ T lymphocytes responses during the course of the disease. The loss of CD4⁺ and $\gamma\delta$ cells through apoptotic cell death has been described for *M. tuberculosis* infection (Koets *et.al.*, 2002)

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Epidemiology of Important Poultry Diseases in Nepal

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ASBTRACT

Despite the rapidly growing poultry industry throughout Nepal, the periodic outbreaks of diseases and infections in poultry birds led to huge production loss. The aim of this study was to identify the top ten poultry diseases in Nepal and an analysis of their seasonal distributions. A cross-sectional study was performed to describe the distributions of major poultry diseases diagnosed from April 2018 to April 2019 at Central Veterinary Laboratory, Nepal. Out of 2358 observations recorded at the CVL registry at that period, only 2271 observations qualified for the final analysis. Among 2271, removing the missing values, only 1915 observations were used to describe bird characteristics such as median age and mean flock sizes. Descriptive analysis and graphical representation was performed in R studio (Version 1.0.143) and MS excel 2010 respectively. The top ten diseases identified with highest to lowest incidence were: colibacillosis 26% (584/2271), mycotoxicosis 13% (301/2271), ascites 10% (232/2271), complicated chronic respiratory disease (cCRD) 9% (196/2271), infectious bursal disease (IBD) 7% (155/2271), Newcastle disease (ND) 7% (148/2271), avian influenza (AI) 3% (76/2271), salmonellosis 2% (40/2271), infectious bronchitis 1% (33/2271), coccidiosis 1% (25/2271) and non-specific diseases accounts for 21% (481/2271). Cases of colibacillosis were predominant all year round. Mycotoxicosis was seen mostly during pre-monsoon and monsoon season. Ascites and IBD were common during spring and winter seasons. cCRD was most common during summer and winter months. AI kept changing the disease outbreak pattern but it was most common during spring and summer. The number of cases of Salmonella, IB and coccidiosis were not sufficient to provide the seasonal trend. Identification of common poultry disease and their seasonal distributions is useful in taking preventive measures such as vaccination and good management practices to minimize their incidence in the future.

Keywords: Outbreaks, Poultry industry, Seasonal distributions

INTRODUCTION

In Nepal, the poultry sector contributes around 4% of GDP. The poultry industry is spreading rapidly throughout the nation; the commercial poultry farming has been practiced in 64 districts out of 77 districts of Nepal (NCPS, 2017). Of the total 70,007,151 poultry birds in Nepal (MoALD, 2018) there are around 93.29% broiler farms, 6.09% layer farms, 0.58% parent farms and 0.04% of giriraja/kuroiler farms in Nepal (NCPS, 2017). The total person employed in poultry sector is about 55,871 and among which 31,330 were male and 24,541 were female. Despite rapid turnover on the poultry business, adoption of an intensive production system, the introduction of new breeds, application of enhanced biosecurity and preventive health measures

are limited in developing countries like Nepal (Biswas *et.al.*, 2009; Permin and Pedersen, 2002). Despite the booming poultry business in Nepal, there is a periodic outbreak of common poultry diseases such as colibacillosis, Newcastle disease (ND), infectious bursal disease (IBD), avian influenza (AI), chronic respiratory disease (CRD), infectious bronchitis (IB) in different parts of Nepal that created a great loss to poultry industry (DLS, 2018). The aim of this study was to perform the descriptive analysis to identify the top ten poultry diseases of Nepal and report their seasonal incidences. Central Veterinary Laboratory (CVL), Kathmandu, Nepal was chosen for the study site because CVL is a national reference laboratory for animal disease investigation in Nepal.

MATERIALS AND METHODS

Study design

A cross-sectional study was performed to analyze the case data recorded in the registry book of postmortem unit, Central Veterinary Laboratory (CVL) from mid-April, 2018 to mid-April, 2019 (Baisakh to Chaitra 2075). All the data in the registry book were entered manually in the excel spreadsheet and the preliminary data cleaning was performed manually. There was a total of 2358 observations in the data with ten variables: age, bird type, flock size, mortality per day, total mortality, source of chicks (name of hatcheries), and source of birds (name of feed company), location of the farm, the date on birds brought for necropsy and tentative disease diagnosed. However, after removing observations with incomplete pieces of information, the total number of cases included in the final study was 2271. The diagnosis of disease in the postmortem units was mostly based on, clinical history, pathological lesions observed at the time of necropsies and the rapid antigen detection tests available for AI, ND, IBD, IB at CVL.

Statistical analysis:

All the data entered in the spreadsheet were first analyzed by Pivot Table in excel to check its consistency. Any discrepancies in the values of the variables were corrected by writing R script and final descriptive analysis was performed in R studio (Version 1.0.143). And, MS excel 2010 was applied to generate the graphical presentation of a monthly and seasonal trend of the cases.

RESULTS

Bird Characteristics:

Of the total 2271 observations, after removing incomplete information, only 1995 observations were used to classify poultry types in calculating their median ages and flock sizes. Among them, the birds were categorized under commercial broilers, commercial layers, commercial parents, kuroilers, giriraja, local chicken, turkey and their median ages (in days) were 26, 89, 80, 52, 59, 65, 105 and 60 respectively. The different bird categories and their average flock sizes along with the proportions of the cases for each category are depicted in Table 1.

Table 1: Different poultry bird categories and their median ages with mean flock sizes.

Bird categories	Median ages	Mean flock size (95% CI)	No of cases	cases (%)
Commercial broilers	26	1708 (1639.4, 1776.6)	1346	70.29
Commercial layers	89	2121 (1938.8, 2303.2)	329	17.18
Commercial parents	80	5629 (1913.9, 9344.2)	27	1.41
Kuroilers	52	748 (506.2, 989.9)	97	5.07
Giriraj	59	690 (425.6, 954.4)	47	2.45
Local chicken	65	965 (581.1, 1348.9)	24	1.25
Turkey	105	124 (41.9, 206.1)	11	0.57
Duck	60	299 (197.1, 400.9)	9	0.47
Others (crow, parrot etc.)			25	1.31
Total			1915	100.00

Classification of Top Ten Poultry Diseases:

Of the total 2271 cases, the top ten diseases diagnosed at CVL in the year 2018-19 is shown in Fig 1. Colibacillosis accounts for 26% (584/2271) of all cases, myotoxicity 13% (301/2271), ascites 10% (232/2271), cCRD 9% (196/2271), IBD 7% (155/2271), ND 7% (148/2271), AI 3% (76/2271), salmonellosis 2% (40/2271), IB 1% (33/2271), coccidiosis 1% (25/2271) and non-specific diseases comprise of 21% (481/2271) of all cases.

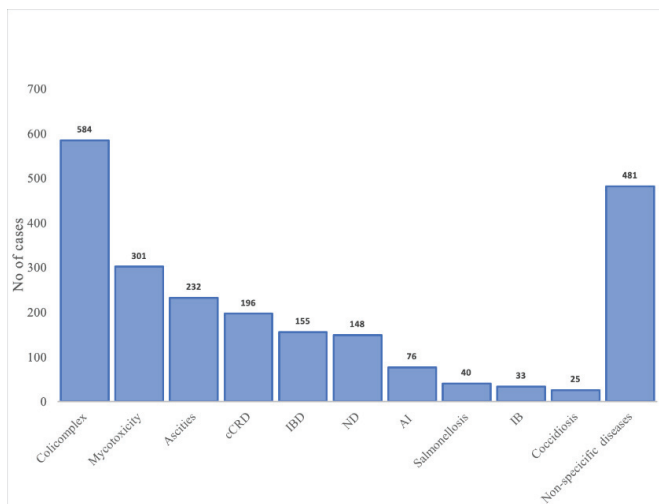


Fig 1. Graphical representation of major poultry diseases diagnosed at CVL, 2018-19.

The Seasonal Trend of Poultry Disease Diagnosed at CVL

Colibacillosis is a predominant disease throughout the year. Myotoxicity is seen mostly during pre-monsoon (Baisakh-Jestha) and continue throughout monsoon season (Ashad to Bhadra). Again, in winter season (Mangsir to Magh) there is a significant rise in cases of mycotoxicosis. Ascities are seen mostly during winter months (Mangsir-Poush) but there is significant number of cases even in spring (Chaitra and Baisakh) too. cCRD is most common during summer (Jestha-Sharawan) followed by winter season (Mangsir to Magh). The cases of IBD are more common during spring (Chaitra-Jestha) and summer (Ashad-Shrawan) which ultimately decline at fall season (Ashoj-Mangsir) and in winter (Poush-Magh). The outbreak time of avian influenza, though keeps changing, is most common during the spring (Falgun-Baisakh) and summer (Jestha-Ashad) (Table 2, Fig 2). The number of cases of salmonellosis, IB and coccidiosis were not sufficient to describe the seasonal trend in this study.

Table 2: Monthly distributions of major poultry diseases diagnosed at CVL from mid April 2018

Months/ Diseases	Colibaci llosis	Mycoto xicosis	Ascites	cCRD	IBD	ND	AI	Salmo nellosis	IB	Cocci diosis
Baisakh	75	30	40	9	32	25	5	4	3	0
Jestha	49	21	13	19	31	31	18	8	1	6
Ashad	43	42	10	17	20	3	7	2	0	6
Shrawan	36	22	11	18	20	19	1	10	5	3
Bhadra	59	29	4	9	6	7	0	4	8	2
Ashoj	37	14	18	9	1	2	0	0	1	3
Kartik	53	23	19	3	6	2	2	0	3	0
Mangsir	73	36	22	20	5	1	4	3	2	1
Poush	25	30	38	40	5	6	3	1	4	0
Magh	43	15	14	32	6	17	3	4	2	1
Falgun	42	17	15	17	4	11	12	4	1	2
Chaitra	49	22	28	3	19	24	21	0	3	1

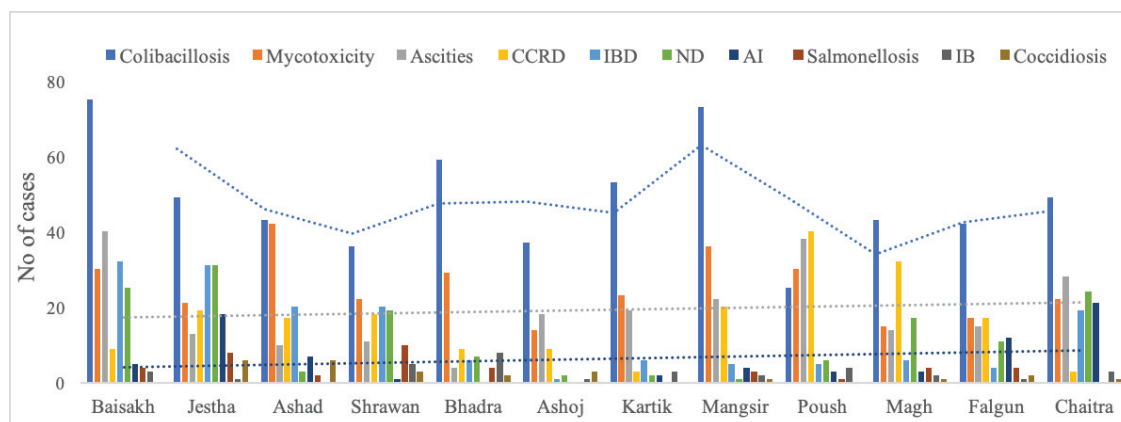


Fig 2: Graphical representation of monthly cases distribution of major poultry diagnosed at CVL during mid-April 2018 to mid-April 2019.

DISCUSSION

Among the major poultry diseases, *E. Coli* infections and their clinical complications in birds is a challenging issue in poultry production system. It is the most common infection in poultry farms (Shrestha and Shrestha 2017; Gautam *et.al.*, 2017; CVL, 2017). This may be due to poor farm hygiene in a poultry house where birds get infected through the accumulation of *E. Coli* in

aerosols and fecal contamination along with egg contaminations at hatcheries (Nolan, 2019; Carli *et.al.*, 2016; Ibrahim *et.al.*, 2019). Colibacillosis is endemic through all seasons in poultry farms, which indicated the poor poultry farm management status in Nepal.

Mycotoxicosis is, the second, leading cause of mortality in poultry that develops mainly by the ingestion of feed with higher concentrations of mycotoxins such as aflatoxins B1 and B2 (Aryal and Karki, 2009; Karki *et.al.*, 2003). This condition is predominant at pre-monsoon (spring) season as the environmental temperature keeps rising above 30°C. And, during monsoon, the humidity of the environment is too high. Both of these conditions are favorable for fungi like *Aspergillus* spp. to produce toxins in them (Amrutkar *et.al.*, 2015; Jurjevic *et.al.*, 2007) (<http://benisonmedia.com/mycotoxin-challenges-in-production-of-poultry-feed-during-rainy-season/>).

Our study revealed that the incidence of ascites was predominant during the winter and spring season than in other seasons. It is because, in winter, in an attempt to keep chicks warm, the ventilation is poorly kept which leads to minimal availability of oxygen inside the farmhouse (Julian *et.al.*, 1989; Acar *et.al.*, 1995; Gupta, A.R., 2011). While in spring, with the rise of temperature there is an increased demand for oxygen in birds that causes ascites in them (Tattori *et.al.*, 1995).

Mycoplasma gallisepticum is also prevalent in Nepal (Khanal *et.al.*, 2018; Mahato *et.al.*, 2015) that is mostly complicated by *E. coli* to develop cCRD. Our study results were similar to the study conducted by Shrestha and Shrestha (2017) in 2016-17 at the National Avian Disease Investigation Laboratory (NADIL), who demonstrated around 10.60 percent of cases of cCRD. However, Sharma and Tripathi (2015) found 18% (401/2229) of cCRD at NADIL, Chitwan during 2014-15.

Our study reveals that cCRD is most common during winter and summer months. The findings were similar to a study by Rajkumar *et.al.*, (2017), who observed the highest incidence of CRD during summer and winter months. It is because, in summer the heat stress and poor availability of oxygen lead to increase susceptibility to CRD (Rajkumar *et.al.*, 2017). The detection of avian influenza at CVL is 3% (76/2271), which is almost similar to the study by Sharma and Tripathi (2015).

The cases of salmonellosis diagnosed at CVL was around 2% (40/2271) which was almost similar (4.5%) to findings by Shrestha and Shrestha (2017) but the result is far lower from the study by Sharma and Tripathi (2015), who recorded incidence of salmonellosis as high as 11.08% (245/229) at NADIL. The variations in the proportions of case detection may be either due to differences in sample sizes or the differences in the diagnostic capabilities of laboratories or the competencies of the veterinary clinicians to identify the cases.

Around 1% of cases at CVL comprised of coccidiosis during the year 2018-19 which was almost similar (0.8%) to study by Shrestha and Shrestha (2017). Detections of infectious bronchitis at CVL all year-round are around 1% of all poultry cases yet any other relevant pieces of literature

related to infectious bronchitis in Nepal were not found. The prevalence of IBD was 7% (155/2271) in our study, which was almost similar to the study by Shrestha and Shrestha (2017) but lower (12.3%) than that of Sharma and Tripathi (2015).

IBD cases were more common found during spring and summer in year 2018-19, which may be due to the stability of IBD virus that can survive even in the peak of summer (Rani and Kumar, 2015). The second reason may be due to the failure of vaccines because of improper storage as IBDv vaccines are ineffective once the environmental temperature rises above 42°C (Rani and Kumar, 2015).

CONCLUSIONS

The poultry sector has an important contribution in uplifting economy and livelihoods among the people of Nepal. Proper identification of major poultry diseases and discovering their seasonal distributions are useful approach in undertaking preventive measures, such as vaccinations, good management practices, which ultimately minimize their incidences in upcoming days.

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Field Verification Trial of ND I-2 Vaccine in Nepal

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ABSTRACT

A study was conducted to assess the overall performance of ND I-2 vaccine in Nepalese context to prevent Newcastle disease (ND) particularly in backyard chicken. The experimental studies were conducted at Animal Health Research Division (AHRD), NARC and field verification were carried out in different eco-zones of the country. Experiments were divided in three segments: evaluation of thermostability, longevity and field verification trial in backyard chickens. The ND I-2 vaccine was prepared at Central Biological Production Laboratory (CBPL), Tripureshwor. In thermostability evaluation antibody titer and mortality percentage showed that ND I-2 vaccine exposed up to 30°C for seven days was found effective in prevention of Newcastle Disease in village chickens. Evaluation of longevity of immunity showed that till 90 days there was protective immunity against ND and in field trials when vaccine delivered without maintaining cold chain, the vaccine gave protective immunity in mid hills and high hills region but not enough immunity in the terai region where the temperature was above 30°C. This study clearly indicated that thermostable ND I-2 vaccine can be used without cold chain in high hills and mid hills in all seasons and only in winter season in the terai region of Nepal (temp. less than 30°C). In summer season, vaccination in the terai region could not give protective immunity (temp. more than 30°C) without maintaining cold chain.

Keywords: EID₅₀, HI, Specific immunity, Thermostable ND I-2 vaccine

INTRODUCTION

Nepal is an agricultural based country, agriculture contribute 28% in national GDP. Livestock shares 12% and poultry sector alone contributes 4% in GDP (ABPSD, 2016). In total population of poultry, backyard chickens comprise 46% and commercial chickens comprise 56% (Parajuli 2008). Newcastle disease (ND) has great economic significance to the poultry industry worldwide. ND is a highly contagious and fatal viral disease that affects birds of all age groups of most species. The disease is frequently responsible for devastating losses in poultry (Alexander *et al.*, 2004). The causative agent of ND in birds is an avian paramyxovirus serotype 1 (APMV-1), which is the member of the genus Avulavirus of the Paramyxoviridae family (Senne *et al.*, 2004). Depending upon the pathotype involved and susceptibility of the flock, the virus causes mortality ranging up to 100%. For this reason, it is included in list A of the Office International des Epizooties (OIE, 2002).

Vaccination is the most cost-effective method for controlling ND and has been used successfully throughout the world (Alexander *et.al.*, 1997). There are two types of vaccines: thermostable vaccine and thermolabile vaccine (FAO, 2002). The ND thermotolerant vaccines are among the attenuated vaccines that offer an appropriate technology for ND control in rural free-range poultry management system where cold chain facilities, logistics management and husbandry factors need consideration (Alder and Spradbrow, 2001). Thermostable ND vaccine exhibit a relative resistance to inactivation and exposure to elevated temperature. Strain of ND virus vary in thermostability. Thermostable vaccines are prepared from a strain of Newcastle disease virus that retains the ability to infect cells after storage outside the cold chain for a short period of times.

In our context, particularly for rural chickens which comprises more than 46% poultry population, thermostable vaccine is more suitable than thermolabile vaccine due to various reasons like poor electrification infrastructures, frequent power cut-offs, scattered and small-scale rearing system and lack of technicians for injectable vaccines. Besides these reasons, thermostable vaccine can be used irrespective of chicken age and longevity of protective immunity, at least three month in Nepalese context (Nyaupane *et.al.*, 2016). Whereas heat labile (thermolabile) vaccines which are being used in commercial chickens right now needs cold chain system. During frequent load-shedding and even without refrigeration facility, heat labile vaccine fails to give protective immunity against ND to chickens. Therefore, thermostable ND vaccine is very useful in Nepal. There are not enough research reports on control of ND in village chicken in Nepal. However, the evaluation of thermostability and longevity of ND, I-2 vaccine conducted at Animal Health Research Division, NARC has showed the very promising result and recommended for field verification (Acharya *et.al.*, 2016).

In this study, field verification trials of ND, I-2 vaccine produced at CBPL, Tripureshwor were performed in backyard chicken in three eco-zones of the country including five districts namely Bara, Sunsari, Kavre, Jajarkot and Jumla. In Jumla and Jajarkot, chicks distributed by Agriculture and Food Security Project (AFSP) were used for sampling, vaccination and sero-monitoring whereas chickens maintained by farmers were used in other locations irrespective of age.

METHODOLOGY

Site of Trials

The field trials were carried out at five locations representing five districts covering three agro-ecological zones. Two districts each from Terai (Madhesa village, Sunasari and Fattepur village, Bara) and mid hills (Tinpihle village, Kavre and Pokhara, Jajarkot) and one district from high hills (Bohara village, Jumla). In Jumla and Jajarkot field trials were conducted on those chicks which were distributed by Agriculture and Food Security Project (AFSP). Trial was conducted in summer season when the temperature of Terai region was above 30°C.

Experimental Design

Ten households having at least 15 chickens were selected randomly for sampling purpose however, the vaccination was carried out in all birds of village irrespective of the age. Vaccine having approximately 10^6 EID₅₀ / 0.1ml were delivered by intra-ocular route at the dose rate of 100 µl/bird. Blood samples were collected at day-one before vaccination (1 DprV) and 30 days-post vaccination (30 DPV). Pre-vaccination and post vaccination sampling were carried out in farmer's field for monitoring antibody to ND by haemagglutination inhibition (HI) test. Results were noted and analyzed for efficacy of ND I-2 vaccine in village chickens in field condition (EID₅₀ calculation, Reed *et al.*, 1938).

Protocol for Field Trial (ACIAR, 2002)

- i. 30 blood samples were collected from each trial site before vaccination for base line antibody evaluation
- ii. 20 households having minimum of 10-15 chicken irrespective of age of chicken (at least 450-500 chicken) were selected for vaccination
- iii. Vaccine reconstituted in given diluents having approximately 10^6 EID₅₀ / 0.1ml which has already calculated while titration of vaccine.
- iv. Vaccine was delivered @ the dose rate of 100 µl / bird intra-ocular using micropipette.
- v. Blood samples were collected 30 days post immunization (30 DPI).
- vi. Sixty blood samples were collected from adult birds of each sites.

Haemagglutination Inhibition (HI) Assay

Haemagglutination (HA) and HI tests were conducted according to OIE Manual, 2012. All the collected sera were stored in - 40°C until use. Before HI assay, sera were inactivated at 56°C for 30 minutes. Sera collected before vaccination were analyzed for baseline antibody titer monitoring. The antibody titer in sera was determined by using 4 HA unit with two-fold serial dilution as recommended by the World Organization for Animal Health (OIE, 2012). Published cut off value was used for the protective HI antibody titer (HI titer $\geq \log 2^3$) (Spradbrow *et al.*, 1993; VanBoven *et al.*, 2008).

RESULTS

Immunization with ND I-2 Vaccines in Birds of Field Verification Site

ND I-2 vaccine delivery, sampling and antibody titers calculation were performed as described in methodology along with experimental protocols. The comparative antibody titers of field birds of different locations are shown in Tables 1 to 5.

Table 1: Antibody titer and protective level of specific immunity to NDV in birds at Tinpipe, Kavre district.

Days of Vaccination	Antibody titer using HI test									Protective Level of specific immunity of NDV
	1:0	1:4	1:8	1:16	1:32	1:64	1:128	1:256	≥1:256	
Pre-vac (1 DPrV)	6	10	11	3	-	-	-	-	-	10 %
Postvac (30 DPV)	-	-	-	-	4	36	14	6	-	100 %

DPrV: Days Pre-Vaccination. DPV: Days Post Vaccination.

Table 2: Antibody titer and protective level of specific immunity to NDV in birds at Fattepur, Bara district.

Days of Vaccination	Antibody titer using HI test									Protective Level of specific immunity of NDV
	1:0	1:4	1:8	1:16	1:32	1:64	1:128	1:256	≥1:256	
Pre-vac (1 DPrV)	16	10	4	-	-	--	-	-	-	0 %
Postvac (30 DPV)	-	16	14	4	22	-	4	-	-	50 %

DPrV: Days Pre-Vaccination. DPV: Days Post Vaccination.

Table 3: Antibody titer and protective level of specific immunity to NDV in birds at Madhesa, Sunasari district.

Days of Vaccination	Antibody titer using HI test									Protective Level of specific immunity of NDV
	1:0	1:4	1:8	1:16	1:32	1:64	1:128	1:256	≥1:256	
Pre-vac (1 DPrV)	20	-	8	2	-	--	-	-	-	6.67 %
Postvac (30 DPV)	-	-	24	-	8	6	22	-	-	60 %

Table 4; Antibody titer and protective level of specific immunity to NDV in birds at Bohara Village, Jumla district.

Days of Vaccination	Antibody titer using HI test									Protective Level of specific immunity of NDV
	1:0	1:4	1:8	1:16	1:32	1:64	1:128	1:256	≥1:256	
Pre-vac (1 DPrV)	8	10	9	3	-	--	-	-	-	10 %
Postvac (30 DPV)	-	-	8	6	6	6	4	30	-	86.66 %

Table 5: Antibody titer and protective level of specific immunity to NDV in birds at Pokhara, Jajarkot district.

Days of Vaccination	Antibody titer using HI test									Protective Level of specific immunity of NDV
	1:0	1:4	1:8	1:16	1:32	1:64	1:128	1:256	≥1:256	
Pre-vac (1 DPRV)	10	12	8	-	-	--	-	-	-	0 %
Postvac (30 DPV)	-	4	5	6	4	8	6	10	17	85 %

DISCUSSION

Village chickens are major component of poultry production in Nepal. However, there is always threat of infectious poultry diseases among which Newcastle disease (ND) is responsible for large economic losses and vaccination is only the effective approach to control.

In this study, birds vaccinated using thermostable ND, I-2 vaccine have shown different protection responses in different locations ranging from 50 to 100% protection (Table 1 to 5). The protective immunity in Kavre district was highest (100%) followed by Jumla (86.6%); Jajarkot (85%); Sunsari (60%); and Bara (50%) respectively. In few birds, there was certain level of immunity detected before vaccination which might be due to previous exposure to ND. This result is similar to other reports which discussed protective immunity at various level (up to 100%) (Acharya *et al.*, 2016; Nyaupane *et al.*, 2016).

In previous study, when evaluating thermostability of ND I-2 vaccine, kept in room temperature (25-30°C) for 7 days had protected ≥80% experimental chicken against challenged field isolate of ND. Likewise, vaccine kept in incubator (37°C) for 7 days could not protect enough against field virus challenge (Acharya *et al.*, 2016). This fact is very much similar to present findings where HI test results have shown 100% protective immunity in Kavre, 86.6% in Jajarkot, 85% in Jumla, 50% in Bara and 60% in Sunsari. In both Terai region the temperature was above 35°C and in mid hill and high hill region (viz. Kavre, Jajarkot and Jumla) temperature was below 30°C range.

In another study about longevity of protective immunity, this vaccine produced specific immunity against ND for at least 90 days (3 month) after vaccination in village chicken (Nyaupane et al, 2016). Whereas another study has claimed that protective immunity lasts for four months and hence it is enough to repeat vaccine in every 4 months (Alders and Spradbrow, 2001). Protective immunity is generally accepted as corresponding to a hemagglutination inhibition titer above 8 ($\log_2^3$) (Bell *et. al.*, 1991). In this results protective HI titer varied in mid hill and hilly areas also, like 100 % immunity seen in Kavre unlike Jumla and Jajarkot. The reasons behind this variation in immune response and longevity of immunity could be due to the immune status and health status of the bird at the time of exposure to the immune stimulus, the nature of the immune stimulus and slight variation in travelling days from research center to trial sites. Generally, in vaccinal immunity if more than 80% protective immunity is achieved in experimental birds, it is considered as effective vaccine to control the disease. Therefore, in this study, vaccine has shown effective response in three districts - Kavre, Jumla and Jajarkot and not protective enough in Bara and Sunsari districts during summer.

Village chickens are not frequently vaccinated against common diseases. Major constraint noticed is the lack of vaccine suited to our village condition. Vaccines which are currently in use for control of ND are targeted to commercial poultry. Manufacturer are providing heat labile ND vaccines in multi-dose vials such as 1000 dose and 2500 doses and need to maintain within cold chain (4°C to 8°C) from manufacture to administration to the chickens which is not easy to maintain cold chain at present condition due to poor electrification network. In contrast, village chickens reared in small scale in scattered form and in free-range system, for these chicken available vaccines are inappropriate. Therefore, to address these various constraints, thermostable ND I-2 vaccine has been used and demonstrated satisfactory protection against velogenic NDV in many Asian and African countries (Vietnam, Malaysia, Tanzania, Malawi, Mozambique, Myanmar, and South Africa). From the studies conducted in Nepal, ND I-2 vaccine seemed to be avirulent, immunogenic and highly protective against virulent isolates of NDV (Shrestha *et. al.*, 2008; Acharya *et. al.*, 2016; Nyuapane *et. al.*, 2016) and hence it may be a suitable vaccine to use in village chickens to protect them against ND.

CONCLUSION

Field trial result showed that ND, I-2 vaccine was effective for village chicken irrespective of age and no necessity of maintenance of cold chain for 7 days during transportation and administration of vaccine where the temperature ranges below 30°C. However, it is recommended that earlier vaccination yields better result, which will certainly assure high vaccinal response. In case of terai region where temperature goes above 30°C, vaccination can be conducted in winter only. This vaccine is potent and adoptable. This vaccine does not give lifelong protection and must be repeated in every three months in our context. Adaptation of this vaccine can be a milestone towards preventing ND in village chicken which will improve the livelihood of rural communities and empower the women farmers along with high value protein supplementation.

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Demonstration of Circulating Antibodies of *Mycobacterium avium* Subspecies *paratuberculosis* in Cattle of Rupandehi District, Nepal

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ABSTRACT

Paratuberculosis, caused by Mycobacterium avium subspp. paratuberculosis, is a chronic intestinal infection of global importance in mainly domestic and wild ruminants. The main objective of the study was to find out the seroprevalence of Paratuberculosis in cattle of Rupandehi district. The research was conducted from October 2016 to December 2016. A total of 184 blood samples were collected from Jugular vein of cattle and tested by Enzyme Linked Immunosorbent Assay (ELISA). The Paratuberculosis Indirect Screening Test Kit was developed by ID. Vet, France. Cattle with history of chronic diarrhoea and emaciation were taken as study population along with other cattle in close association with them. Overall seroprevalence in Rupandehi district was found to be 4.89%. No significant relation of paratuberculosis was found with age, breed, parity, body condition score and location. Higher prevalence was found in cattle of older age and low body condition score. The result of this study reports the presence of bovine paratuberculosis in cattle of Rupandehi district.

Keywords: ELISA, Paratuberculosis, Seroprevalence

INTRODUCTION

Paratuberculosis, also known as Johne's disease, is a chronic intestinal infection of global importance in mainly domestic and wild ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). This infection can cause significant economic losses in cattle primarily related to decreased value at slaughter (Kudahland Nielsen, 2009), reduced milk production and premature culling (Raizman *et.al.*, 2009), and eventual death (Kudahl *et.al.*, 2007). Other economic losses resulting from the presence of paratuberculosis in cattle herds include decreased fertility, decreased fat and protein content in milk, reduced feed efficiency, and increased incidence of mastitis (NielsenandToft, 2009).

The diagnosis of paratuberculosis is divided into two parts- the diagnosis of clinical disease and the detection of subclinical infection- and the latter is essential for control of the disease at the farm, national or international level. In cattle, clinical cases can be diagnosed without difficulty because chronic diarrhea in adult animals is indicative of the disease. In the laboratory, a diagnosis can be made by isolating the agent from feces or necropsy materials, by histological study of the lesions, and polymerase chain reaction (PCR) assays. By using the Ziehl-Neelsen staining method (ZN-test), acid-alcohol resistant bacteria are observed in fecal smears and at the

end portions of the small intestine, however, serum-ELISA has been referred as superior to the ZN-test to confirm the presumptive diagnosis of clinical paratuberculosis in cattle (Weber *et.al.*, 2009). Subclinical cases can be diagnosed by isolating the bacteria from the feces, serological tests, or allergy tests (Lilenbaum *et.al.*, 2007). Hendrick *et.al.*, (2005) found that serum- and milk-ELISAs may be potentially useful and convenient methods in detecting subclinical paratuberculosis in lactating dairy cattle, with sensitivities of 73.6% (95% CI = 61.9-83.3%) and 61.1% (95% CI = 48.9-72.4%), and specificities of 87.5% (95% CI = 84.7-90.0%) and 94.7% (95% CI = 92.6-96.3%), respectively, compared to fecal culture.

Intradermal skin tests carried out in cattle of organized farms of Kathmandu and Pokhara valley showed an overall prevalence of 3.22% (0.9-10.13%) (Annual Reports of Central Animal Disease Investigation and Research Laboratory, Kathmandu, 1982-1988). Absorbed ELISA was conducted in 115 serum samples from buffaloes and 98 serum samples from cattle of different districts of Western Nepal which revealed 39 samples (33.9%) and 27 samples (27.5%) positive to antibodies against paratuberculosis (Joshi and Joshi, 1999). Similarly, rectal scrapings were collected from 28 buffaloes and 26 cows and 4 scrapping smears and 3 cultures were positive for acid fast organisms (Jha, 2003/2004). In recent years (2006-2008), studies on paratuberculosis were conducted by using different diagnostic tests conducted at Animal Health Research Division, NARC, Khumaltar and overall prevalence of 7.10 percent (3.5-9.09%) were detected in DTH, 4.55 percent (4.25-4.85%) in fecal smear, 0.70 percent in fecal culture and 5.34 percent (4.54-5.82%) in AGID in cattle of Kathmandu and Chitwan district. Also, Acharya (2016), found 12.17 percent animal level prevalence and 19.36 percent herd level prevalence of paratuberculosis in cattle of Western Chitwan.

Johne's disease shows some histopathological analogies with Crohn's Disease (CD), a human chronic inflammatory bowel disease of still unresolved etiology, and MAP appears to be related to the etiology of CD as either a causative agent or one of the factors of a multi-etiological syndrome (Atreya *et.al.*, 2014). This association has aroused great scientific interest since the 1980s, when Chiodini *et.al.*, (1984) isolated MAP and its DNA from biological tissues in CD patients. Based on these observations, the link between MAP and CD has been widely investigated through direct and indirect methods for MAP detection (Chiodini *et.al.*, 2012).

MAP prevalence estimation is a key element to assess the disease impact, and for the design of control programs (Verdugo *et.al.*, 2014), therefore, the objective of this study was to determine the seroprevalence of MAP infection using serology from cattle from Rupandehi district and compare it according to age, sex, breed, parity, location etc. These findings will be used by the concerned government and non-government bodies and organizations to monitor the disease, make necessary plans and policies and intervention programs.

Although Johne's disease has been reported in various districts of Nepal, no previous studies have been carried out to estimate its prevalence in Cattle of Rupandehi District. No study has been conducted to determine the associated risk factor for Johne's disease in smallholder dairy cattle in Rupandehi District. Rupandehi is at high risk due to import of animals and animal products from free border of India. MAP prevalence estimation is a key element to assess the disease impact and for the design of control programs.

MATERIALS AND METHODS

A cross sectional study was carried out in Rupandehi district, Nepal, a total of 184 serum samples were collected purposively from female cows between October 2016 and December 2016. Blood was collected from jugular vein and serum was separated and stored at -20°C. ELISA test was performed according to the manufacturer's protocol (ID Screen® manufactured by ID Vet, France). Data entry, management and analysis was done using program Microsoft Office Excel 2007. The difference in prevalence according to location, age, parity, breed, body condition score parameters was compared statistically by a Chi-square (χ^2) analysis and Fisher's Exact analysis using software OpenEpi version 3 with significance level defined at the $p < 0.05$.

RESULT

Seroprevalence of MAP

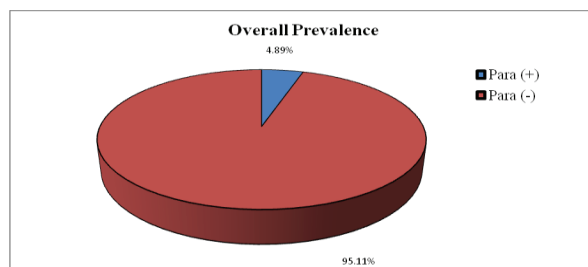


Figure 1: Seroprevalence of MAP in Rupandehi District

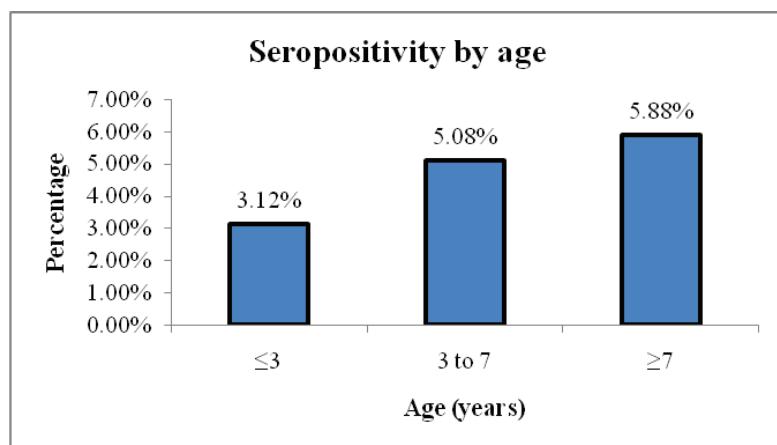
Prevalence by Breed

Table 1: Prevalence of MAP by Breed

Breed	Total sample	Positive cases	Seropositivity%
Jersey and Jersey Cross	135	6	4.44%
Others	49	3	6.12%

Out of 135 serum samples of Jersey and Jersey Cross, 6 serum samples (4.44%) were positive whereas in other breeds (Holstein, Holstein Cross, Brown Swiss, Sindhi, Local), 3 serum samples were positive (6.12%) out of 49 samples. There was no significant difference in prevalence according to breed ($p=0.891$; $p>0.05$).

Prevalence by Age



Out of 32 serum samples of less than equal to 3 years age group, 1 serum sample (3.12%) was positive, out of 118 samples between 3 to 7 years age group, 6 serum samples were positive (5.08%) and 2 serum samples (5.88%) were positive of greater than equal to 7 years age group out of 34 samples. There was no significant difference in prevalence among the age groups ($p=0.8625$; $p>0.05$).

Figure 2: Prevalence of MAP by Age

Prevalence by Parity

Table 2: Prevalence of MAP by Parity

Parity	Total sample	Positive cases	Seropositivity%
<5	147	7	4.76%
≥5	37	2	5.40%

Out of 147 serum samples of cattle of parity less than fifth, 7 serum samples (4.76%) were positive whereas in cattle of parity more than equal to fifth, 2 serum samples were positive (5.40%) out of 37 samples. There was no significant difference in prevalence according to parity ($p=0.754$; $p>0.05$).

Prevalence by BCS

Out of 122 serum samples of cattle having BCS less than 2.5, 7 serum samples (5.74%) were positive whereas in cattle having BCS more than equal to 2.5, 2 serum samples were positive (3.22%) out of 62 samples. There was no significant difference in prevalence according to BCS ($p=0.72$; $p>0.05$).

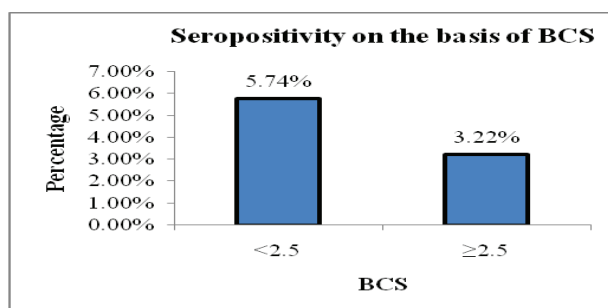
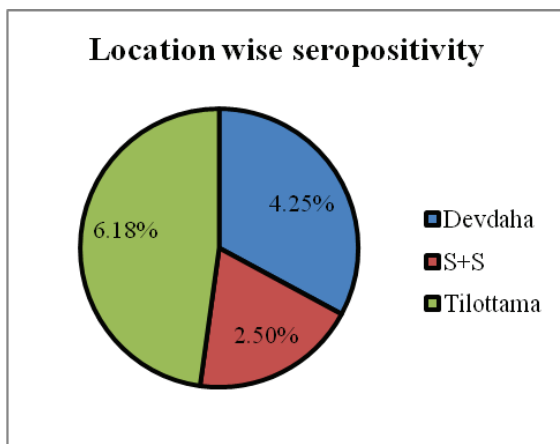


Figure 3: Prevalence of MAP by BCS

Prevalence by Location



Out of 97 serum samples from Tilottama Municipality, 6 serum samples (6.18%) were positive, out of 47 samples from Devdaha Municipality, 2 serum samples were positive (4.25%) and only 1 serum sample (2.50%) was positive from Sainamaina Municipality and Saljhandi VDC (S + S) out of 40 samples. There was no significant difference in prevalence among location ($p=0.643$; $p>0.05$).

Figure 4: Prevalence of MAP by Location

DISCUSSION

Paratuberculosis causes important economic losses in ruminants (Nielsen and Toft, 2009; Coelho *et. al.*, 2007). Although the presence of paratuberculosis has been known for a long time in Nepal, there are a few studies related to prevalence of paratuberculosis. To our knowledge this is the first report on estimation of sero-prevalence of MAP in Rupandehi district. In the present study, overall seroprevalence of Paratuberculosis in Rupandehi district was found to be 4.89%. This finding is greater than overall prevalence of 3.32% in cattle of organized farms of Kathmandu and Pokhara valley done by Intradermal Skin Test as reported in Annual Reports of Central Animal Disease Investigation and Research Laboratory, Kathmandu, 1982-1988. However, this result is less than findings of Acharya, 2016 *i.e.*, 12.17% prevalence at individual animal level in cattle of Western Chitwan. In Western Nepal, Joshi and Joshi (1999) found 37.5% (27/98) samples positive to antibodies against MAP using absorbed ELISA. In recent years (2006-2008), studies on paratuberculosis were conducted by using different diagnostic tests conducted at Animal Health Research Division, NARC, Khumaltar and overall prevalence of 7.10 percent (3.5-9.09%) were detected in DTH, 4.55 percent (4.25-4.85%) in fecal smear, 0.70 percent in fecal culture and 5.34 percent (4.54-5.82%) in AGID, in cattle of Kathmandu Valley and Chitwan District. The differences in prevalence may be due to the geographic variations, location of farms, sampling procedures and the use of different detection methods. This is supported by research of McKenna *et.al.* (2004) which regards that prevalence of paratuberculosis can be affected by the factors such as climate, nutrition, region and housing conditions and diagnostic methods.

No significant relation of Paratuberculosis was found with age, breed, parity, body condition score and location. Higher prevalence of Paratuberculosis was found in cattle of Tilottama municipality of Rupandehi district than in other locations. Cattle of older age were found to have higher seropositivity against Paratuberculosis than those of younger age and higher prevalence was found in cattle with low body condition score. The observed greater prevalence with higher parity is in line with the chronic nature of the infection which implies that the cows are relatively

old (typically 2-5 years old), before showing symptoms of the disease (Chiodini *et.al.*, 1984). This result is similar to the findings of Jakobsen *et.al.*, (2000) in Danish dairy cattle, Higher prevalence in other breeds can be observed than in Jersey or Jersey cross breeds, which differs from finding of Jakobsen *et.al.*, (2000) which shows the Jersey cattle having higher risk of being seropositive against Paratuberculosis.

CONCLUSION

This study is the first to report and estimate the seroprevalence of paratuberculosis in cattle of Rupandehi district and hence will certainly contribute in the management and eradication of the disease from this region. This study shows no any significant relation between the disease and various factors such as age, breed, parity BCS, location etc.

However, the validity and accuracy of this research could be challenged through increased sample sizes. Enhanced surveillance using confirmatory techniques with sufficient sample size is a prerequisite to confirm the findings. Further epizootiological investigations on Paratuberculosis in other farm animals at the country level are important to monitor and determine the magnitude of Paratuberculosis infection in order to estimate its economic impact on animal industry.

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Study on Status of Fish Diseases in Nepal

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ABSTRACT

Fisheries play an important role in increasing the Nepalese economy as well as sustaining livelihood of some ethnic groups of our country. With the increased demand of fish, pisciculture have also increased to a great extend. Due to the rise in fish culture, there has been also rise in fish diseases. The study aims to investigate different parasitic, bacterial, fungal diseases in fish and to suggest treatment to control the diseases in four different fish farm of Nepal. A cross-sectional qualitative method was used to collect data from four selected fish farm (Kakani, Trishuli, Begnas, Mirmi) of Nepal. Infected fishes were transferred to the lab in oxygen filled plastic bags and further tested for bacterial, fungal and parasitic infection. The result of the study indicates that Epizootic Ulcerative Syndrome was the most common bacterial-fungal disease that had a significant impact on common carp fish especially in Trishuli, Begnas and Mirmi. Coccidiosis caused by Eimeria spp was found to be a growing problem in rainbow trout farming (Kakani, Nuwakot) infecting intestine, liver, gut and skin causing yellow diarrhea and skin lesions. Trichodina was observed number one problematic parasitic in carp culture not only in government farm like Begnas and Mirmi, but also in commercial farms in most of the fishery areas of the country. Fin rot were more commonly reported during winter months affecting common carp, Silver carp, Bighead carp, Fingerling Fish causing fin erosion in them. Ascites in brood fishes (Trishuli) was noticed which may be due to bacterial infection and due to nutritional deficiency. Besides this, white spot in the gills (a protozoan parasitic disease) was perceived and to control it Neodox with Formalin (150ppm) was used and was found to be very effective. This study also identified some fish health management related problems in fish farms of Nepal such as lack of assistance, poor technical knowledge, and lack of suitable diagnostic laboratory and their proper use.

Keywords: Fish farming, Fish diseases, Economical loss, Health management, Nepal

INTRODUCTION

Nepal lying in between India and China is a small land locked country having three eco-zones, terai in the southern plain, hill in the middle and the mountains in the north. It is rich in term of water resources which makes Nepal a country with great potential in fish farming. In Nepal, 200 fish species are available in which around 190 are indigenous species and remaining are exotic species (Sharma and Sharma, 2008). Commercial fish farming commenced since 1947 with the introduction of pond culture of Indian carps (FAO, 2012). Today, there are many varieties of fishes produced commercially in different parts of the country majority being Rohu, Naini, Bhakur, Carps (Common, silver, grass, and bighead), Tilapia, Puntius, Rainbow trout etc. Among the indigenous fishes, Asla, Katla, Sahar etc. are captured from ponds, lakes and rivers and marketed in the local markets.

Different system of fish culture is practiced and followed in different region of the country. Mostly Indian major carps, Chinese major carp and Cat fish are cultured in earthen ponds, cemented ponds in intensive or semi-intensive way. Besides these, cage culture, pen culture and paddy cum fish culture are also under practice. Government agencies and progressive farmers have initiated cage culture in Phewa, Rupa, Begnas and Kulekhani lakes and dams but preliminary cage culture in lakes and reservoirs with herbivorous carps was initiated with the support of FAO/UNDP and later the International Development Research Center (IDRC) Canada in the 1970s (Karki, 2016). Farmers are also practicing integrated farming system which includes livestock, horticulture along with fisheries. Some ethnic groups (Tharu, Majhis, Kumal, Kewat, Mushar, Boteetc) sustain their livelihood by catching and selling of captured fishes from rivers, ponds and lakes of the country (Budhathoki and Sapkota, 2018).

With increased demands of fish, piscicultures have also increased to great extent. Big commercial fish farmers have practiced fish farming not only in terai region but also it is coming up in the lower hills of the country. The intensive fish culture involves higher rates of stocking and supplementary feeding which has substantially enhanced the incidence of diseases in fishes in our country. Fishes go through wide varieties of diseases of infectious and non infectious etiology in their natural environment. Several literatures are available on fish mortality and morbidity due to bacteria, parasites, fungus, nutritional deficiencies, metabolic disorders and faulty environmental conditions like oxygen deficiency, high or very low water temperature, chemical pollutants, overcrowding, mal-nutrition, algal blooms etc (Mukherjee, 2002). It is estimated that the annual loss in production due to diseases range from 25 to 30% (Anon, 1996).

Bacterial fish diseases are very common and are one of the most difficult health problems to deal with. Bacterial diseases have been frequently encountered in eggs, fry, fingerlings of fish, causing heavy mortality. These microorganisms are essentially opportunistic pathogens which invade the tissues of a fish host rendered susceptible to infection by stress factors (Sandeep.P, 2016). The bacterial diseases commonly encountered are columnariosis, farunuclosis, tail rot/fin rot, bacterial gill diseases, aeromoniasis, edwardsiellosis, vibriosis, eye disease, pseudomoniasis and enteric red mouth disease (Mishra *et al.*, 2017). Compared to other diseases, the parasitic disease alone causes 8-12% loss in production (Anon, 1996). Fish parasites multiply rapidly under favourable conditions, there by affecting the health of fishes, often leading to high mortality. Parasites interfere with nutrition of hosts, disrupting the metabolism and secretory

functions of alimentary canal and damage nervous system (Farhaduzzaman et al, 2010). Parasitic diseases commonly reported in fish culture are Argulidae (Carp lice), *Dactylogymus* (Gill fluke), ichthyophthiriasis (White spot), trichodiniasis, gyrodactylosis (Skin fluke) and myxosporidiasis (Mishra SS *et al.*, 2014).

Contrasting to bacterial and parasitic diseases, a very few number of fungal species are known to be pathogenic to the fish. Mostly these are present in water and under unfavourable conditions; they attack the fish causing skin lesions. Most fungal infections recorded in carp culture are those caused by species belonging to the oomycete fungi, *Saprolegnia*, *Achlya* and *Aphanomyces*. Diseases caused by these fungi are collectively called “saprolegniasis”. The important fungal induced disease in fish culture of high economic importance is Epizootic Ulcerative Syndrome (EUS). It is widely occurred in freshwater aquaculture causing a great loss. It is a severe and economically important disease affecting farmed freshwater fish and listed as a notifiable disease. Other fungal diseases like Branchiomycosis (gill rot), ichthyophonosis, aspergillomycoses are also commonly encountered in fish culture (Mishra, 2017).

The study aims to investigate different parasitic, bacterial, fungal diseases in fish and to suggest treatment to control the diseases in four different fish farm (Kakani, Trishuli, Begnas, Mirmi) of Nepal.

MATERIAL AND METHODS

Selection of Study Area

The study was conducted in four selected fish farms of Nepal i.e. (Kakani, Trishuli, Begnas and Mirmi) due to abundance of fish resources (fish rearing farmers, societies, fish rearing and availability of hatcheries).

Data Collection and Sampling

A combination of participatory and qualitative methods was used for data collection. Data were collected from 2072 to 2075. A traditional survey method of direct interview from the selected fish farmers was followed, using pre-tested questionnaire. After collecting primary data, focus group discussion with farmers and cross-check interviews with key informants were carried out to justify the previously collected data. Fishes were examined for external symptoms and health conditions at the site of the farm. Infected fishes were transferred to the lab in oxygen filled plastic bags.

Test for Parasite

Intestines of yellow diarrheal syndrome affected rainbow trout were sampled. Collected trout intestine samples were stored in vials of normal saline solution for coprological observation and species identification. The sampled intestines were opened in longitudinal direction and the mucus and fecal material were drawn off. Qualitative fecal examination was performed by McMaster technique with some modification.

Test for Fungi

The fish samples were disinfected from surface to prevent secondary contamination with airborne spores by dipping fish in 1% formaldehyde for 1 to 5 min. Then the sample was

transferred to 70% alcohol and finally several washings were given in sterilized distilled water. The fungi were isolated from infected organs of fish with sterile needle, inoculated on Potato dextrose agar (PDA). The agar plates were incubated at 28-30° C and fungal growth was observed after 5-7days. Slides were prepared by taking material from each colony and stained with Lacto-phenol cotton blue. The slides were observed under microscope and photographed. The fungal identification was done with the help of identification keys and literature.

Test for Bacteria

Fish samples were collected aseptically. These samples were first enriched then inoculated in respective selective medias then immediately sub-cultured in nutrient agar and incubated at 37°C for 24-48 hours and the colony characters were recorded. The colonies obtained on the agar surface were stained with Gram's stain for morphological studies and subjected to conventional biochemical tests for identification of the organisms.

RESULTS

According to this study, coccidiosis caused by *Eimeria* spp. was found to be a growing problem in rainbow trout farming (Kakani, Nuwakot) infecting intestine, liver, gut and skin causing yellow diarrhea and skin lesions. Trichodina was observed number one problematic parasitic diseases in carp culture not only in government farm like Begnas and Mirmi, but also in commercial farms in most of the fishery areas of the country. Epizootic ulcerative Syndrome was noticed in Trishuli, Begnas and Mirmi in some breeding fishes which mainly infect the skin causing skin lesions which further expand to form ulcers followed by the development of granulomas on the internal organs and death. It was prevalent mostly during winter months.

Fingerling mortality in Mirmi was mostly due to low oxygen level in water and due to overcrowding. Fin rot was more commonly seen during winter months affecting Common carp, Silver carp, and Bighead carp. Ascites in brood fishes (Trishuli) was noticed which may be due to bacterial infection and due to nutritional deficiency. Besides this, white spot in the gills (a protozoan parasitic disease) was perceived and treated with Neodox with Formalin (150ppm) which was very effective. In slow growth, addition of protein and essential amino-acid in the feed was suggested.

Table 1: Survey lab finding and treatment of different fish diseases.

In slow growth, addition of protein and essential aminoacid in the feed was suggested.

*Vime-Anticoc (200gm)	*Prolium-K (150gm)
Sulfadimidin sodium	Amprolium + Vit K3
Diaveridine HCL	
Mendione Na	

The other parasitic diseases Argulosis caused by metazoan parasite (*Argulus*) was found widely in Indian major carps, especially in brood fish. Maximum argulosis problem was found in Mirmi, Syangja.

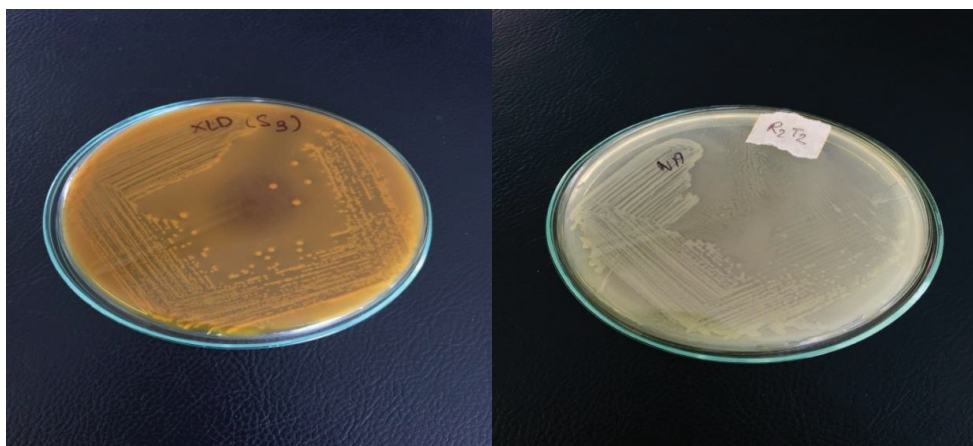


Figure 1: *E.coli* (Left) and *Salmonella* (Right) in XLD and Nutrient agar isolated from infected fish sample

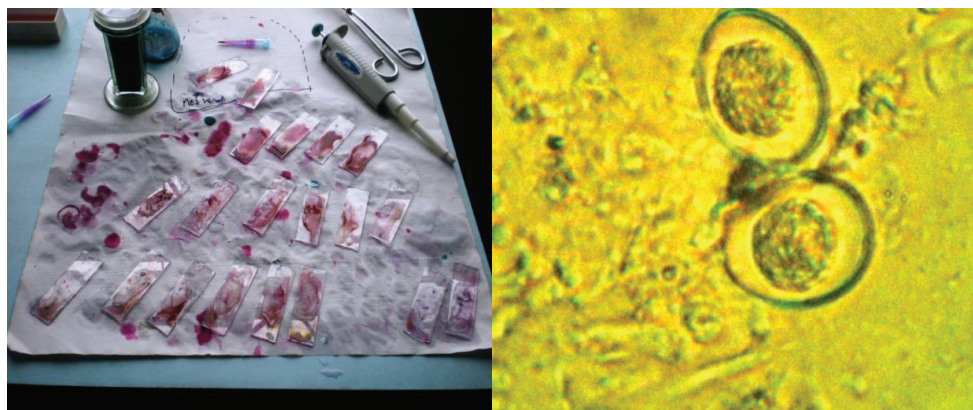


Figure 2: Processing of fecal sample (right) and *Eimeria aurati* (left) isolated from intestine of rainbow trout viewed under a microscope

Table 2: Feed formulation for trout (breeding stock)

Ingredients	Percentage (%)
Prawn	35
Soya cake	25
Wheat	15
Rice bran	13
Mustard cake	10
Vitamins	1
Minerals	1
Total	100%

Table 3: Other fish diseases that have been reported in Nepal (R. Thakur *et al.*,)

Other diseases reported in Nepal					
Protozoan	Crustacean	Bacterial	Fungal	Viral	Environmental
-Costiasis or Blue Slime Disease - Ichthyophthiiasis or White spot disease	-Lernaeasis	-Tail Rot or Fin Rot -Dropsy	-Saprolegniasis - Branchiomycosis Or Gill rot -Epizootic Ulcerative Syndrome (EUS)	-Spring Viremia of carp	-Gas Bubble disease or Gas Embolism in hatchlings -Asphyxiation

DISCUSSION

Persistent occurrence of disease is one of the major curtailments to fish farming in Nepal that may eventually become a limiting factor for its development. The increasing development of aquaculture activities and with intensification of fish culture, has led to increasing number of infectious diseases. The study identified different types of diseases in fish farming in Nepal. A cross-sectional qualitative method was used for data collection. The most commonly reported diseases was Epizootic Ulcerative Syndrome, followed by trichodiniasis, fin rot, gill rot, coccidiosis, white spots, argulosis and dropsy.

The present study showed that Epizootic Ulcerative Syndrome was the most common bacterial-fungal disease that had a significant impact on common carp fish especially in Trishuli, Begnas and Mirmi of Nepal. The pathogenic fungus, *Aphanomyces invadans* and bacteria *Aeromonas* spp was identified as the causative agent of the disease. In a study conducted by Bagum *et al.*, (2013) revealed that EUS was the most common disease that had a significant impact on major carps fish (*C. catla*, *L. rohita* and *C. cirrhosus*) especially in Sylhet (32%) and Rajshahi (35%) districts of Bangladesh. Mukherjee (2002) have reported the role of *Aeromonas hydrophila* in ulcerative disease of fish and identified the biochemical differences among the various strains of these organisms. In most of the case *A. hydrophila* was found to be isolated although several other bacterial forms (*Pseudomonas* spp, *Vibrio* spp and *E.coli*) were also present as secondary invaders that mainly infect the skin causing skin lesions which further expand to form ulcers followed by the development of granulomas on the internal organs and death. Most of the farmers called it as fish “Ulcers” and can easily recognize the diseases. According to the survey, this EUS usually appeared at the beginning of the winter season and caused severe mortality of the fish. Besides this other fungal infections like saprolegniasis and brachiomycosis (Gill rot) was also observed.

Parasitic infestation has also been the major cause of concern and causing significant setback to freshwater aquaculture in Nepal. According to Anon (1996), it has been estimated that parasitic diseases alone cause 8-12% loss in production. The fish parasites have the ability to multiply rapidly under poor water quality conditions, affecting fishes and leading to high morbidity. In this study, the fish parasites mostly responsible for the infection were *Trichodina* spp. and helminth parasite (cestode and nematode). *Trichodina* spp. attack mostly the gills and skin,

damaging the host tissue and consuming the resulting dead tissues. Parasites interfere with nutrition of fish, disrupt the metabolism and secretory functions of alimentary canal and often damage the nervous system, thereby reducing growth rate and even causes mortality, as a result causes substantial economic loss in fish culture system.

The other parasitic diseases Argulosis caused by metazoan parasite (*Argulus*) was found widely in Indian major carps, especially in brood fish. Maximum argulosis problem was found in Mirmi, Syangja. In the study conducted by Kter, Ossain, and Edwanur (2007), highest mortality of carp fingerlings were reported from nurseries infected with protozoan and monogenean parasites. The species *Eimeria* are considered as significant pathogens of cultured rainbow trout in Kakani, Nuwakot. *Eimeria* positive rainbow trout showed sluggish behavior, blackish in color, anorexia and yellow diarrheal syndrome. The two anti-coccidial drugs (Vime-Anticoc and Prolium-K) were suggested for the treatment of *Eimeria* and were found to be effective. A. Rayamajhi *et. al.*, (2011) suggested a high incidence of eimeriosis in rainbow trout, in semi intensive raceway farming system of Nepal and concluded that Supercox and Ccotreat-EP was effective for *Eimeria* infection.

A wide variety of bacterial pathogens are also responsible for the major losses to the freshwater aquaculture. These microorganisms are essentially opportunistic pathogens which invade the tissues of a fish host rendered susceptible to infection by stress factors. Bacterial diseases like motile *Aeromonas* septicaemia, *Pseudomonas* septicaemia, bacterial gill disease, Fin rot and Dropsy were often reported in various fish culture in Nepal. The poor, abnormal water quality acts as a predisposing factor making the cultured fish susceptible to bacterial opportunistic pathogens. According to this study, most of the diseases mainly occurred during winter season during which the water level drops to minimum and the quality becomes very poor. During low temperature, the immune system of fish suppresses making them more susceptible to diseases. (Ahmed, Akter, Nipa, and Hossain, 2009) reported that the immune system of fish suppressed this time due to low temperature and fish become more susceptible to fish disease. A number of chemotherapeutics were reported for controlling fish disease. The two anti-coccidial drugs Vime-Anticoc (100 gm in 200 kgs feed), Prolium-K (30 gm in 50 kgs of feed) was suggested for the treatment of *Eimeria* (Yellow diarrhea) and for the treatment of other diseases Neodox with Formalin (150 ppm) was suggested and was found to be effective. In slow growth, addition of protein and essential amino-acid in the feed was suggested. Faruk *et.al.*, (2012) reported that mostly lime was exceptionally used in treating brood and health management. Lime is very effective in maintaining pH, water color, and turbidity, increase the rate of decomposition and to treat disease fishes. Hence farmers could be suggested to take these preventive measures at the beginning of winter months. Economic losses from diseases are likely to increase as aquaculture expands and intensifies. The estimated economic loss due to fish disease may vary from farm to farm based on the intensity of infection and the management practices adopted by farmers. The lack of awareness about fish diseases and reporting places and diagnostic laboratory, can lower the reporting of the fish diseases by the farmers.

CONCLUSION

The present study has helped in demonstrating different fish diseases (parasitic, fungal and bacteria) as an alarming problem to the fish farming in Nepal. In this study, the cross-sectional

qualitative survey was done to determine the status of fish diseases in Nepal. And from this conducted research, it has demonstrated that Epizootic Ulcerative Syndrome was the most common bacterial-fungal diseases followed by Trichodiniasis, Fin rot, Coccidiosis, Argulosis, White spot, Dropsy etc. And among the four places, the fishes in Trishuli, Begnas, Mirmi were highly infected with different parasitic, bacterial and fungal diseases. Chronic mortalities and poor growth caused by disease are generally not recognized. In order to quantify disease losses, farmers should be able to identify disease as the reason for production loss, slow growth or poor harvest. Rural farmers are mostly resource poor with little or no knowledge of health management and have inadequate opportunities to improve management skills. Therefore, it is important to train farmers to carry out field-level diagnosis and assess the likely impacts of diseases.

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Toxoplasmosis in Cats and Its Zoonotic Potential in and Around Bangladesh Agricultural University Campus

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ABSTRACT

Toxoplasmosis is a zoonotic disease, occurs in almost all warm blooded animals including human beings and is caused by a global protozoan intracellular parasite Toxoplasma gondii. It is estimated that one third of the world human population have been infected by this parasite. This protozoan causes a significant public health problem in humans and imposes considerable economic losses and damages to livestock. The final host is cat, Feliscatus, accounts for all of these significant burdens. Hence the present study was designed to determine the prevalence rate of T. gondii infection in cats and also to analyze the associated factors that were potential for human beings and livestock as well in the period from September 2014 to August 2016. Altogether 254 faecal samples from cat and 390 water samples were collected from Bangladesh Agricultural University (BAU) campus, farm and residential areas of BAU and were examined for presence of T. gondii oocysts at Department of Parasitology, BAU Mymensingh. The overall prevalence of T. gondii was 4.03% (26/644). Presence of T. gondii oocysts in faecal and water samples were 5.5% (14/254) and 3.08% (12/390), respectively. Adult cats had higher (6.7%) prevalence than young cats (3.8%). Summer season was found high prevalence (6.7%) of oocysts in cat faeces. Similarly drain water found higher positivity (6.67%) of oocysts. Therefore, this study concluded that the cats are contaminating the environment by shedding oocysts and then other animals can get the infection from grazing of contaminated grasses and drinking water. So eating undercooked goat, sheep and cattle meat; eating contaminated vegetables and drinking contaminated water are major sources for getting toxoplasmosis to human beings. Hence this disease has great zoonotic importance around BAU campus and therefore it is essential to create public awareness.

Keywords: Toxoplasmosis, Cat, Zoonotic potential, Prevalence

INTRODUCTION

Toxoplasmosis is a major parasitic zoonotic disease of almost all warm blooded animals including human beings. This disease is caused by an obligate intracellular protozoan parasite *Toxoplasma gondii*. *T. gondii* affects over 350 species of vertebrates. Based on serological investigations, it is estimated that one third of the world's human population has been exposed to this widespread zoonotic agent (Innes 2010; Pereira *et al.*, 2010; Montoya and Liesenfeld, 2012). In Bangladesh, seroprevalence of toxoplasmosis in human was 38.5% (Ashrafunness *et al.*, 1998), 12.4% in blood donors, 11.8% in pregnant women, 50% in butchers (Samad *et al.*, 1997)

and 15.89% in women (Samad *et.al.*, 1993). *T. gondii* is recognized as an important opportunistic pathogen of fetuses, newborns and patients with a variety of primary and secondary immunodeficiency (Petersen *et.al.*, 2001). In Mymensingh district, seroprevalence for toxoplasmosis were also recorded in cattle-12%, sheep-40% and goats-32% (Shahiduzzaman *et al.*, 2011) and cattle-16.10%, sheep-17.65% and goats-12.09% (Samad *et al.*, 1993).

The ingestion of tissue cysts from undercooked meat and consuming food as well as water contaminated with oocysts are the two most important modes of transmission of *T. gondii* in humans. Felids play a pivotal role for *T. gondii* as definitive hosts, because they excrete environmentally resistant oocysts in faeces and contaminate soil, food and water.

Cats are the most popular pet in the world, and are now found in almost every place where humans live (Driscoll *et.al.*, 2009). No doubt Bangladesh has unknown number of domestic, stray and wild cats. One report showed 33.33% seropositive of *T. gondii* in cats in Bangladesh (Samad *et.al.*, 1997). Usually cats do not show the clinical signs even during shedding of oocysts. It means subclinical form occurs in cat. So cats have a key and crucial role in the epidemiology of toxoplasmosis, so expanding the basic knowledge about *T. gondii* infection in cats is a matter of importance. A little is known of toxoplasmosis in cats in Bangladesh and there is no information on the excretion of *T. gondii* oocysts by cats in Bangladesh. Therefore, here we emphasize to report the presence of *T. gondii* oocysts in cat faeces and contaminated water for the first time in Bangladesh.

MATERIALS AND METHODS

Examination of cat feces for *T. gondii* oocysts

Altogether 254 samples of cat feces were collected from Mymensingh (in and around BAU campus) in the period from September 2014 to August 2016. People having cats were requested to collect cat faeces in plastic bag and were dispatched to Department of Parasitology, BAU. Faeces were collected cats from student residential place and staff quarters areas of BAU. The cat feces were examined for the presence of *T. gondii* oocysts using Sheather's sugar flotation as was previously described by Dubey and Beattie (1988); Barutzki and Schaper (2003); Dubey *et. al.*, (2006); Schares *et.al.*, (2008); Dubey JP (2009). Briefly, feces (2-10 gm) of each cat was floated in sucrose solution (454 gm sugar, 355 ml water and 6 ml formalin; specific gravity, 1.203), filtered through gauze and centrifuged in a 15 ml tube at 1500 rpm for 10 min. A drop of the float from the meniscus was examined microscopically between cover slip and glass slide at 200X magnification. Oocyst was subspherical to spherical in shape, 10-12 µm in diameter in size, thick walled structure, containing cellular structures, as there is no maturation to sporozoites at this stage. Measurements of oocysts were taken with the aid of an ocular micrometer.

Examination of Water Samples for *T. gondii* Oocysts

A total of 390 water samples were collected from different water sources (tap/tube well, pond, drain, stagnant water) at Bangladesh Agricultural University (BAU) campus and adjacent villages like Sheshmore, Sutiakhali, Digerkanda, Fakirkanda, Bhagnabari, Chaurkalibari and

Kewatkhali of Mymensingh from September 2014 to August 2016. Sample size was estimated by using the following formula according to Thrusfield (2007):

where n is required sample size; 1.96 is the z value for 95% confidence interval; The P_{exp} (expected prevalence) of water samples is assumed 50% if prevalence is unknown; d is desired absolute precision=5%.

Samples after pre-filtration step were brought to the Department of Parasitology, BAU for further processing and examination. Water samples were examined for the presence of *T. gondii* oocysts as described by Kourenti *et.al.* (2003). Briefly, about 30-50 litres each water samples were collected and were filtered with successive decreasing mesh sieves (pre-filtration step) could be incorporated to remove large particles. Then filtration was again performed with one micron pore size polyester filter bag (Duda). The filtrate was washed with 0.5% Tween-20 (laureth-12, polyoxy-ethylensorbitan-monooleate) into 15 ml testtube (Figure 1. a, b, c, d). The oocysts were floated with sucrose solution (specific gravity: 1.203) by centrifuging at 1500 rpm for 10 min. A drop of the float from meniscus was placed on a clean glass slide, was covered with coverslip and was examined for oocysts microscopically.

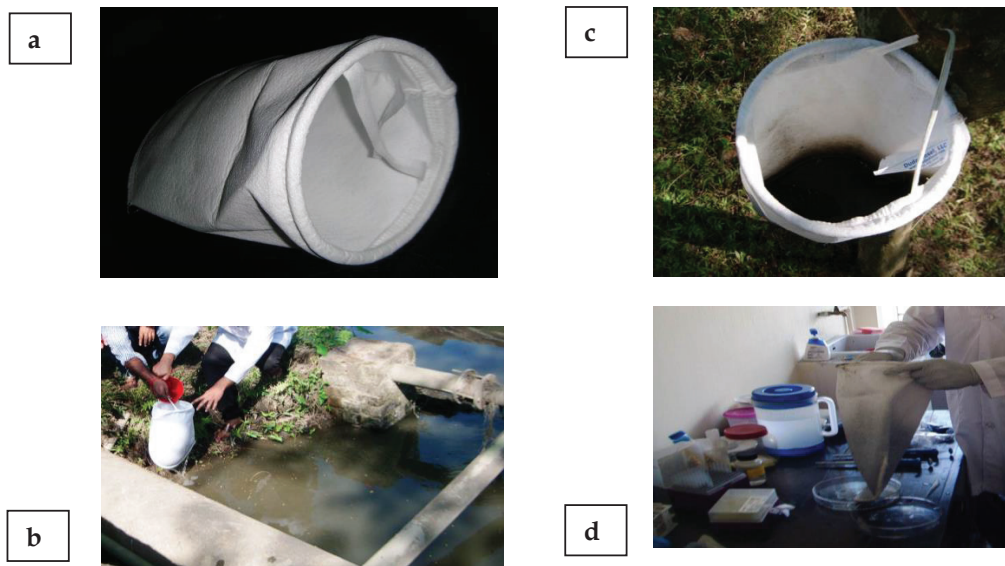


Figure1. Collection and processing of water samples. a) One micron polyester filter bag (Duda), b) Collection of water sample from waste water body, c) Filtration of water sample by filter bag, d) Collection of concentrated sample in filter bag with dispersion detergent Tween-20

Analysis: In this study domestic environmental conditions of the cats were defined as: household cats (indoor only) and street cats (both indoor and outdoor). Age of cat was categorized as young (<4 months) and adult (≥ 4 months). Season was divided into three: Summer (Mar-Jun), Rainy (Jul-Oct) and Winter (Nov-Feb). Obtained data were compiled in Microsoft Excel and analyzed.

RESULT

Study was being performed for two years. After examining the samples (cat faeces- 254 and water samples- 390), the overall presence of *T. gondii* oocysts was found 4.03% (26/644).

Detection of *T. gondii* oocysts in faecal samples

A total of 254 cat faeces were examined and the prevalence of *T. gondii* oocysts was 5.5% in cat faeces (Table 1, Figure 2).

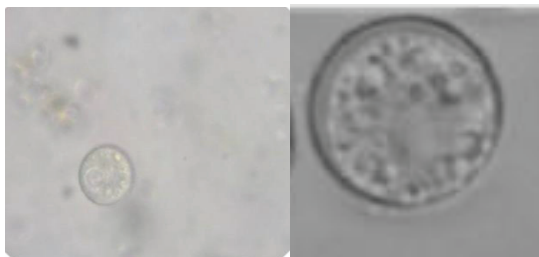


Figure 2. *T. gondii* oocyst (40X; right one magnified)

Table 1. Distribution of *T. gondii* oocysts in fecal samples based on age of cat and season

Category	No of sample	Positive for oocyst	Percentage (%)
Overall	254	14	5.5
Age			
Adult cat	150	10	6.7
Young cat	104	4	3.8
Season			
Summer (Mar-Jun)	134	9	6.7
Rainy (Jul-Oct)	50	2	4.0
Winter (Nov-Feb)	70	3	4.3

Detection of *T. gondii* Oocysts in Environmental Samples

The prevalence of *T. gondii* oocysts was 3.08% (12/390) in water samples as shown in Table 2.

Table 2. Distribution of *T. gondii* oocysts and other oocysts in water sample based on different water sources and seasons

Category	No of sample	Positive for <i>T. gondii</i> oocyst	Prevalence %	Positive for oocysts other than <i>T. gondii</i>
Overall	390	12	3.08	66 (16.92%)
Water sources				
Tap water/tube well	120	0	0	0 (0%)
Pond	90	3	3.33	15 (16.67%)
Drain	90	6	6.67	34 (37.78%)
Stagnant water in field	90	3	3.33	17 (18.89%)
Season				
Summer (Mar-Jun)	130	3	2.31	
Rainy (Jul-Oct)	150	6	4.0	
Winter (Nov-Feb)	110	3	2.73	

Except tap/tubewell, all three water sources were found to be contaminated with *T. gondii* oocysts. Drain water was found highly contaminated in this study. In addition to take water samples, the presence of cats in those areas and their activities were observed in this study (Figure 3).

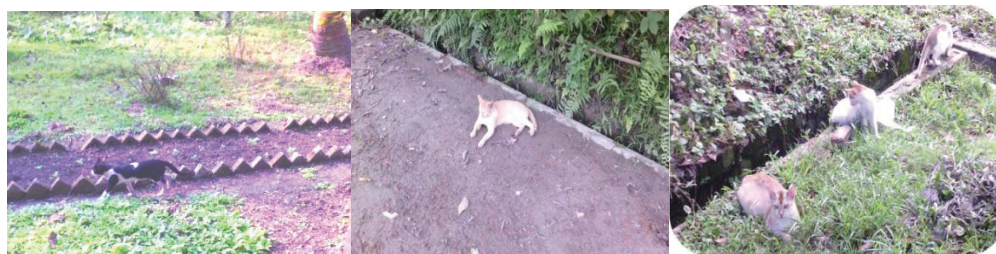


Figure 3. Cats wandering and contaminating environment at BAU area

All of the noticed cats were free roaming but with limited access to households and none had specific owner. It was also noticed that people offered left over feed to cats. Additionally, it was found that cats were living in close contact with human dwellers and animal houses.

DISCUSSION

Detection of *T. gondii* oocysts in Faecal Samples

The finding of this study was very high from reports of other authors (Dabritz *et.al.*, 2007; Bizhga B, 2017). In Bangladesh, there are limited organized animal farms and cats are wandering everywhere in studied areas contaminating the grass or pasture. So, cattle, sheep and goats are usually kept in pasture for grazing, and therefore they have an increased risk of infection due to contamination of the environment with sporulated oocysts shed by cats.

Hence, people may get infection after eating undercooked meat of these animals which is rare due to food habit of Bangladeshi people. People can also get infection by eating unwashed

vegetables, fruits due to contamination of the environment or kitchen garden with sporulated oocysts. People can get accidental infection when they are working in garden and field etc.

Summer season (March to June) showed higher prevalence. In fact, soil contamination by *T. gondii* oocysts is not a stationary event because it can vary depending on the presence of feline excretion of oocysts in faeces and on environmental conditions such as temperature and moisture (Dubey 2004; Casartelli-Alves *et.al.*, 2015). Several studies have confirmed a link between toxoplasmosis outbreaks and water contamination with oocysts (Cook *et.al.*, 2000; Dubey, 2004). After contamination of soil with oocysts, oocysts may reach into water source by wind; rain etc. and then water become contaminated.

Adult cats were affected more than young (<4 months old) by this protozoa. It is expected that with increasing age, exposure of cats to *T. gondii* infection also increases (Miro *et.al.*, 2004; Alvarado *et.al.*, 2012). This may be due to feeding cats with raw meat and infrequent cleaning of the litter box (Besne *et.al.*, 2008). Young cats might get infection via congenital transmission from infected dam or from milk.

All of the studied cats were free roaming but with limited access to households and none had specific owner. In general street cats are more prone to *T. gondii* infection compared to household cats because street cats could acquire the infection through catching the rodents, birds, reptiles and raw food scraps.

Detection of *T. gondii* Oocysts in Eenvironmental Samples

The prevalence of *T. gondii* oocysts was 3.08% in water samples in this study. Transmission of *T. gondii* via drinking water has been reported in the context of outbreaks and as a risk factor in toxoplasmosis-endemic areas (Jones and Dubey 2010, Karanis *et.al.*, 2013, Krueger *et.al.*, 2014). However, estimation of the levels of host exposure to *T. gondii* oocysts water is a complex and multifactorial subject.

Absence of *T. gondii* oocysts was found in tap/tubewell water sources. It is because of no chance for contamination of such water with oocysts. That is why such water sources can be assumed as Toxoplasma safe. Such water can be contaminated outside after taking out from tap/tubewell.

Among four water sources, drain water was found to be more contaminated with *T. gondii* oocysts. Drain water contains water from many places like kitchen, hotel, animal sheds, gardens etc and these places are liked by cats for food, shelter and play.

The study showed pond water can be contaminated with this protozoan. This might be due to washing of contaminated vegetables in pond water before taking to market (Bangladeshi trend) and cleaning kitchen utensils near the pond. During cleaning, few kitchen leftover materials like fish, fish spines, meat etc were left aside and these materials are good attraction for coming cats there and thereby pond water become contaminated after shedding oocysts.

The present study showed that stagnant water can be the source of infection. Lack of proper drainage facility enhances the water stagnant everywhere in the field. Generally, cats are wandering everywhere in the country and they are contaminating the environment with sporulated oocysts. Animals like cattle, sheep and goats are usually kept in pasture for grazing, and therefore they have an increased risk of infection due to drinking of contamination water.

T. gondii oocysts were found higher in rainy season (July to October). In rainy season oocysts might come up from soil and grazing areas or pasture to the water sources due to flow of water and thereby make contamination to water sources.

CONCLUSION

There is presence of *T. gondii* oocysts in the environment (cat faeces, water), which might be hazardous for both public and animal health. Hence this disease has great zoonotic importance around BAU campus and therefore needs to develop public awareness.

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Controlled Internal Drug Release (CIDR) Based Hormonal Protocols Effect upon Estrus Response and Pregnancy Outcome in Anestrous Cows

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ABSTRACT

Controlled internal drug release (CIDR) based hormonal protocols CoSynch + CIDR, OvSynch + CIDR and CIDR + PGF2 α were applied to 25 crossbred anestrous Holstein and Jersey cows to improve the fertility at the farm of National Cattle Research Programme, Rampur, Chitwan. All three protocols were found equally effective ($P>0.05$) with 100% estrus expression rate, out of which, 80% (8/10), 85.7% (6/7) and 75% (6/8) had estrus expression with well cervix open at fixed time artificial insemination in CoSynch + CIDR, OvSynch + CIDR and CIDR + PGF2 α protocols respectively and rest had estrus expression with partial cervix open. Statistically non-significant ($P>0.05$) pregnancy outcome that is 14.3 % (1/7) in CIDR+PGF2 α group and 12.5 % (1/8) in OvSynch group while none in CoSynch + CIDR group were pregnant following fixed time artificial insemination. Poor pregnancy outcome of the anestrous cows in all protocols might not only have hormonal problem but also could have various other reasons which was beyond the objective of this research findings. Hence, study concludes anestrous cows respond well to the CIDR based hormonal protocols to revive the estrus.

Keywords: Fixed time artificial insemination, Intra-vaginal insert device, Progesterone, Prostaglandin analogue and synchronization

INTRODUCTION

Reproductive efficiency is dependent upon optimization of management, health, and physiology of cows (Lucy, 2001, Sartori *et.al.*, 2010). It is important that cows become pregnant at a biologically optimal time and produces offspring at defined calving interval (Sakaguchi M, 2011), otherwise leads to Infertility or Sub-fertility. Major physiological aspects that affect fertility in dairy cows are behavioral estrus or physiological anestrous, timely ovulation, quality semen, and oocytes, uterine environment, maternal recognition of pregnancy, release time and concentration of progesterone and negative energy balance (Lucy, M C, 2007; Walsh *et.al.*, 2011).

Anestrous condition is characterized by absence of estrus behavior (Peter, A T *et.al.*, 2009). During postpartum period negative energy balance may cause anovulation which leads to physiological anestrous (Kumar *et.al.*, 2014). It is obvious for the most of the cows to remain in anestrous during 10 to 12 weeks postpartum due to state of negative energy balance and thereafter animal may be cyclic but again due to incompetent oocytes, smaller dominant follicle,

abnormal progesterone profiles and poor embryo survival may affect the fertility (Peter, A T *et. al.*, 2009).

Cows having concentrations of progesterone <1.0 ng/ml in both of the first 2 blood samples at least 10 days apart were classified as anestrus (Chebel *et.al.*, 2006). Adams G P *et.al.*, 2008 reported that oocytes that developed under a prolonged-low-progesterone level (*i.e.*, mimicking persistent oversized follicles) failed to ovulate. Hence, it can be regarded as progesterone hormone has significant role in the estrus expression and ovulation in the dairy cows.

This research work focuses on the management of anestrus cows through application of hormonal approach. Increased progesterone hormone during the growth of follicular wave increases fertility more than 10% to the subsequent timed AI. Also, many manipulative studies have reported improvement in ~5-7% pregnancy using CIDR in OvSynch hormonal protocol prior to artificial insemination (Wiltbank *et.al.*, 2012) and similarly 10 % more pregnancy with co-synch protocol alone (Lamb *et.al.*, 2001).

A new reproductive tool—the CIDR (controlled internal drug release) was approved by Food and Drug Administration in 2002 for the synchronization of estrus. The CIDR, an intra-vaginal progesterone insert device can be used in conjunction with prostaglandin analogue (Darrel J K, 2002). Hence the objective of this experiment was to apply the CIDR as a source of progesterone hormone with various protocols upon prolonged anestrus cows for their effect on estrus expression and pregnancy outcome following fixed timed artificial insemination.

METHODOLOGY

Twenty five crossbred dairy anestrus cows of Holstein and Jersey that had not expressed heat for at least last six months were selected from the farm of National Cattle Research Programme, Rampur, Chitwan during February 2017 to July 2018. Parity of selected cows was in between 1 to 4 and milk yield less than two liters a day per cow. Selected cows were applied to three different CIDR based hormonal estrus synchronization protocols. Hormones used in the protocols were progesterone (P4), gonadotropin releasing hormone (GnRH) and prostaglandin (PGF2 α). Eazi-Breed CIDR[®] cattle devices each of 1.9 gram in each cow was used as a source of progesterone which was intra-vaginally inserted, Cloprochem 0.025% (Cloprostenol) as a prostaglandin analogue at the rate of 2 ml intramuscular injection and Gynarich (Busereline 4mcg/ml) as a source of GnRH at the rate of 2.5 ml intramuscular injection was used in protocol I: OvSynch+CIDR in 7 cows, protocol II: CoSynch+CIDR in 10 cows and protocol III: CIDR insert for 10 days + PGF2 α 8 cows as shown in the Figures 1, 2 and 3.

Fixed time artificial insemination was done in cows of all groups as per the protocols where protocol III received double fixed time artificial insemination. Estrus expression signs like mucus discharge, swollen vulva, pinkish vaginal mucus membrane and status of cervix (well open or partial open) at the time of artificial insemination was observed and recorded accordingly. Estrus expression response in the cows due to the effect of various protocols were divided into two sub-groups namely estrus signs with well open cervix and estrus signs with partial open cervix.

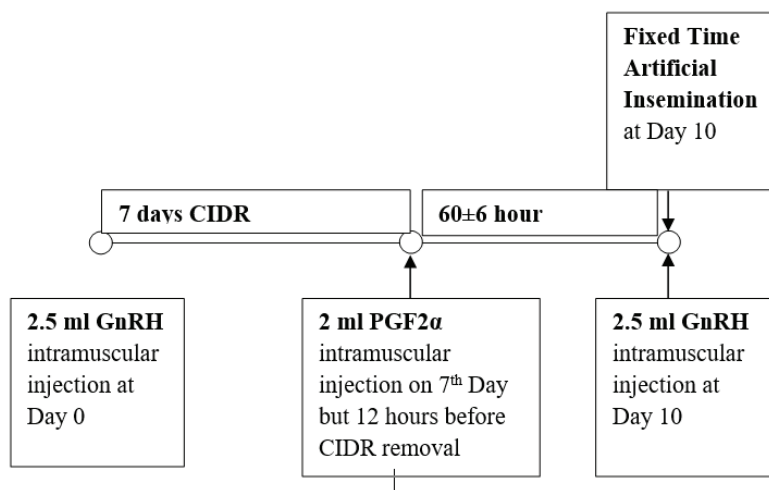


Figure 1. CoSynch + CIDR protocol

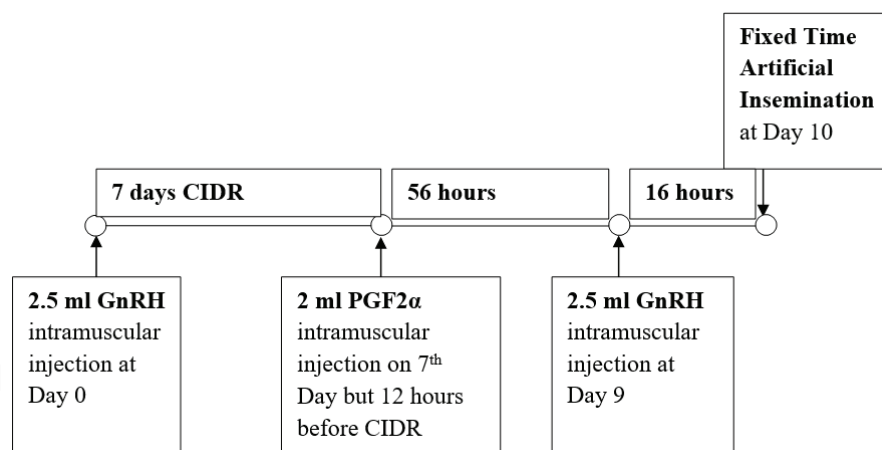


Figure 2. OvSynch + CIDR protocol

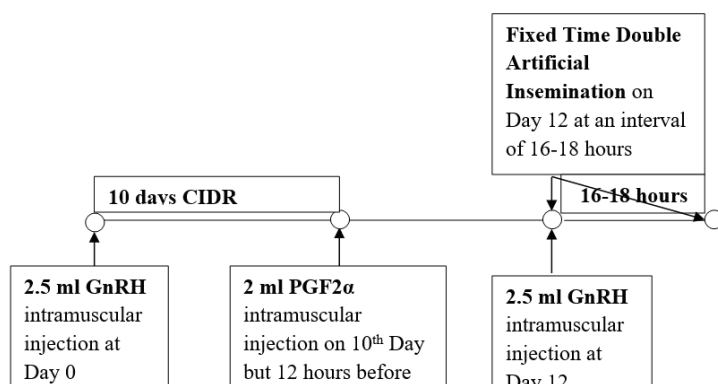


Figure 3. CIDR + PGF2α protocol

Pregnancy diagnosis was done after 60 days post artificial insemination and recorded as positive and negative results for all the cows under experiment. Data on the pregnancy outcome and estrus response outcomes were entered in the IBM SPSS version 20 and applied Fisher's exact test to find the association of pregnancy outcome and estrus response outcome with respect to various hormonal protocols at the confidence interval (CI) 95% and significance value $P = 0.05$.

RESULTS

Effect of Hormonal Protocols

100% estrus expression observed by the time of fixed time artificial insemination all protocols. However, there were only 80.0% (8/10), 85.71% (6/7) and 75.0% (6/8) estrus response with well open cervix and rest with estrus response with partial cervix open at the time of fixed time artificial insemination in CoSynch + CIDR, OvSynch + CIDR and CIDR + PGF 2α protocols respectively as shown in Figure 4. Estrus expression were statistically non-significant ($P=1.00$) among all the protocols applied.

CIDR based hormonal protocols effect upon estrus response in aneustrous crossbred cows

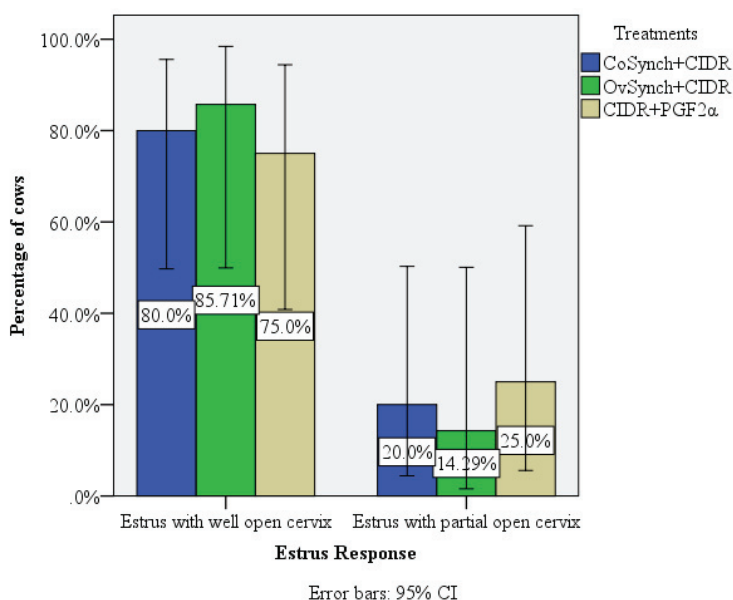


Figure 4. CIDR based hormonal protocols effect upon estrous response in aneustrous crossbred cows

Pregnancy Outcomes Following Fixed Time Artificial Insemination

Statistically non-significant ($P=0.50$) pregnancy outcome was found following the fixed time artificial insemination in all protocols with only 14.3 % (1/7) in OvSynch + CIDR protocol followed by 12.5 % (1/8) in CIDR + PGF 2α and with no pregnancy outcome in CoSynch + CIDR as shown in Table 1. The results of pregnancy outcomes were found to be non-significant ($P=0.50$) with respect to three protocols applied in the experiment.

Table 1: CIDR based hormonal protocols effect upon pregnancy outcome in crossbred anestrous cows

CIDR Based Hormonal Protocols	Pregnancy Outcome (%)
Protocol I: CoSynch + CIDR (N=10)	0
Protocol II: OvSynch +CIDR (N=7)	14.3 (1/7)
Protocol III: CIDR + PGF2 α (N=8)	12.5 (1/8)
P=0.50 (Non-significant)	

DISCUSSION

Result of 100 % estrus expression is found to be consistent with the results reported by Dhami, *et.al.*, 2015; V Kumaravel and S Sendur Kumaran, 2017 but in contrast to other reports like Chebel *et.al.*, 2006, Kalwar Q *et.al.*, 2015 where 30 and 76.47 % estrus expressions were reported respectively. After progesterone withdrawal, anestrous cows experience increased pulse frequency and mean concentrations of lutenizing hormone (LH), increased numbers of LH receptors in the granulosa and theca cells of follicles (Inskeep *et.al.*, 1988) there by increased estradiol production by the follicles, and an estradiol-stimulated LH surge and ovulation (Rhodes *et.al.*, 2003; Gumen and Wiltbank, 2005).

Poor pregnancy outcome in this experiment is not in agreement with most of the studies where at least 26 % pregnancy rate by CIDR + PGF2 α protocol was reported by Lucy *et.al.*, 2001 to higher conception rates like 52.94% in OvSynch + CIDR (Kalwar Q *et.al.*, 2015), 53.33 % by Kumaravel and Kumaran, 2017, and conception has been reported up to 80 % in CoSynch + CIDR by A J Dhami, *et.al.*, 2015.

High progesterone near at the time of fixed time artificial insemination also leads to decreased fertility (Wiltbank *et.al.*, 2012) as high progesterone produces incompetence oocyte whereby poor fertilization or poor embryo quality production and finally reduced pregnancy which could have happen with this experiment work. Hence, in such researches hormonal assay of progesterone, follicle stimulating hormone, estradiol and lutenizing hormone is also required to correlate and interpret the results in a more precise way. Besides, conception is governed by many factors like semen quality, maternal recognition pregnancy, and early embryonic deaths etc. which were beyond the objective of this research findings.

CONCLUSION

CIDR based protocols OvSynch + CIDR, CoSynch + CIDR and CIDR + PGF2 α are equally effective in estrus induction of anestrous dairy cows with poor pregnancy outcome where reason for poor conception rate need further research findings.

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Study on Calf Morbidity and Mortality on Farm Condition with Special Emphasis on Colibacillosis

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ABSTRACT

Diarrhoea in farm animals, especially in neonatal calves is one of the most challenging clinical syndromes encountered by practicing large animal's veterinary practitioners throughout the world. Therefore looking into the complexity of early age calf diarrhea primarily due to E. coli, the present study was conducted in different dairy farm in Sylhet Sadar in order to determine the major cause and risk factor associated with calf morbidity and mortality due to colibacillosis. The main result revealed that out of 121 calves different ages were selected where calf manifesting the characteristics clinical sign of colibacillosis. From thirteen dairy farms in Sylhet sadar out of 121 samples 75 were observed affected with colibacillosis. The overall morbidity of calf colibacillosis was 61.98% and mortality were 00.00%. Data analysis showed that the calf colibacillosis in dairy farms due to many factors such as age was the only risk factor (calf factor) which was highly significantly ($\chi^2 = 13.003$, P-value = 0.000) washing of udder just before sucking milk to calf was statistically highly significant ($\chi^2 = 7.373$, P-value = 0.007) which means washing of udder just before sucking milk to calf lowers the calf morbidity due to colibacillosis. Milk replacer sanitation was statistically significant ($\chi^2 = 6.5$, P-value = 0.011) water supply to the calf was statistically significant ($\chi^2 = 4.247$, P-value = 0.039) which means hygienic water supply to calf lowers the calf morbidity due to colibacillosis. Proper drainage system ($\chi^2 = 4.069$, P-value = 0.044) and daily use of disinfectant in farm premises ($\chi^2 = 6.163$, P-value = 0.013). The mortality of calf due to colibacillosis was null because of proper treatment, knowledge of management of diseased calf and availability of veterinarians. Morbidity and mortality of calf colibacillosis in different dairy farm at Sylhet sadarUpazila were 61.98% and 0.00% respectively.

INTRODUCTION

Calf morbidity and mortality is the major cause of economic losses in livestock production. It is roughly estimated that calf morbidity and mortality can reduce net profit to 38 per cent (Blood and Rhodostits 1989). Calf management plays an important role in the development of the dairy sector of the country. The success of the dairy industry depends on appropriate calf management. Calf-care is not only essential for sustenance of the dairy industry but is also essential for preserving and maintaining our good quality germ plasm. Important aspects in the calf rearing are the health management and proper nutrition (Mehmood, 1991). Calf morbidity and mortality are persistent problems in farm condition worldwide (Heinrichs and Radostits, 2001) occurring mainly due to Colibacillosis caused by entero-pathogenic *Escherichia coli*, a gram-negative, aerobic, rod-shaped, flagellated, motile, oxidase negative, non-spore forming, small sized (2-3 x 0.6 μ) and toxin producing bacteria (endotoxin), under the family Enterobacteriaceae (Buxton and Fraser, 1977) and characterized by watery white or yellowish diarrhoea, septicemia, rise of body

temperature, general weakness, dehydrated, lack of appetite, dry skin coat, sunken eyeballs and high mortality (Runnel *et.al.*, 1986). This organism (*E. coli*) is widely distributed in nature; being present in soil, surface water, animal and human faeces. So, calves are easily get exposure and infected with *E. coli* mostly at the age of 1-3 days and 3-8 weeks (Abraham *et.al.*, 1992). In Colibacillosis affected calves, the morbidity rate ranges from 30-75% and mortality rate varies from 10 to 50% depend on the level of clinical management (Radostits *et.al.* 2000).

The prevalence of morbidity and mortality in calves due to Colibacillosis are caused by several possible reasons which include improper housing system, non-cemented floor type, poor ventilation, contaminated feed, unclean and dirty water, poor biosecurity, large sized herd, method of colostrum feeding, house cleanness, amount of colostrum provision, the amount of milk fed daily, sex, age and parity of the dam (Kaper *et.al.*, 2004). Calf morbidity and mortality causes huge economic loss to the farmers arising from death, treatment cost, decreased lifetime productivity and survivorship. It also causes the loss of genetic material for herd improvement and decreases the number of dairy, beef and breeding calves available for herd replacement and expansion (Waltner-Toews *et.al.*, 1986).

The objectives of this study is to to determine the morbidity and mortality of calf due to Colibacillosis at Sylhet Sadar Upazila and to determine the risk factors associated with morbidity and mortality of calf due to Colibacillosis at Sylhet SadarUpazila.

MATERIALS AND METHODS

The research work was conducted in the Department of Medicine, Sylhet Agricultural University (SAU) Sylhet-3100 during the period of November 2014 to June 2015.

Collection of Samples: A total of 121 faecal samples were collected aseptically from the rectum of different calves in each farm after collection, the samples were transported to the laboratory of Microbiology and Immunology, faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet, as soon as possible for bacteriological examinations and further characterization.

Table 1. Summary of samples of different farms

S.No.	Name of the farms	No. of Sample Collected	Total
1.	Sylhet Dairy Farm	12	121
2.	BulBul Dairy Farm	10	
3.	Aftabul Dairy Farm	12	
4.	Mamun Mia Dairy Farm	5	
5.	AlhadMokleshwor Rahman Dairy Farm	18	
6.	Baby Dairy Farm	8	
7.	Home Fresh Dairy Farm	20	
8.	Rahman Dairy Farm	8	
9.	Dream Dairy Farm	4	
10.	Jalalabad Dairy Farm	6	
11.	Aaksha Dairy Farm	5	
12.	Taskiya Dairy Farm	10	
13.	Husna Dairy Farm	3	

Collection of Data: The data was collected from the farmer's record books and also from a survey using a well design questionnaire. Owners of the selected farms were interviewed face to face for recording farms and calf information regarding calf morbidity and mortality due to calf colibacillosis.

Examination of Faecal Sample: A fresh faecal sample were placed in nutrient broth and incubated for overnight at 37°C for the growth of the organisms. After primary culture of the organisms, a small amount of inoculum from nutrient agar was sub-cultured in the nutrient agar and MacConkey agar to observe the colony morphology. Characteristic colony morphology of the organisms indicating the features of *E. coli* was selected for subculture on EMB agar (Carter, 1986). Morphological characteristics shape, size, surface texture, edge and elevation, color, opacity etc. of the suspected colonies on different agar media developed within 18 to 24 hours of incubation were carefully studied and recorded.

Data Management and Analysis: Data related to the major causes and risk factors regarding calves morbidity and mortality were analyzed using International business machine Statistical package for social sciences (IBM SPSS) version 20.

RESULT AND DISCUSSION

This study was conducted in different dairy farms in Sylhet sadarupazila in order to determine the calf morbidity and mortality due to colibacillosis and risk factors associated with calf colibacillosis in dairy farm. This study recorded that the overall calf morbidity and mortality due to colibacillosis in different dairy farm at Sylhet sadarupazila were 61.98% and 0.00% respectively (Table 2). But the calf morbidity due to colibacillosis was 66.67% in Sylhet Dairy Farm, 80% in BulBul Dairy Farm, 75% in Aftabul Dairy Farm, 60% in Mamun Mia Dairy Farm, 66.67% in AlhadMokleshwor Rahman Dairy Farm, 62.5% in Baby Dairy Farm, 75% in Home Fresh Dairy Farm, 25 % in Rahman Dairy Farm, 25% in Dream Dairy Farm, 50% in Jalalabad Dairy Farm, 60 % in Aaksha Dairy Farm, 50% in Taskiya Dairy Farm, 33.33% in Husna Dairy Farm. Among them, calf morbidity due to colibacillosis was highest 80% in BulBul Dairy Farm and lowest 25% in Rahman Dairy Farm and Dream Dairy Farm. In contrast to the findings of this study, (Hossain *et.al.*, 2013) recorded that morbidity of calf colibacillosis was 53.33% in Sirajganj and 40% in Mymensingh. (Navade *et.al.*, 2000) noticed morbidity of calf colibacillosis was 53.37% in Mathura and (Gupta *et.al.*, 2006) observed morbidity of calf colibacillosis was only 23.72% in Mathura.

But there was not any calf mortality percentage due to colibacillosis in any farm in Sylhet sadarupazila because of proper treatment, knowledge of management of diseased calf, proper calving supervision and management of newborn calves.

Table 2. Overall calf morbidity and mortality due to colibacillosis in calf in different dairy farm at Sylhet Sadar Upazila

S.No.	Name of the farms	No. of Sample Collected	Positive	Morbidity %	Mortality%
1.	Sylhet Dairy Farm	12	8	66.67	0
2.	BulBul Dairy Farm	10	8	80	0
3.	Aftabul Dairy Farm	12	9	75	0
4.	Mamun Mia Dairy Farm	5	3	60	0
5.	AlhadMokleshworRahman Dairy Farm	18	12	66.67	0
6.	Baby Dairy Farm	8	5	62.5	0
7.	Home Fresh Dairy Farm	20	15	75	0
8.	Rahman Dairy Farm	8	2	25	0
9.	Dream Dairy Farm	4	1	25	0
10.	Jalalabad Dairy Farm	6	3	50	0
11.	Aaksha Dairy Farm	5	3	60	0
12.	Taskiya Dairy Farm	10	5	50	0
13.	Husna Dairy Farm	3	1	33.33	0
	Total	121	75	61.98	0

A range of possible risk factors were tested for their association with calf morbidity and mortality due to colibacillosis in dairy farms. Age was the important risk factor found to affect calf morbidity and mortality due to colibacillosis in dairy farms. Age was the only risk factor (calf factor) which was highly significantly ($\chi^2 = 13.003$, P- value = 0.000) associated with risk of morbidity due to colibacillosis in all calves which agreed with earlier study. Another calf risk factors affecting calf morbidity and mortality due to colibacillosis in dairy farms was sex which was statistically insignificant ($\chi^2 = 0.287$, P- value = 0.592). This means calf colibacillosis can occurs in any sex either male or female calf (Table 3).

Table 3. Potential risk factors associated with morbidity due to calf colibacillosis

S.no.	Variables	χ^2	P-value
	Calf factors	13.003	0.000*
	Age		
	Sex	0.287	0.592
	Managemental factors		
	Ventilation	0.624	0.429
	Washing of udder	7.373	0.007*
	Milk replacer sanitation	6.5	0.011*
	Daily use of disinfectant in farm premises	6.163	0.013*
	Water supply	4.247	0.039*
	Proper drainage system	4.069	0.044*
	Disinfectant used in footbath daily	2.654	0.103
	Floor type	2.654	0.103
	Calf kept together	2.340	0.126
	Milk feeding just after milking	1.491	0.222
	Barn clean properly	1.387	0.239
	Footbath	0.872	0.350
	Sanitary condition of calving site	1.516	0.469
	Weaning of calves	0.010	0.921
	Instrument properly cleaned	0.005	0.941

Note: *Significance level at 0.05

Among Managemental factors, washing of udder just before sucking milk to calf was statistically highly significant ($\chi^2 = 7.373$, P- value = 0.007) which means washing of udder just before sucking milk to calf lowers the calf morbidity due to colibacillosis. milk replacer sanitation was statistically significant ($\chi^2 = 6.5$, P- value = 0.011) which means good sanitation of milk replacer in weaned calf lowers the calf morbidity due to colibacillosis. water supply to the calf was statistically significant ($\chi^2 = 4.247$, P- value = 0.039) which means hygienic water Supply to calf lowers the calf morbidity due to colibacillosis. Proper drainage System ($\chi^2 = 4.069$, P- value = 0.044) and daily use of disinfectant in Farm Premises ($\chi^2 = 6.163$, P- value = 0.013) were statistically significant which were significantly associated with calf morbidity due to colibacillosis. These risk factors are closely associated with calf morbidity due to colibacillosis which agreed with (Curtis *et.al.*, 1988) who found that cleanliness of the calving area, calf housing, and feeding equipment and sanitation of milk replacer reduced the risk of neonatal colibacillosis in the calf.

Other managemental factors including ventilation ($\chi^2 = 0.624$, P- value = 0.429),disinfectant used In footbath daily ($\chi^2 = 2.654$, P- value = 0.103),Floor type ($\chi^2 = 2.654$, P- value = 0.103),calf kept together ($\chi^2 = 2.340$, P- value = 0.126), milk feeding just after milking ($\chi^2 = 1.491$, P- value =0.222),barn clean properly ($\chi^2 = 1.387$, P- value = 0.239),footbath ($\chi^2 = 0.872$, P- value = 0.350), sanitary condition of calving site ($\chi^2 = 1.516$, P- value = 0.469), weaning of calves ($\chi^2 = 0.010$, P- value =0.921), instrument properly cleaned ($\chi^2 = 0.005$, P- value = 0.941) were

insignificantly associated with calf morbidity due to colibacillosis. These risk factors are not associated with calf morbidity due to colibacillosis.

CONCLUSION

In this study, the morbidity of calf colibacillosis was higher in Sylhet sadarupazila than other region of the Bangladesh which causes huge loss to farm due to increase of treatment and medicine cost but the mortality of calf due to colibacillosis was Null because of proper treatment, knowledge of management of diseased calf and availability of veterinarians. Morbidity and mortality of calf colibacillosis in different dairy farm at Sylhet sadarupazila were 61.98% and 0.00% respectively. Morbidity of calf colibacillosis was highest 80% in BulBul Dairy Farm and lowest 25% in both Rahman Dairy Farm and Dream Dairy Farm. Management of calf and farm plays an important role in morbidity of calf colibacillosis. There is poor management of calf and farm in BulBul Dairy Farm but good Management of calf and farm in both Rahman Dairy Farm and Dream Dairy Farm. As a result, there is high morbidity of calf colibacillosis in BulBul Dairy Farm and low in both Rahman Dairy Farm and Dream Dairy Farm.

From this study, it has been found that there were some risk factors such as age, washing of udder just before sucking milk to calf, milk Replacer Sanitation, water supply, drainage System and daily use of disinfectant in Farm Premises significantly associated with morbidity of calf colibacillosis. Therefore, implementation of improved calf management practices is greatly suggested to reduce the high level of morbidity of calf colibacillosis in the study herds and also in other areas with similar management system which will reduce the loss of farms and will get healthy calf for replacement stock.

RECOMMENDATION

Efforts should be made to increase calving supervision, improve management of newborn calves, and prevent diseases. In addition, we recommend implementation of calves' vaccination programs to protect calf diarrhea caused by viruses and activating health laws aimed at reducing the spread of diseases through proper disposal of dead calves and employing of veterinarians at dairy farms. Likewise, extension services among dairy farms owners and labors are urgently needed on proper dairy farm practices such as record keeping, sanitary practices and cleaning programs.

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Prevalence of Helminths in Wild Asian Elephant and Indian Rhinoceros in Chitwan and Bardia National Park, Nepal

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ABSTRACT

The Nepalese elephant (Elephas maximus maximus) and rhinoceros (Rhinoceros unicornis), are an important part of Nepal's heritage, culture and wildlife conservation. Despite its importance, not much is known about the helminth parasites that affect elephant and rhinoceros. This study investigates the prevalence of helminth parasites in wild Asian Elephant and wild Indian Rhinoceros. A study was conducted from November 2011 to April 2012 to screen helminth parasites of wild Asian elephant and wild Indian Rhinoceros at Chitwan and Bardia National Park of Nepal. Total of 80 samples, 40 each taken from the Wild Asian Elephants and Indian Rhinoceros. The study showed 88.75% overall prevalence of helminthes. The prevalence in Wild Asian Elephant and Indian Rhinoceros was 95% and 82.5% respectively. 97.37% and 72.73% infection were mixed infections in elephant and rhinoceros respectively. Among 38 positive samples of the elephant of CNP and BNP, 9 different types of helminth eggs were found. 15 (39.47%) were positive for Fasciola Spp. With 450 EPG count, 11 (28.95%) for Paramphistomum spp. with 600 EPG, 27 (71.05%) for Schistosoma spp. with 500 EPG, 3 (7.89%) for Dicrocoelium spp. with 900 EPG, 12 (30.16%) for Moniezia spp. with 433.3 EPG, 17 (44.74%) for Oesophagostomum spp. with 1025 EPG, 10 (26.31%) for Chabartia spp. with 1141.65 EPG, 17 (44.74%) for Strongyloides spp. with 15558.335 EPG and 23 (60.53%) for Strongylus spp. with 1700 EPG. Similarly out of the 33 positive samples of Rhinoceros of CNP and BNP, 8 different types of helminth eggs were found. 11 (33.33%) were positive for Fasciola Spp. With 558.34 EPG, 10 (30.30%) for Paramphistomum spp. with 525 EPG, 7 (21.21%) for Schistosoma spp. with 475 EPG, 4 (12.12%) for Moniezia spp. with 650 EPG, 6 (18.18%) for Strongyloides spp. with 1466.67 EPG, 4 (12.12%) for Strongylus spp. with 1625 EPG, 9 (27.27%) for Toxocara spp. with 699.98 EPG and 20 (60.61%) for Trychostrongylus spp. with 1149.98 were found to be positive.

Keywords: Wild Asian Elephant, Wild Indian Rhinoceros, Helminths, Qualitative and Quantitative Examination

INTRODUCTION

Nepal is remarkable for an abundance and variety of wildlife which have been famous throughout the world for their uniqueness, gameness, strength, agility and beauty. The mammals of Nepal have intrinsic and biological value. The study of ecology, behavior and health of mammals in nature and captivity can be a great tool in their preservation. Many pristine forms are now verging towards extinction (Shrestha, 1997). The need of wildlife habitat improvement,

health improvement, preservation of biological diversity, protection of natural environment and monitoring pollution are the fundamental of wildlife conservation.

The Asian Elephant is an endangered species. Although there are few accurate data on historical population size, from what is known about trend of habitat loss/degradation and other threats including poaching, disease condition, an overall population decline of at least of at least 50% over the three generation time (estimated to be 20-25 years) seems realist. The western populations (*Elephas maximus asurus*) were probably extinct by 100BC and the main Chinese population disappeared sometime after 14th century B.C. (IUCN, 1986).

With a few notable exceptions, population of Asian elephant and rhino over the world have experienced major declines over the past few decades as a result of habitat loss, fragmentation and poaching. Large animal like elephant and rhino and elephants are wide ranging and require extensive areas to support viable population. It is possible that rhino and elephant populations in several of Asia's relatively small protected areas have reached caring capacity and the areas have inadequate ecological resources to support larger populations (WWF, 2011).

In Nepal, Elephants were once widespread in the low land terai, but now restricted too, few protected areas along the border with India: CNP, Parsa Wildlife Reserve, BNP and Suklaphanta Wildlife Reserve and their environs. Greater one horned rhinoceros is also one of the IUCN red listed threatened species (Talukdar, 2008). The populations are increasing overall due to strict protection especially in India. However some populations are decreasing, especially in Nepal and parts of northeastern India. Currently, the Indian rhinoceros exist in a few small sub-populations in West Bangal, Uttar Pradesh and Aasam of India and in three protected areas of Nepal. In late 1960 an estimated 65 Indian rhinoceros survived in Nepal, but due to increased conservation efforts, the total population was up to 612 in 2000. A total of at least 91 animals were poached in 2000-2003 and since 2000, numbers have decline. In CNP, the number of individuals has declined 544 individuals in 2000 to 372 individuals in 2005, the decreased being due to increased pouching following political instability in Nepal and habitat changes. In BNP reintroduced rhinos were approximately 40 individuals in 1997 and 35 animals in 2007 while in suklaphanta Wildlife Reserve the re-introduced population is only six individuals (Martin, 2006 cited by Sapkota, 2009). Recent study found population of the rhino 527 (99 more than it was counted in 2008) at CNP and BNP (DNPWC 2011).

Elephant and Rhino are possibly the most well known member of the animal kingdom. The enormous size, unusual anatomy and longevity of elephants have fascinated humans for millennia. Elephant are mainly raised in wildlife reserves for security purpose and patrolling. Most free living organism harbors parasites of several species which can adversely affect host health, fecundity and foraging and may also modify host behavior to fascinate parasites transmission (Hamilton *et.al.*, 1996). Asian Elephant *Elephas maximus* are susceptible to gastrointestinal parasitic infection in wild and in captivity are often confined to small enclosure and/or maintained in isolation (Vanitha *et.al.*, 2011)

MATERIALS AND METHODS

Site of Study

The study areas were CNP and BNP. CNP is the first National Park at the terai (Siwalik) of mid Nepal in the south-east part of the Chitwan District, Narayani Zone. It covers an area of 932 sq. km. The park covers the part of Parsa, Makawanpur, Nawalparasi and Chitwan districts, encloses Rapti Valley, Churea hill, Ox-bow Lake, rivers like Narayani, Rapti, Reu and Sal dominated forest, grassland with the flood plain. BNP located at the Bardia district of Midwestern Nepal in the bank of Karnali River and covers an area of 969 sq. km and established at 1975. It is low land park at Terai (Siwalik) region. Population of elephant and rhinoceros in both National Parks are 527 (DNPWC 2011) and 278 (ECAPN-2008).

Sampling of Animal

Opportunistic fresh fecal samples were taken. The samples were collected on the plastic bags. Ice box and 3% formalin are used for the transportation of the samples from national Park to the Laboratory. The examined samples of CNP were collected from November 1st 2011 to January 15th 2012. Similarly examined sample of BNP were collected from January 16th to March 14th 2012. Numbers of sample taken from National Parks are shown in table 1. Due to the risk of the wild animal during sample collection, captive elephant was used for travelling within the National Park.

Table 1: Numbers of sample from the different sites

Wild Animal	CNP	BNP	Total
Elephant	20	20	40
Rhinoceros	20	20	40
Total	40	40	80

Identification of the Sample

The detail qualitative coprological parasitic investigation was assessed through direct microscopic examination and sedimentation floatation method as per Soulsby (1978). Egg and larvae identifications were made as per the atlas of Soulsby (1978). Qualitative examination was done by direct smear method, Sedimentation method and differential floatation Method.

Quantitative investigation was done by egg per gram count (EPG) to know the number of parasite eggs in the animal by using McMaster counting.

RESULT AND DISCUSSION

Result

The study showed the overall prevalence of helminthes to be 88.75%. The prevalence in wild Asian Elephant and Indian Rhinoceros was 95% and 82.5% respectively. 97.37 and 72.73% infection were mixed infections in elephant and rhinoceros respectively. Location wise prevalence in elephants of CNP and Elephant of BNP, Rhinoceros of CNP and rhinoceros of BNP were 95%, 95%, 75% and 90% respectively. The detail of helminthes in elephant and rhinoceros are illustrated in table 2.

Table 2. Overall Prevalence of helminthes parasites

	CNP		BNP		Total
	Elephant	Rhinoceros	Elephant	Rhinoceros	
Total	20	20	20	20	80
Positive	19 (95%)	15 (75%)	19 (95%)	18 (90%)	71 (88.75%)

Species of Parasites Identified in Elephant and Rhinoceros of CNP and BNP

Species of Helminthes in Elephant

Among 40 fecal samples of the elephants of CNP and BNP, 38 (95%) sample were positive and 9 different types of helminthes egg were identified. Among 38 positive samples of the elephant of CNP and BNP, 9 different types of helminth eggs were found. 15 (39.47%) were positive for *Fasciola* Spp. With 450 EPG count, 11 (28.95%) for *Paramphistomum* spp. with 600 EPG, 27 (71.05%) for *Schistosoma* spp. with 500 EPG, 3 (7.89%) for *Dicrocoelium* spp. with 900 EPG, 12 (30.16%) for *Moniezia* spp. with 433.3 EPG, 17 (44.74%) for *Oesophagostomum* spp. with 1025 EPG, 10 (26.31%) for *Chabartia* spp. with 1141.65 EPG, 17 (44.74) for *Strongyloides* spp. with 15558.33 EPG and 23 (60.53%) for *Strongylus* spp. with 1700 EPG. The detail of parasites and their prevalence are illustrated in Figure 1. *Dicroclium* spp. was not found in BNP elephant.

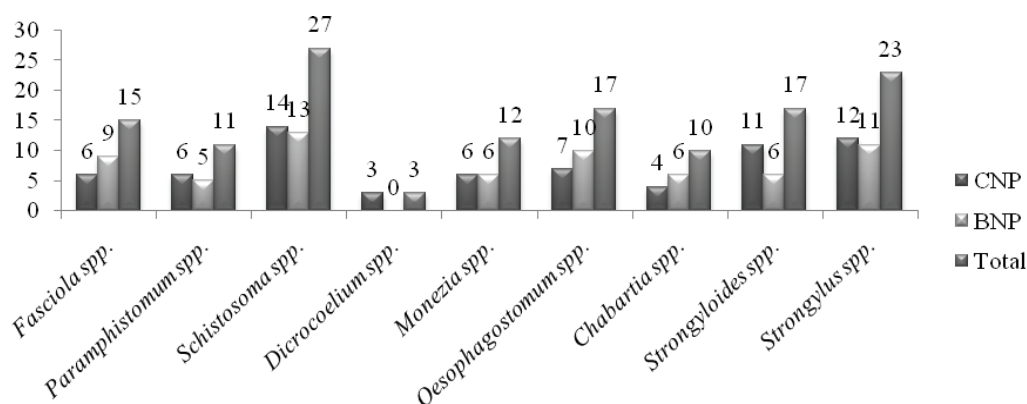


Figure 1: Number of samples positive for helminth in elephant with percentage out of total positive samples

Species of Helminthes in Rhinoceros

sample of the CNP. The detail of parasites and their prevalence are illustrated in figure 2. Out of 40 fecal samples of the rhinoceros of CNP and BNP, 33 (82.5%) were positive and 8 EPG, 6 (18.18%) for *Strongyloides* spp. with 1466.67 EPG, 4 (12.12%) for *Strongylus* spp. different types of helminth eggs were found. 11 (33.33%) were positive for *Fasciola* Spp. With 558.34 EPG, 10 (30.30%) for *Paramphistomum* spp. with 525 EPG, 7 (21.21%) for *Schistosoma* spp. with 475 EPG, 4 (12.12%) for *Moniezia* spp. with 650 with 1625 EPG, 9 (27.27%) for *Toxocara* spp. with 699.98 EPG and 20 (60.61%) for *Trychostrongylus* spp. with 1149.98 were found to be positive. But *Monezi* spp. and *Strogylus* spp. were not found in rhino sample of BNP. Similarly *Strongyloides* spp was not found in

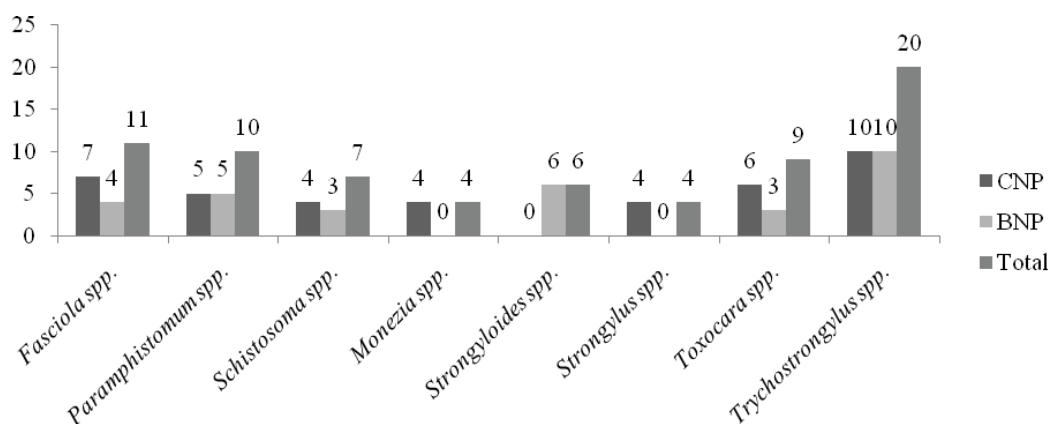


Figure 2: Number of samples positive for helminth in Rhinoceros with percentage out of total positive samples

Prevalence Based on the Spectrum of Helminth Infections

Wild Asian Elephant

Out of the 38 positive fecal samples of the elephant, 37 (97.37%) revealed mixed parasitic infection and 1 sample (2.63%) had single infection (figure 3)

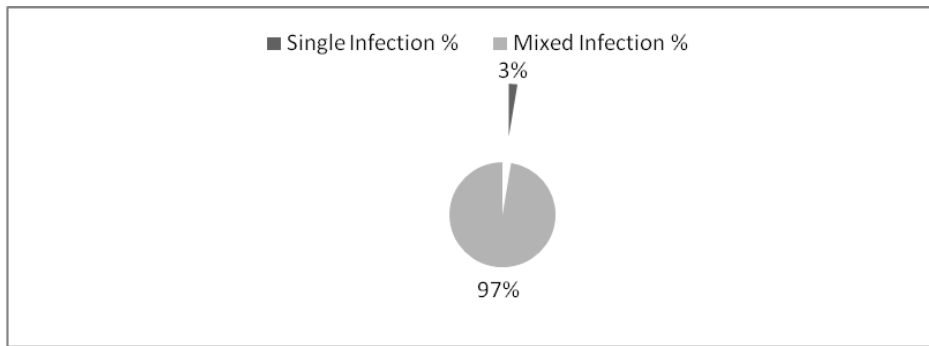


Figure 3: Prevalence based on the spectrum of helminth infection in elephant

Indian Rhinoceros

Out of the 33 positive fecal samples of the elephant, 24 (27.27%) revealed mixed parasitic infection and 9 samples (72.73%) had single infection. The photos of parasite eggs are given in Annex X.

Prevalence Based on Classes of Helminth Parasites

Wild Asian Elephant

Out of the 38 positive fecal samples from elephants of the both national parks, 34 (41.46%) sample have trematodes infection, 36 (43.90%) samples had nematode infection, 12 (14.63%) samples had cestodes infection.

Indian Rhinoceros

Out of the 33 positive fecal samples from elephants of the both national parks, 23 (44.23%) sample have Trematodes infection, 25 (48.08%) samples had nematode infection, 4 (7.69%) samples had cestodes infection.

Egg per gram (EPG) Count

1. Wild Asian Elephant

Out of the 40 fecal samples of the elephant from both National Parks, 20 (11 from CNP and 9 from BNP) samples were analyzed randomly for EPG. The results obtained are presented on figure 4.

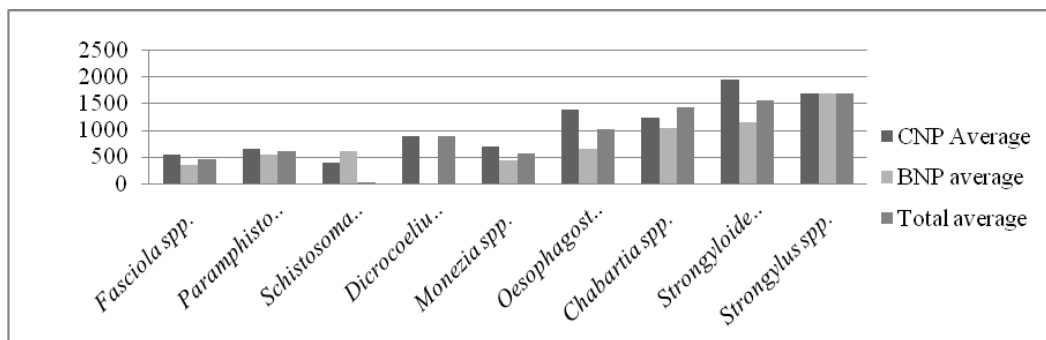


Figure 4: EPG count of different helminth parasites of wild Asian Elephant of CNP and BNP

Wild Indian Rhinoceros

Out of the 40 fecal samples of the rhinoceros from both National Parks, 19 (10 from CNP and 9 from BNP) samples were analyzed randomly for EPG. The results obtained are presented on figure 5.

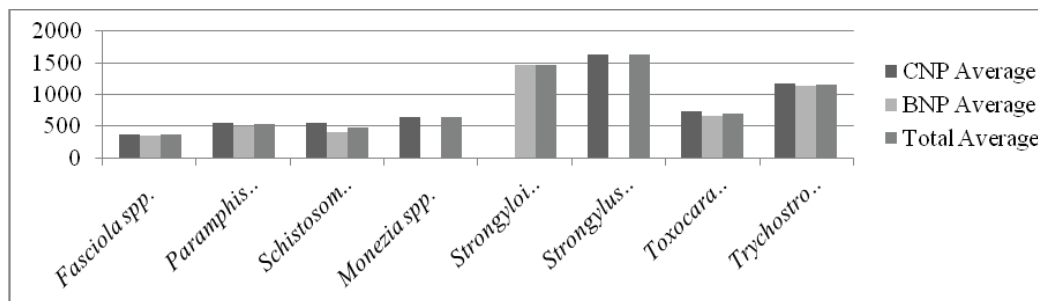


Figure 5: EPG count of different helminth parasites of wild Indian Rhinoceros of CNP and BNP

DISCUSSION

Karki and Manandhar (2007) reported 100% prevalence of the internal parasites in captive elephant of the National Park and Wildlife reserve but this study in wild elephants showed lower prevalence of internal parasites than their study. It may be due to the lower sample size and seasonal variation. They also reported that in Captive elephant of Nepal 30% *Fasciola jacksoni*, 10% *Paramphistomum*, 75% *Oesophagostomum*, 90% *Schistosomoses*, 18% *Chabertia*, 5% *Dicrocoelium*, 5% *Moniezia* species prevalence. The infection of *Oesophagostomum*, *Schistosomoses* and *Chabertia* species were found high but infection of *Fasciola*, *Paramphistomum*, *Dicrocoelium* and *Moniezia* species were found less than this study. Also parasites like *Strongyloides* and *Strongylus* species were not reported (Karki and Manandhar 2007) in captive elephant. That might be due to management practice of elephant and other. Location wise samples showed that samples from CNP were for *Fasciola*, *Paramphistomum* and *Oesophagostomum* spp. (Karki and Manandhar 2007). But in our finding 6 more parasites are reported in CNP elephants, they are *Schistosoma*, *Dicrocoelium*, *Moniezia*, *Chabertia*,

Strongyloides and *Strongylus* species. That may be due to the wild state of elephant and great sample size.

In February 94 proglottides of *Anoplocephala gigantean* were reported in the feces of rhino of Nepal (Rietschel, 2000) but this species was not observed in this study. Li. *et.al.*, (1993) recovered *Choniangiumepistomum*, *Murshidiafalcifera*, *M. murshida*, *Chabartia* spp. from *Elephas maximus* in China. Except *Chabartia* spp. other parasites are not reported in this study that might be due to geographic and climatic variation, type of methodology adopted etc.

DNPWC, Annual Report 2011 shows the death of 18 rhino in a period of 10 month (2067-05-02 to 2068-3-30) in Nepal, out of which 14 (77.78%) death were natural. Thus, this death may indicate that wild rhinoceros are also dying from disease condition, which might be due to the parasitic Load. Therefore extensive research of the health problem including helminth load should be carried out along with the treatment protocol of wildlife.

Dung samples collected from 44 and 55 elephants during 2000 and 2002 respectively and examined for the presence of helminths ova. Of the 99 elephants, 17 (17.17%) were positive for helminthes. The incidence of helminth infection is decreased from 22.73% in 2000 to 12.73% in 2002. Among the positive dung samples, 10 (10.10%) had *Strongylidae* and 7 (7.07%) had *Digeneaspp.* (Saseendran, *et.al.*, 2004). This study shows the lower prevalence of helminth than our finding. That might be due to the low incidence of helminth infection among captive elephants in the studied area may be captive elephant.

Qufiloniatravancra and *Bathmostomum sangeri* were reported for the first time in Malaysia (Cheah *et.al.*, 1993) but these species are not reported in Nepal. This may be due to the lack of research seasonally and periodically. Chakraborty and Ishlam, 1993 recorded *Kilulumagoodeyi*, *Charbertia* spp., *Necator ammericans*, *Bonostomum* spp., *Paramphistomum* spp., *Anoplocephala* spp. and hydatid cyst. Some of these parasites are not found in our study.

Of the 84 fresh rhino fecal samples, avoided at different dung heap by individual rhinoceros 61.90% was positive and different parasites reported were of genera *Paramphistomum* (39), *Strongylus* (17) and *Anoplocephala* (2) (Chakraborty and Islam, 1993). This report is lower than our finding that might be due to ecology, climate or season etc.

The result showed that the *Fasciolidae* were present in 25% of Rhinoceroses, *Oxyuris* spp. were present in 25% of rhinoceros, the *Paramphistomum* were present in 64.86% of elephants and the *Acaridae* were present in 2.7% of elephants (Muryani *et.al.*, 2008). *Fasciolidae* infection is similar to our study but prevalence of the *Paramphistomum* in elephants higher than the study, which may be due to the climatic variation, host-species difference etc. Kashid *et.al.*, 2003 studied that fecal of various species of wild and zoo animal for the presence of gastrointestinal helminthes and concluded that there are many helminth parasites that have common host or might have public significance.

There are so many parasites that are not reported in Indian Rhinoceros of Nepal but reported once from other species of rhinos are: from the stomach of the black Rhinoceros, from the Umfolozi Game Reserve, South Africa (Gibbons *et.al.*, 1996), *Kilulumagoodeyi*, *Charbatia* spp., *Necator ammericans*, *Bonostomum* spp., *Paramphistomum* spp., and *Anoplocephala* Spp., (Chakarborty and Ishlam 1995). Nematode Specimens collected from feces of the Indian one horn Rhinoceros (*Rhinoceros unicornis*) in the Alipori Zoological Garden, Calcutta, India are diagnosed as a new species under the genus *Probstmayria* and named as *Probstmayria bangaliensis*. These all Parasites not reported in Nepal's Indian rhino but further study and larvae culture for the nematode identification is needed.

CONCLUSION

Parasitic infection decreases the production and productivity in the animals mainly in the reduction of the body weight or failure to gain weight or even increase the mortality in acute cases. Being the both Asian elephant and Indian rhinoceros IUCN red listed wildlife, conservation of those species should be our national objective. Poaching and habitat loss and climate changes are the main causes of population decline but emphasis should also be given to wildlife health aspect which may contribute to save the species by intervention on disease ecology.

A coprological study was conducted to determine the prevalence of helminth parasites in the wild Asian elephant and Indian rhinoceros of CNP and BNP, Nepal. The study showed the overall prevalence of helminth to be 88.75%. The prevalence in Asian elephant and Indian rhinoceros was 95% and 82.5% respectively. The mixed infection found in elephant and rhinoceros were 97.37% and 72.73% was to be found among the 38 and 33 out of the 40 samples respectively. Thus we can conclude that there is a high load of the helminth parasites in wild elephant and rhinoceros, which also may be the causes of death. So, development of treatment protocols for wildlife is essential for wildlife conservation.

RECOMMENDATION

This study revealed many parasites of zoonotic importance from wild animals which indicates human and animal populations of the buffer zone area is in the risk of parasitic infestation. We recommend to further conduct extensive research year-round to monitor the prevalence of helminths to explore epidemiology and prevalence of other infectious transmissible diseases in livestock and wildlife. For the wildlife health problem, specific policy should be formulated to cover overall area of the wildlife including veterinary sectors. Research on anthelmintic property of herbs and shrubs should be prioritized. Also, we suggest performing regular deworming programme of livestock in buffer zone and captive elephant.

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Annexes

Annex I: Population of the Asian elephant and rhinoceros in CNP and BNP

	Rhinoceros	Elephant	
		Captive	Wild (overall)
BNP	24	21	107-145
CNP	503	112	
SOURCE	DNPWC annual report 2011	ECAPN-2009	Yonzon- 2008

Annex II: Number of samples from the different sites

Species	Chitwan National Park	Bardia National Park	Total
Elephant	20	20	40
Rhinoceros	20	20	40
Total	40	40	80

Annex III: Identification of feces of rhinoceros and elephant

S.N.	Identification tool	Rhinoceros	Elephant
1	Color	Generally black and dark green	Dark grey and yellowish
2	Site of defecation	Defecate in the same place by number of individual	Defecate on the walking way
3	Consistency	Less fibrous than elephant's feces	More fibrous
4	Fibers in feces	Thin and short	Thick and long
5	Foot print	Observe nearby feces	Large
6	Age difference	Different size of the stool mass in a place	Different size of the stool mass in different place
7	Time of defecation	Feces become moist and appear fresh within 24 hours	Feces become moist and appear fresh within 24 hours

Annex IV: Number of sample positive for helminth in wild Asian elephant with percentage out of total positive samples

S.N.	Helminths parasites	CNP	BNP	Total	%
1	<i>Fasciola</i>	6	9	15	39.47
2	<i>Paramphistomum</i>	6	5	11	28.95
3	<i>Schistosoma</i>	14	13	27	71.05
4	<i>Dicrocoelium</i>	3	0	3	7.89
5	<i>Moniezia</i>	6	6	12	30.16
6	<i>Oesophagostomum</i>	7	10	17	44.74
7	<i>Chabertia</i>	4	6	10	26.31
8	<i>Strongyloides</i>	11	6	17	44.74
9	<i>Strongylus</i>	12	11	23	60.53

Annex V: Number of sample positive for helminth in Indian rhinoceros with percentage out of total positive samples

S.N.	Helminths parasites	CNP	BNP	Total	%
1	<i>Fasciola</i> spp.	7	4	11	33.33
2	<i>Paramphistomum</i> spp.	5	5	10	30.30
3	<i>Schistosoma</i> spp.	4	3	7	21.21
4	<i>Moniezia</i> spp.	4	0	4	12.12
5	<i>Strongyloides</i> spp.	0	6	6	18.18
6	<i>Strongylus</i> spp.	4	0	4	12.12
7	<i>Toxocara</i> spp.	6	3	9	27.27
8	<i>Trychostrongylus</i> spp.	10	10	20	60.61

Annex VI: Single and mixed infection in wild Asian elephant and Indian rhino

Type of infection	Wild Asian elephant				Wild Indian rhinoceros			
	CNP	BNP	Total	%	CNP	BNP	Total	%
Single	1	0	1	2.63	2	7	9	27.27
Mixed	18	19	37	97.37	13	11	24	72.73

Annex VII: EPG count of different helminths parasites of wild Asian elephant of CNP and BNP

S.N.	Helminth Species	EPG average		
		CNP average	BNP average	Total average
1	<i>Fasciola</i> spp.	550.00	350.00	450.00
2	<i>Paramphistomum</i> spp.	650.00	550.00	600.00
3	<i>Schistosoma</i> spp.	400.00	600.00	500.00
4	<i>Dicrocoelium</i> spp.	900.00	-	900.00
5	<i>Moniezia</i> spp.	700.00	433.30	566.65
6	<i>Oesophagostomum</i> spp.	1400.00	650.00	1025.00
7	<i>Chabertia</i> spp.	1233.30	1050.00	1141.65
8	<i>Strongyloides</i> spp.	1966.67	1150.00	1558.34
9	<i>Strongylus</i> spp.	1700.00	1700.00	1700.00

Annex VIII: EPG count for different helminths parasites of wild Indian rhinoceros of CNP and BNP

S.N.	Helminth species	EPG Average		
		CNP average	BNP average	Total average
1	<i>Fasciola</i> spp.	366.67	350.00	358.34
2	<i>Paramphistomum</i> spp.	550.00	500.00	525.00
3	<i>Schistosoma</i> spp.	550.00	400.00	475.00
4	<i>Moniezia</i> spp.	650.00	-	650.00
5	<i>Strongyloides</i> spp.	-	1466.67	1466.67
6	<i>Strongylus</i> spp.	1625.00	-	1625.00
7	<i>Toxocara</i> spp.	733.33	666.67	700.00
8	<i>Trychostrongylus</i> spp.	1166.67	1133.33	1150.00

Annex IX: Prevalence based on the types of class of helminth in Wild Asian elephant and Indian rhinoceros

	Wild Asian elephant				Wild Indian rhinoceros			
	CNP	BNP	Total	%	CNP	BNP	Total	%
Trematodes	16	18	34	41.46	12	11	23	44.23
Nematodes	17	19	36	43.90	12	13	25	48.08
Cestodes	6	6	12	14.63	4	0	4	7.69
Total	39	43	82	100	28	24	52	100

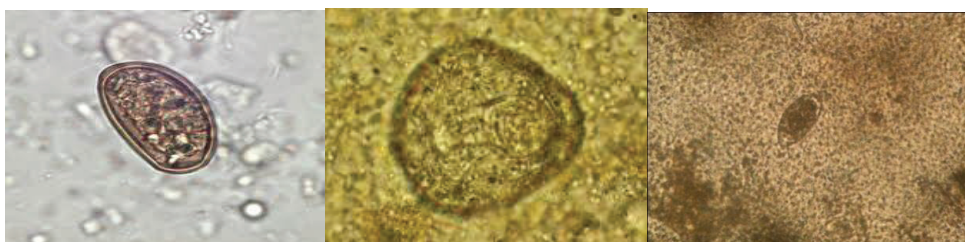
Annex X: Photos of the different eggs of helminth



1: *Fasciola* spp.

2: *Paramphistomum* spp.

3: *Schistosoma* spp.



4: *Dicrocoelium* spp.

5: *Moniezia* spp.

6: *Chabertia* spp.



7: *Oesophagostomum* spp.

8: *Strongyloides* spp.

9: *Strongylus*

Welfare Status and Type of Floor Associated with Hock Lesions in Dairy Cattle of University, Research and Private Farm of Chitwan District.

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ABSTRACT

This research is an investigation of the prevalence of, and factors associated with, hock lesions on dairy cattle of University, Research and Private farm of Chitwan district. Cemented floor (n = 141), Muddy floor (27), Floor with Mat (35) and brick paved floored animals (n = 33) were visited. Cows were scored for hock (tarsus) lesions on a 3 point scale combining the attributes of hair loss, broken skin, and swelling. Type of farm (University, Research and Private) and type of floor measures were taken which were hypothesized to be risk factors for lesions. On university (AFU), research (NARC) and private farms the mean level prevalence of hock lesions was 1.827 ± 0.384 , 1.4 ± 0.4905 and 1.465 ± 0.68 respectively. On cemented floor, muddy floor, floor with rubber mat and brick paved floor, the mean prevalence of hock lesions was 1.63 ± 0.489 , 1.0165 ± 0.577 , 1.343 ± 0.481 and 1.83 ± 0.44 respectively. In conclusion muddy floor was associated with reduced hock lesions scoring compared to cemented, brick and rubber mat

Keywords: Hock lesion score, Welfare, Floor types

INTRODUCTION

Intensive farming systems are now common practice to meet the increasing demand for milk in different parts of the world. This has led to the introduction of dairy cows to an environment arbitrarily different from the cows' natural habitat, thereby triggering a range of welfare consequences. An animal is said to be in good welfare when it is able to express its innate behavior, free from distress and fear, in the absence of pain, and in good health (Duncan and Fraser, 1997). However, these fundamentals of optimal welfare are often lacking with the advent of confining cows and persistent demands for high milk yield. As a result of these practices, outcomes such as chronic pain, discomfort, increased susceptibility to infectious disease and metabolic or physical fatigue are now common in dairy cows within intensive farming systems (Cook *et.al.*, 2016). Lameness is a multifactorial condition and the most important welfare problem in dairy cows. Lameness is also regarded as a cause of economic loss owing to a reduction in milk yields, lowered reproductive performance and an increased risk of culling (Sogstad *et.al.*, 2006). Farmers are often reported to underestimate the prevalence of lameness, thereby prompting a low perception of its impact on cow welfare, health and production (Horseman *et.al.*, 2016). With the rising occurrence of lameness in dairy herds globally, attempts to reduce the impact on welfare and production are needed. However, certain animal-based measures such as body condition scoring (BCS), hock condition and leg hygiene have been employed in assessing cow welfare, with recent findings suggesting vital associations

with lameness. Poor hock condition have been reported to have a higher likelihood of becoming lame (Randall *et.al.* 2015). Housing design is vital for the maintenance of good welfare in dairy cows. Floor type and its influence on locomotion performance in dairy cattle were first suggested by Albright in 1997. Subsequently, floor features such as abrasiveness and hardness leading to insufficient friction and traction—as present in concrete floors (CF) were suggested to negatively impact the claw health and locomotion of dairy cows (Van der Tol *et.al.*, 2005).

Although claw lesions remain among the major causes of lameness in dairy cows (Manske *et.al.*, 2002), hock lesions and injuries are becoming a persistent problem in intensively managed dairy farms (Zaffino *et.al.*, 2014). The term “hock lesion” is used to describe various anomalies such as hair loss, visible wounds, broken skin, and localized and general swelling of the hock (Kielland *et.al.*, 2009). In dairy cows, the absence of fatty tissues and muscles around the hock makes the region prone to trauma and damage to the skin. Consequently, the development of hock lesions is directly influenced by the nature of the lying surface of hard and abrasive (Kester *et.al.*, 2014). In welfare assessment, the lateral aspect of the hock is often examined and suggested to be the most affected area. Poor hock conditions are often manifested as hair loss, swelling or ulceration (Potterton *et.al.*, 2011).

The hock condition score (HCS) measures the severity of hock lesions on various scoring scales based on features ranging from normal to substantial injuries. The assessment is important in free-stalls and loose cubicle housing, as such provisions encourage movement and interaction with stall designs. This makes it important to investigate the dairy animals for hock lesions and associated risk factors under field conditions. Keeping this in mind the present study was designed to determine the incidence of hock lesions as Welfare status in the study area and type of floor associated with hock lesions in dairy cattle of University, Research and Private farm of Chitwan district.

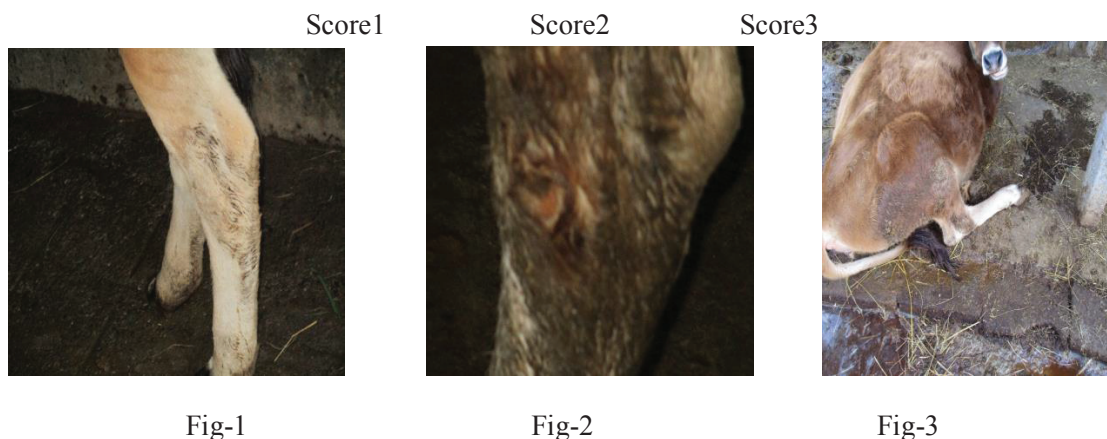
METHODOLOGY

The present study was carried out in Chitwan District, Nepal during December 2015 to May 2016. Majority of farmers and university and research farm in the study area were rearing Holstein Friesian (HF) crossbred and Jersey crossbred dairy cattle. Number of animal maintained per household was two to five mature cows along with their progenies. Number of animals maintained at research (NARC) farm and university farm (AFU) was 71 and 29 respectively. The floor type in university farm was completely cemented where as in research farm, it was in combination of mat and cemented. However in private farm it was in combination of brick, mud and cemented floor. Therefore, all the mature animals were investigated for once during the study period to determine the incidence of hock lesions and floor types associated with incidence of hock lesions by recording of animal level and farm level factors associated with hock lesions using standard observation methods. A total of 250 crossbred dairy cattle were investigated during the study period regardless of their lactation status.

Hock lesions scoring was assessed using a 3 point ordinal scale where 1 scores (fig.1) were considered clinically normal hocks, 2 (fig. 2) with skin loss and 3 (fig. 3) with swelling and ulceration scores (Lombard *et.al.*, 2010). The floor type which was prevalent in area were categorized into cemented, muddy, rubber mat and brick paved.

Statistic

Data obtained was entered in MS Excel 2007 and then loaded into OpenEpi software. Basic descriptive analysis was done and the ANOVA was performed to find out the association among various type of farms and various types of floor associated with the hock lesions. A p value less than 0.05 was considered significant.



RESULT

Results are shown in table 1 and table 2. The average hock lesions score in university farm was significantly higher in comparison to research and private farm. Although non-significant the hock lesion score was less in muddy floor as compared to cemented, brick paved and rubber mat floor.

Table 1. Overall distribution of mean hock lesion score with S.D. among different types of farm.

Type of Farm	Number	Mean hock lesions score $\pm$ S.D.
University (AFU)	29	1.827 $\pm$ 0.384
Research (NARC)	71	1.4 $\pm$ 0.905
Private farm	150	1.465 $\pm$ 0.68

P-value=0.00168295.

Table 2. The overall distribution of mean hock lesion score with S.D. among different types of floor.

Type of Flooring	Number	Mean Hock lesion score $\pm$ S.D.
Cemented	141	1.63 $\pm$ 0.489
Mud	27	1.0165 $\pm$ 0.577
Rubber Mat	35	1.343 $\pm$ 0.481
Brick Paved	33	1.83 $\pm$ 0.44

P-value=0.525189

DISCUSSION

Our research showed that university livestock farm having more hock lesion score compared to research and private farm. It might be due to prolonged standing and walking on hard or abrasive surfaces in university farm. At research farm although animals are also maintained on cemented floor but some animals are maintained on rubber mat. At private farm the animals are maintained on combination of cemented, brick and muddy floor that might result into less hock lesion score compared to university farm. From our results it has been shown that less hock lesion score, when animals are maintained on muddy floor. Fjeldaas *et.al.*, (2011) reported that the risk of higher lameness score (LS) was three times higher in cows on cemented floor (CF) compared to rubber floor (RF). Several studies have demonstrated the inter-relationship between occurrence of hock lesions and lameness in dairy cow (Cook *et.al.*, 2016, Whay *et.al.*, 2002, Rutherford *et.al.*, 2009).

The level of comfort from the lying surface might influence the severity of hock lesions as well as increase the risk of lameness (Brenninkmeyer *et.al.* 2013). Hence, the pathogenesis of hock lesions and the direction of the event as related to lameness need to be investigated. Severe hock lesions could initiate painful sensations leading to lameness, while a prolonged duration of lying down in lame cows on hard and abrasive surfaces might precipitate hock injuries. Another aspect that might contribute to the occurrence of severe hock injury is floor slipperiness. A recent study reported higher odds (Odds ratio, OR = 2.0) of cows being lame and with hock lesions (OR = 1.4) when reared on slippery floors compared to non-slippery floors (Solano *et.al.*, 2015). Telezhenko *et.al.* (2017), in a recent study involving gait analysis and skid resistance of different flooring systems in dairy housing, showed that rubber mats had the highest coefficient of friction and skid resistance values compared to concrete and mastic asphalt floors. This further depicts lower slipping tendencies in cows when housed on rubber mats or floors. Our observation also showed that university farm having dung soiled cemented floor which become slippery that might lead to more hock lesions score.

Overall, aforementioned events and our results show that preventive measures for hock lesions have the potential of reducing lameness incidence contributing to general improvements in cow welfare. Mostafa and Maharan (2016) have reported higher hock injury in a tie stall barn with concrete floor (11.9%). Higher hock injury in the tie-stall barn can be attributed to confinement of the animal at one place due to which their movements are confined which have an effect on hock injury and may be the reason behind more stress on hock in tie-stall barns.

CONCLUSION

Our research showed that muddy floor produce less hock lesion score compared to cemented, rubber mat, and brick paved floor in dairy animals.

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Studies on Factors Affecting Pregnancy Rate after Treatment of Anestrus Buffaloes in Chitwan District

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ABSTRACT

This study was designed to determine the effects of age, parity, feeding practice, body condition score (BCS), blood nutritional parameters, types of anoestrus, and length of anoestrus on pregnancy rate of anoestrus buffaloes after their treatment with ovsynch protocol followed by fixed time artificial insemination. Blood samples were collected on the day ovsynch protocol and analyzed. The mean values of the nutritional parameters between different groups were compared by Students t test and pregnancy rate was compared by Fisher's exact test or Chi-square test. The overall pregnancy rate in buffaloes was 28.6%, when checked on day 41 and 64.3% on day 102 of FTAI. Blood glucose, cholesterol, calcium and phosphorus level was found to be significantly higher ($p < 0.05$) in pregnant than in non-pregnant buffaloes. Pregnancy rate was higher in silent estrus buffaloes. Buffaloes with $BCS \geq 3$ had higher pregnancy rate than those with $BCS < 3$. Similarly, pregnancy rate was higher in lactating than in non lactating buffaloes. While young buffaloes of age < 10 year responded better than old buffaloes of age ≥ 10 year. Buffaloes which were free from gastrointestinal parasitic infection had higher pregnancy rate. In conclusion, ovsynch protocol produces a good pregnancy in anoestrus buffaloes; however state of true anoestrus, older age and poor BCS with poor nutritional management adversely affect the pregnancy outcome. The role of blood nutritional parameters requires further clarification.

Keywords: Pregnancy, Anestrus, Buffalo, Nutrition

INTRODUCTION

Reproduction is an important factor in economics of animal production. The buffalo (*Bubalus bubalis*) is given the name "Black Gold of South Asia" where more than 95% of the buffalo milk is produced (Javaid *et.al.*, 2009). Buffaloes play a prominent role in overall social development by maintaining sustainable food producing system and power for agricultural operations in developing countries (Nanda and Nakao, 2003; Barile, 2005).

Absence of estrus/anoestrus is the most common single cause of infertility observed in rural buffaloes. It is also a big reproductive problem in modern dairy cow production worldwide. Anoestrus and inactive ovaries lead to longer postpartum interval in buffaloes. In Nepal, it has commonly recognized that buffaloes have severe reproductive problems (Sah and Nakao, 2010). Majority of cases of infertility in buffalo presented in infertility camps were of anoestrus condition (Sankhi, 1999). Infertility in buffalo is mostly due to anoestrus condition (Devkota, *et.al.*, 2012). True anoestrus and silent estrous conditions are prevalent among Nepalese

anestrous buffaloes (Sah and Nakao, 2010). Incidence of anestrous is higher in the buffaloes than in cattle. Anestrous accounted 52.7% cases in cattle and 69.4% cases in buffaloes indicating dysfunction of ovaries (Singh and Sahni, 1995).

Ovsynch is a protocol (Figure) consisting the first injection of GnRH (20mcg) I/M on randomly selected day of oestrus cycle which either causes ovulation of matured follicle and start new follicular wave or there may be follicular wave present spontaneously, followed by PGF_{2α} injection (25mg) I/M on 7th day which induces luteolysis of newly formed CL and the second injection of GnRH given 9th day induces ovulation of mature follicle (Pursley, Mee and Wiltbank, 1995).

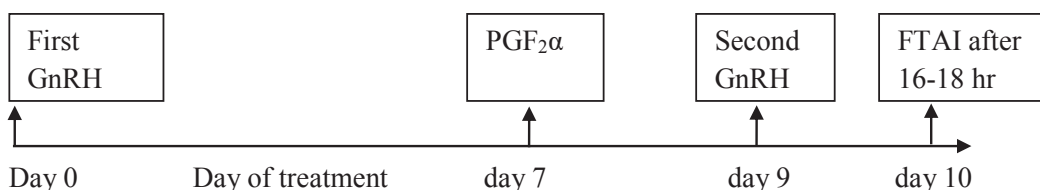


Figure. Schematic representation of ovsynch protocol

Anestrus buffaloes are conventionally treated with vitamin-mineral mixture (Vit-M) supplements with the variable effects. But nowadays, more veterinarians have started to use hormones, such as PGF_{2α} and GnRH, for the treatment of anestrus in buffaloes in Nepal. But treatment of anestrus using hormones only is not enough to get satisfactory level of pregnancy rate. There are many factors which directly affect pregnancy rate in livestock that are herd-level management factors (such as methods of husbandry, feeding, estrus detection, semen handling and transition cow management), environmental factors (such as season, temperature, humidity) and cow-level factors (such as age, BCS, parity, blood nutritional parameters, post parturient problem, disease events, milk yield and genetics) (Lucy, 2001). Tesfaye *et.al.*, (2015) stated that BCS and age of the cow, management system and AI service performance affect the reproductive performance of the smallholder dairy farms. Thus, to fulfill the demand of milk and meat production from dairy sector or buffalo, increased reproductive performance/pregnancy is mandatory and to increase the pregnancy rate or fertility in livestock it need to understand the factors affecting it. So, this study reveals about the factors which affect the pregnancy rate in buffalo such as hormonal disorders, nutritional causes, environmental causes, infectious conditions, BCS and parity after the various heat synchronization treatment.

MATERIAL AND METHODS

Study Area

This study was carried out in Chitwan District of Nepal with Average annual maximum temperature of the district is 32.2°C and minimum temperature 18°C with relative humidity 83%. AFU livestock farm of Sharadanagar VDC and private farms of adjoining Shivanagar VDC of Chitwan district were selected for research study. This area is part of tropical section of Chitwan district. Number of buffalo (118,955) is higher than that of cattle (74, 609) among which 48,932 are milking in Chitwan district (DLSO, 2014)

Hormones Used

Consultan injection 10 ml (a product of ASKA Japan) as depicted in Plate 1:
Each ml of Consultan injection contains 50 microgram fertirelin.

Zenoadin C 20 ml (a product of ZENOAQ, Japan) as depicted in plate 1:
Each ml of Zenoadin C contains 0.250 mg cloprostenol.

Data Collection and Classification of Buffaloes

A data record sheet was developed covering reproductive, managemental and nutritional aspects, as presented in appendix 1 and record sheet was filled, data were entered in Microsoft excel 2007. From history record of anoestrus, 6 buffaloes from IAAS farm and 8 buffaloes from shivanagar VDC were selected randomly and examined by trans-rectal palpation of genital organs. Informations based on rectal palpation were verified with per rectal ultrasonography. On gynaecological examination, colour of vulva, diameter of cervix, its contraction and consistency, diameter of uterine horns, its contraction and consistency and size of both ovaries, its follicular condition, presence or absence of CL etc were examined and cattle were classified on the following basis.

True Anoestrus: Those buffaloes which have ovaries without detectable corpus luteum and without follicles larger than 5 mm.

Subestrus (silent oestrus): Those buffaloes which have large follicles and/or detectable CL on the ovaries.

Length of Anestrus Period: Parous buffaloes were classified into 2 groups

Length of Anoestrus period	<10 months
	≥ 10 months

Body Condition Score (BCS): Buffaloes were classified into following two groups based on the BCS recorded following the procedure as presented in website of Elanco Animal Health.

BCS (Body condition score	Buffaloes with BCS < 3.0
	Buffaloes with BCS ≥ 3.0

Milking Status: Buffaloes were categorized into following two groups

Milking Status	Lactating
	Non-lactating

Parity: Buffaloes were classified into two groups:

Parity	Heifers
	Parous

Managemental Condition: Buffaloes are classified into 2 groups

Managemental condition	University management (Fed with straw and little green grass)
	Farmer's management (Fed with green grass ,straw and concentrate)

Age: Buffaloes were classified into 2 groups:

Young - <10 years
Age Old - ≥10 year

Blood Collection and Biochemical Analysis:

Blood samples were collected in chilled heparinized tubes of buffaloes under the investigation (10 mL, BD Vacutainer; Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) on the first day of ovsynch protocol by vein-puncture through the jugular vein. Tubes were centrifuged (3000 rpm for 15 min) and serum was harvested according to the standard methods (Coles, 1986) and 12 × 75 mm polypropylene tubes and stored at -20 °C to get clear serum. Serum samples were drawn in dry clean capped tubes and sent to medical lab to determine the parameters (i.e. Glucose, Total protein, Cholesterol, Ca and P) using specific kits that were analyzed by automatic biochemical analyser. Glucose was measured after oxidizing it to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide was added that reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoxaline dye complex. Intensity of the colour formed was measured that was directly proportional to the amount of glucose present in the sample. Calcium was measured in an alkaline medium that combines with o-cresolphthalein complexone to form a purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample. Total proteins were measured in the alkaline medium that binds with cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample. For measuring the cholesterol level, esterase was added that hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the colour formed was measured that is directly proportional to the amount of cholesterol present in the sample. Phosphorus was determined spectrophotometrically by applying molybdenum blue using Coral clinical systems (Tulip group) B-2440 Geel, Belgium.

Fecal Examination

Sedimentation procedure is used for fecal examination. It is primarily used to detect eggs or cysts that have too high a specific gravity. Two grams of fecal is mixed with tap water in a cup or beaker. Strain the mixture through a tea strainer into a centrifuge tube. Centrifuge tubes and centrifuge is balanced the sample at about 1500 cycle/ minute or (rpm). If a centrifuge is unavailable, allow the mixture to sit without disturbance for 20-30 minutes. The liquid is poured off in the top of the tube without disturbing the sediment at the bottom. A small amount of the top layer of sediment is transferred to a slide. If the drop is too thick, dilute it with a drop of water. A coverslip is placed to the drop and examined under the microscope.

Fixed Time Artificial Insemination at Tenth Day of Ovsynch Protocol

Fixed time artificial insemination was done at 7 am of tenth day of Ovsynch protocol 18 hours after second injection of Consultan and 63 hours after Zenoadin C injection. Frozen semen of Murrah breed from regular government supply by NLBC was used through DLSO, Chitwan for AI. AI gun was easily passed in some of the buffaloes due to good relaxation of cervix and not in others. Semen was deposited in body of uterus in those animals in which gun was easily passed. In buffaloes in which passing of gun was difficult, semen was deposited in between the cervical rings.

Pregnancy Diagnosis at 41 Days of AI

Pregnancy was diagnosed at forty one day after AI by ultrasonographic scanning by using 7.5 MHz rectal probe.

Pregnancy Diagnosis at 102 Days of AI Including Breeding of Subsequent Natural Sstruses

All buffaloes were scanned for pregnancy with ultrasonograph and rectal palpation for confirmation of maintenance of pregnancy in previously pregnancy positive diagnosed buffaloes and for diagnosis of pregnancy of subsequent breeding in previously pregnancy negative diagnosed buffaloes.

Statistical Analyses

Non-parametric test i.e. χ^2 test and Fisher's exact test in IBM SPSS Statistics version 20 were utilized to know the effect of different factors on pregnancy rate. Students' t-test assuming equal variance, at 5% level of significance was used to compare mean value of different blood nutritional parameters of pregnant and non-pregnant animals using MS-EXCEL-2007. Similarly, MS-EXCEL-2007 was used for data entry and making different diagrams.

RESULT

Pregnancy Rate

Among total 14 buffaloes, 4 (28.6%) buffaloes were found to be pregnant when checked at day 41 of FTAI i.e. on 1st time AI, while 9 (64.3%) buffaloes were found to be pregnant when checked at day 102 of FTAI i.e. on 2nd time AI, which include both positive at 41 day and second time AI of negative pregnancy at 41 day of FTAI.

Blood Nutritional Parameters in All Buffaloes (n=14) Used in the Experiment

Blood nutritional parameters of all buffaloes used in the experiment was given in the Table 1. The range of different nutritional parameters obtained from this experiment was very similar with range explained by Abd Ellah *et.al.*, (2014). Abd Ellah *et.al.*, (2014). Serum biochemical and haematological reference intervals for water buffalo (*Bubalus bubalis*) heifers.

Table 1 Blood nutritional parameters in all buffaloes used in the experiment

Parameters	Mean $\pm$ SD	Range
Glucose (mg/dl)	75.8 $\pm$ 19.8	38-95
Total protein (mg/dl)	6.6 $\pm$ 0.4	6-7.6
Cholesterol (mg/dl)	60.1 $\pm$ 9.1	40-70
Calcium (mg/dl)	8.3 $\pm$ 2.1	4-11.1
Phosphorus (mg/dl)	6.2 $\pm$ 2.3	2-9
Ca:P	1.5 $\pm$ 0.5	0.8-2.5

Comparative Study of Blood Nutritional Parameters

The mean ($\pm$ SD) values of various nutritional parameters of pregnant and non-pregnant buffaloes are shown in Table 2. It was found that blood glucose level, cholesterol level, calcium level and phosphorus level of non-pregnant buffaloes were significantly lower than pregnant buffaloes. Protein level was also lower in non-pregnant buffaloes than pregnant ones but not significantly lower. When these nutritional parameters of pregnant and non-pregnant buffaloes were compared separately with the average value of all experimental buffaloes then no parameters were found to be significantly different.

Table 2. Comparative study of blood nutritional parameters between pregnant and non-pregnant buffaloes

Parameters (mg/dl)	Mean $\pm$ SD			P value a and b	P value a and c	P value b and c
	Mean of total n=14 (a)	Pregnant n=9 (b)	Non-pregnant n=5 (c)			
Glucose	75.8 $\pm$ 19.8	83.7 $\pm$ 11.8	61.6 $\pm$ 24.6	0.3	0.25	0.04**
TP (g/dl)	6.6 $\pm$ 0.4	6.3 $\pm$ 1.5	5.2 $\pm$ 1.6	0.6	0.4	0.25
Cholesterol	60.1 $\pm$ 9.1	63.6 $\pm$ 6.3	53.8 $\pm$ 10.6	0.3	0.2	0.05**
Calcium	8.3 $\pm$ 2.1	9.3 $\pm$ 1.4	6.5 $\pm$ 2.2	0.2	0.1	0.007***
Phosphorus	6.2 $\pm$ 2.3	7.1 $\pm$ 1.6	4.5 $\pm$ 2.2	0.3	0.15	0.02**
Ca:P	1.5 $\pm$ 0.5	1.4 $\pm$ 0.5	1.6 $\pm$ 0.5	0.7	0.6	0.5

Note: ** Significantly different ($P \leq 0.05$)

*** Highly significantly different ($P \leq 0.01$)

Effect of Various Factors on Pregnancy Rate after treatment with Ovsynch Protocol

Among various factors, no factors were seen significantly associated with the pregnancy outcome. It might be due to small sample size. When we see the odd ratios of different factors with pregnancy outcome at 42 days of FTAI, It can be said that milking status and management conditions are more associated with pregnancy outcome than other factors. So it can be said that lactating condition and farmers management/good feeding management played major role in pregnancy outcome. But the odd ratios of different factors with pregnancy outcome at 102 days of FTAI, then we found age factors was more associated with pregnancy outcome than other factors i.e. young age/ <10 year played major role in pregnancy outcome. Then after parity (parous buffaloes) was found to be associated with pregnancy outcome (Table 3).

Table 3. Effect of various factors on pregnancy rate when checked at 41 day and at 102 day of FTAI

Basis of categorization	No. of Animal	Pregnancy rate (No.) at 41 day	Odd ratio	P value	Pregnancy rate (No.) at 102 day	Odd ratio	P value
Type of anestrous							
True	9	22.2 (2)	0.43	0.58	55.6 (5)	0.31	1.00
Silent	5	40 (2)			80 (4)		

Length of anestrus							
< 10 months	9	33.3 (3)	2	1.00	55.6 (5)	0.31	1.00
≥ 10 months	5	20 (1)			80 (4)		
BCS							
< 3	6	33.3 (2)	1.5	1.00	50 (3)	0.33	0.58
≥ 3	8	25 (2)			75 (6)		
Milking status							
Lactating	8	37.5 (3)	3	0.58	75 (6)	3	0.58
Non lactating	6	16.7 (1)			50 (3)		
Parity							
Heifer	3	33.3 (1)	1.33	1.00	33.3 (1)	0.19	0.5
Parous	11	27.3 (3)			72.7 (8)		
Age							
Young	9	33.3 (3)	2	1.00	77.8 (7)	5.25	0.27
Old	5	20 (1)			40 (2)		
Mgmt. condition							
University mgmt.	6	16.7 (1)	0.33	0.58	50 (3)	0.33	0.58
Farmers mgmt.	8	37.5 (3)			75 (6)		
Total	14	28.6 (4)			64.3 (9)		

Effect of Blood Nutritional Parameters on PregnancyRate

When blood nutritional parameters were analyzed with pregnancy rate, It was found that blood total protein level have greater tendency to be associated with pregnancy outcome than other factors according to, p-value and when pregnancy was checked at 42 days of FTAI, but it was found that blood phosphorus level have greater tendency to be associated with pregnancy outcome when pregnancy was checked at 102 days of FTAI but they were not significantly associated.

When we use the odd ratios to describe the relationship then it was found that blood total phosphorus level ≥ 4.5 (mg/dl) had played major role in pregnancy outcome than all other factors in both of time of pregnancy checked. Then after blood total protein ≥ 7 (g/dl) and blood glucose level ≥ 75 (mg/dl) respectively were found to be responsible for pregnancy outcome when pregnancy was checked at 42 days of FTAI but when pregnancy was checked at 102 days of FTAI it was found that after blood phosphorus level, blood cholesterol level ≥ 60 (mg/dl), glucose level ≥ 75 (mg/dl) and blood total protein ≥ 7 (g/dl) and blood calcium level ≥ 8.5 (mg/dl) were found to be responsible for pregnancy outcome respectively. The detail description was given in Table 4.

Table 4. Effect of blood nutritional parameters on pregnancy rate checked at 42 and 102 day of FTAI

Basis of categorization	Number of animal	Pregnancy rate (No) at 42 day	Odds ratio	P value	Pregnancy rate (No.) at 102 day	Odds ratio	P value
Glucose							
<76 (mg/dl)	5	20 (1)	0.5	1	40 (2)	0.19	0.26
≥ 76	9	33.3 (3)			77.8 (7)		
Total protein							
< 7 (g/dl)	9	11.1 (1)	0.08	0.09	55.5 (5)	0.31	0.58
≥ 7	5	60 (3)			80 (4)		
Cholesterol							
< 60 (mg/dl)	3	33.3 (1)	1.3	1	33.3 (1)	0.18	0.5
≥ 60	11	27.3 (3)			72.7 (8)		
Calcium							
<8.5 (mg/dl)	6	33.3 (2)	1.5	1	50 (3)	0.33	0.58
≥ 8.5	8	25 (2)			75 (6)		
Phosphorus							
< 4.5 (mg/dl)	4	0 (0)	0	0.25	25 (1)	0.08	0.095
≥ 4.5	10	40 (4)			80 (8)		
Ca:P							
< 1.5	7	50 (2)	1	1	57.1 (4)	0.8	1
≥ 1.5	7	50 (2)			71.4 (5)		

Influence of Gastrointestinal Parasite on Pregnancy Rate

Among all 14 anestrus buffaloes, 8 were found to be negative and 6 were found to be positive on gastrointestinal parasite infection when their fecal examination was done. Among negative, 6 (75%) were found to be pregnant after ovsynch treatment of anestrus while among positive only 2 (33.33%) were found to be pregnant and it was not significantly lower than negative/non-infected buffaloes. This showed that there was not significant relationship between gastrointestinal parasite infection and pregnancy rate.

Table 5: Influence of gastrointestinal parasite on pregnancy rate of *anestrus* buffaloes within treatment

Parasitic infection	No. of buffalo treated	No. of buffalo pregnant (%)	P-value
Negative	8	6 (75)	0.27
Positive	6	2 (33.33)	

DISCUSSION

It was found that there was low pregnancy rate i.e. 28.6% on 1st AI, checked on 41 day of FTAI while pregnancy rate was greater i.e. 64.3% on 2nd AI, checked on 102 day of FTAI. This finding was similar to the finding of Rabidas and Gofur (2017), who found 31.96% (31/97) pregnancy rate in indigenous river type buffaloes of Bangladesh after treatment with ovsynch protocol in 1st AI. Yotov *et al.* (2012) found 37.5% pregnancy rate on anestrus buffaloes of Bulgaria with

ovsynch protocol. Low pregnancy rate on 1st AI of ovsynch of this research might be due to true anoestrus buffalo which is not fertile and hormone induces conjunctive fertile oestrus cycle in next cycle.

In this study blood serum glucose level, cholesterol level, Calcium and Phosphorus level of pregnant and non-pregnant buffaloes were found to be significantly different ($P < 0.05$) i.e. higher in pregnant buffaloes than in non-pregnant buffaloes while total protein and Ca:P level were not found to be significantly different. The similar finding was obtained by Yotov *et al.* (2012), who also found significantly higher blood glucose, cholesterol, Calcium and Phosphorus ($p < 0.05$) in pregnant buffaloes than non-pregnant buffaloes in Bulgaria. Present study showed that glucose, calcium, phosphorus and total cholesterol levels in blood could have an impact on the subsequent reproductive performance of buffaloes after the ovsynch treatment.

Present study found that silent estrus buffaloes which were cyclic and contain palpable CL in their ovary, showed higher pregnancy rate than true anoestrus which were non cyclic and contain no palpable CL in their ovary. It is generally accepted that malnutrition and an excessive negative energy balance are the main causes of true anoestrus/inactive ovary and delayed ovarian function after calving (Butler, 2000; Lucy *et al.*, 2008) which can't response properly to the hormones given externally. While silent estrus mostly have good BCS and can response to the hormones given externally and become pregnant. This study showed that pregnancy rate was higher in buffaloes having more than 10 month of anoestrus period than less than 10 month of anoestrus period when pregnancy was checked at 102 days of FTAI. Similar finding was obtained by Devkota *et al.*, (2013) who found highest pregnancy rate (37.5%) in buffaloes showing 7-12 months of anoestrus and found lowest pregnancy rate (12.5%) in buffaloes having 2-6 months of anoestrus and >12 months of anoestrus. Similar finding was obtained by Haque *et al.*, (2014), in cattle in Bangladesh.

BCS In this study it was found that pregnancy rate was higher in buffaloes having BCS ≥ 3 than having BCS < 3 but it was not significantly higher when pregnancy was checked at 102 days of FTAI. Similar finding was obtained by Devkota *et al.*, (2013) who found highest pregnancy rate (40%) in buffaloes with BCS=2.5-3.5 and lowest pregnancy rate (0%) in buffaloes with BCS < 2.5 ($p < 0.05$). Similar findings was obtained by Anzar *et al.*, (2003) in Pakistan in cattle and buffaloes who found conception rate was significantly influenced by BCS ($P < 0.05$), being higher in animals with better condition than in animals having poor condition. The pregnancy rate was found to be higher in parous buffaloes than heifers when pregnancy was checked in 102 days of FTAI but no significantly different. Similar finding was obtained by Haque *et al.*, (2014) in cattle in Bangladesh who found highest pregnancy rate (57.4%) in cows of parity 1-2 and lowest pregnancy (45.0%) in cows of parity 0/heifer. However, the variation in pregnancy rates among different parity of cows was not significant ($p > 0.05$). Similarly, Devkota *et al.*, (2013) found that buffaloes with ≥ 4 parity have highest pregnancy rate (30%) than 1-3 parity (20%) and lowest in the heifer (10%) after treatment with PGF₂ α in Nepal. Similarly, Khan *et al.*, (2015) reported higher conception rate in cows at second and third parity than that of cows at zero parity (nulliparous).

In this study, pregnancy rate was found to be higher in lactating/milking buffaloes than non-lactating but it was not significantly higher. Similar finding was obtained by Anzar *et al.*, 2003,

in Pakistan in cattle and buffaloes who found that conception rate was affected significantly by the lactation state of animals ($P<0.05$). It was lowest in dry animals i.e. 6.5% and highest in those which were in milking and were being suckled i.e. 31.3%. But there are other studies showing that conception rate is lower in high milk producer (Nebel and McGilliard, 1993). Haque *et.al.*, (2014) in cattle in Bangladesh found that the pregnancy rate in cows yielding >2-5 L milk was the highest (62.1%) and the pregnancy rate in cows yielding 1-2 L milk was the lowest (49.4%). However, the variation in pregnancy rates among cows of different milk yields was not significant ($p>0.05$). Similarly, Sarder (2001) found no variation in pregnancy rates between high and low yielding indigenous cows. In contrast, conception rates are higher in cattle with higher milk production than that with lower milk production (Shamsuddin *et.al.*, 2006). The positive effect of high milk yield on conception rate may be explained by the fact that the high yielding cows received more attention of owners, balanced diet and reared under good management programme (Shamsuddin *et.al.*, 2006). In present study, the pregnancy rate was found to be higher in young buffaloes with age <10 year than with age $\geq$ 10 year. Haque *et al.* (2015) in cattle in Bangladesh found that cows of 3-5 years of age showed the highest (56.1%) pregnancy rate while the cows of 2-3 years of age showed the lowest (47.1%).

Gastrointestinal parasite infection has been common problem to cause poor production and sub fertility in buffaloes. Nearly a half of buffaloes referred to the infertility camps were positive for helminthes. In present study the infected buffaloes showed a lower pregnancy rate than those without infection. Similar result was obtained by Devkota *et.al.*, (2013) who found significantly higher pregnancy rate in buffaloes with negative gastrointestinal parasitic infection than infected ones (76.9%).

CONCLUSION

This study showed the pregnancy rate obtained in buffaloes of AFU farm and Shivanagar VDC after their treatments with ovsynch protocol. It has compared the blood nutritional parameters of pregnant and non-pregnant buffaloes to check whether they have any role on pregnancy outcome and it has analyzed various management and individual factors to check whether they affect on pregnancy rate. The current study revealed that ovsynch protocol produces a good pregnancy in anoestrus buffaloes, however state of true anoestrus, older age and poor BCS with poor nutritional management adversely affect the pregnancy outcome. The role of blood nutritional parameters requires further clarification.

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Seroprevalence and Risk Factors of Bovine Viral Diarrhea in Improved Cattle of Chitwan, Nawalpur and Rupandehi Districts of Nepal

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ABSTRACT

This study was aimed at assessing the prevalence of bovine viral diarrhea (BVD) in improved dairy cattle of Chitwan, Nawalpur and Rupandehi Districts of Nepal. Altogether 92 blood samples were aseptically collected from jugular vein of cattle and transferred to clot activator tube and transported to National Cattle Research Program, Rampur, Chitwan. This cross sectional study was carried out from Mangsir to Magh of 2075 B.S using purposive sampling. Serum separation was done by centrifugation @3500 for 5 minutes. The serum was stored at -20°C till ELISA test was done. Serological analysis was done according to the protocol of ID.vet BVD Ab ELISA kit. Data were analyzed using Ms excel and SPSS version 20.0. The seroprevalence of BVD Virus was found to be 7.76%. Provided that no history of vaccination against BVD in cattle was done in the study area, seropositive was due to natural infection. Geographic location as well as sampling method might have contributed to this result. The result indicated lower prevalence along with no statistical significance to breed and age risk factors undertaken during the research. However significant association was observed between abortion history and seropositivity. Hence, there is a need of further research to identify the disease burden and its impact in the agricultural gross domestic products (AGDP).

Keywords: Bovine viral diarrhoea, Seroprevalence, ELISA, Dairy cattle

INTRODUCTION

Bovine viral diarrhea (BVD) is an infectious and highly contagious viral disease of cattle. It is cosmopolitan in distribution and Office International des Epizootic (OIE) has listed it as notifiable disease. In Nepal, infertility problem in crossbreed and exotic cattle has been reported to be the most prioritized problem in pocket area in the country (Jha, 2000). It is caused by Pestivirus of family Flaviviridae which is characterized by alimentary, respiratory and reproductive disorder along with haemorrhagic syndrome and thrombocytopaenia (Baker, 1995). BVD virus (BVDV) is divided into two biotypes cytopathic (CP) and non-cytopathic (NCP) based on cytopathic effect on cell culture (Vanroose *et al.*, 1998). NCP can cross the placenta and is thus considered important. Noncytopathic crosses through the placenta to establish a persistent infection (PI). If the fetus is infected and survives after birth, BVD virus can severely affect the reproductive and nervous system (Saliki JT 2004). Reproductive problems like abortion and infertility are of major concerns in the dairy farming of Nepal. Very limited information is available regarding the causes of bovine reproductive problems in Nepal.

The prevalence of persistent BVDV infection in dairy cattle in two commercially important livestock districts i.e. Chitwan was 0.7% and Kavrepalanchowk was 3.4% as individual animal prevalence (Gaire T.N *et.al.*, 2016). Import of most of the BVDV infected cross-breed dairy cattle from India makes Tarai of Nepal more susceptible to BVDV. The BVDV is transmitted by direct contact to animals, by transplacental route, by discharges from reproductive tract of an infected cow (Meyling *et.al.*, 1990). The prevalence of persistent BVDV infections ranges from 0.5-2% in cattle populations from different countries of the world (Charleston B *et.al.*, 2001). In India, 15.3% cattle and 23.2% buffaloes were reported to be BVDV positive (Sudharshana K.J. *et.al.*, 1999).

MATERIALS AND METHODS

The study was carried out from December 2018 to February 2019 at National Cattle Research Program, Rampur, Chitwan. The 92 cattle serum samples were collected from eastern areas of Chitwan (Ratnanagar municipality), Nawalpur (Gaidakot and Kawasoti Municipality) and Rupandehi (Sunwaland Devdaha municipality). Animals were selected on the basis of the history in relation to their reproductive problems. The test was carried out in accordance to the manual of the ID Vet France @ BVD Ab ELISA for in vitro detection of antibodies. The ELISA used was of ID Vet-BVD P80 Antibody detection Lot/Ch.B: 693-008. The sample size necessary for detection of BVDV was calculated from EpiTools Epidemiological Calculators by Ausvet, in assumption of overall 2.2% of prevalence of BVDV infection in dairy cattle of Nepal (Manandhar S *et.al.*, 2018) computed with expected precision of 5% and 95% of confidence level. The data generated were stored in the MS excel 2010 and overall prevalence was calculated from MS excel. Questionnaire survey was also employed that included general description, herd history and individual animal history. Association between the categorical variables and outcomes variable was assessed by using IBM SPSS statistics 20.

RESULT AND DISCUSSION

Overall Seroprevalence of BVD

The study reveals overall seroprevalence of 7.76% from 92 samples tested, 7 samples being positive. Also, there is no any practice of vaccination against BVD in Nepal, the seropositivity must be attributed in response to natural infections. The samples detected positive by ELISA can be subjected to total RNA extraction and PCR amplified gene of BVDV for further confirmation with greater accuracy. Lower prevalence than this study was reported by Manandhar S (2018) in western Chitwan of Nepal (2.2%). This study is lower than the prevalence found 13.20% in Tamil, Nadu (Krishna K.S. *et.al.*, 2017). In contrast to this study, higher seroprevalence were found in different areas like in Europe, comprising more than around 50% of the isolates in North American 90% of the isolates (Ridpath J.F., 2005), 51.1% in Bangladesh (Uddin MA *et. al.*, 2017), 33.2% in cattle of Selangor, Malaysia (Daves L. *et.al.*, 2016), 15.29% in cattle of 16 states of India (Sudharshana K. J. *et.al.*, 1999). The higher seroprevalence in other countries might be due to vaccination against BVD.

Table 1: Seroprevalence of BVD determined by using ID.vet, France Ab ELISA

	No. of positive	No. of Negative	Prevalence	True prevalence
Total serum sample examined				
92	7	85	7.76%	

There is no significant association between districts and occurrence of BVDV ($p>0.05$). But Chitwan (9.37%) and Nawalpur (12.9%) have more prevalence percent than Rupandehi (0%). This might be affected by geographical location, sampling method and management practices. Sampling technique may have major role in missing out the positive samples.

Table 2: Districtwise seroprevalence of BVD

Districts	Total tested	Positive	Negative	Prevalence %	P-value
Chitwan	32	3	29	9.37	0.152
Nawalpur	31	4	27	12.90	
Rupandehi	29	0	29	0	

The study found no significant difference ($p>0.05$) between age and prevalence. Statistically it is not significant which indicates that both age groups have equal probability of getting BVD. This result is in contrast with Daves L. *et.al.*, 2016 whose finding shows that the prevalence positivity increases with age.

Table 3: Age wise Seroprevalence of BVD

Age	Positive	Negative	Total	Prevalence	P-value
3 and less (young)	4	45	49	8.16	0.830
4 and more (adult)	3	40	43	6.97	

There is no significant difference ($P>0.05$) between breed and prevalence. The present study indicates prevalence percent higher in Holstein, statistically it is not significant indicating that both breeds have equal chance to be infected with BVD. The prevalence percent was higher in Jersey cross (7.76%) than Holstein-Friesian cross (0%). This might be due to the higher number of Jersey crosses in tested sample.

The result is in agreement with S. Manandhar (2018) findings which shows prevalence of Jersey cross (2.4%) than Holstein-Friesian (1.7%) and with (Daves L. *et.al.*, 2016) which shows that higher prevalence is in Jersey breed of cattle than Holstein Friesian.

Table 4: Breed wise prevalence of BVDV

Breed	Positive	Negative	Total	Prevalence %	P-value
Jersey cross	7	65	72	9.72%	0.147
Holstein cross	0	20	20	0%	

Seroprevalence of BVDV with Abortion History

Seroprevalence of BVD between abortion history and result was found to be significant ($P=0.015$, i.e. $P < 0.05$) Odd Ratio: 6.333, The chance of BVD with history of abortion is 6.333 times more than the no history of abortion. This results is in agreement with S.Y. Derdour 2017 which findings shows that there was significant association between abortion and seropositivity (OR= 14.21).

Table 5: Abortion history wise seroprevalence

Abortion history	Positive	Negative	Total tested	Prevalence	P-value
Yes	3	9	12	25%	0.015
No	4	38	42	9.523%	

CONCLUSION

The result of the present study suggests that our dairy cattle have been infected with BVDV. This study found no significant association between seropositivity with district, age and breed with p-value 0.152, 0.830 and 0.147 respectively. However significant association was observed between abortion and seropositivity with p-value 0.015 and Odd ratio 6.333. Different tools like systematic control, culling PI animals, restriction of movement of infected herds should be used to contain this disease. There is a need of further research in molecular level to better understand the different factors causing the BVD.

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***Taenia solium* Prevalence in Nepal: A Retrospective Review**

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ABSTRACT

Taenia solium is a zoonotic cestode parasite which causes cysticercosis in human and porcine cysticercosis in pigs. The infective stage of *T. solium* develops in pig while that of *T. saginata* develop in cattle and buffalo. The adult stages of *T. solium* are obligatory intestinal parasites for man. In humans the cysticercus larvae commonly encyst in the brain, causing neurocysticercosis. The disease was first described in pig by Aristophanes and Aristotle in 3rd century B.C. In Nepal, *Taenia* cysts were observed for first time in pork meat slaughtered in Kangeswari, Kathmandu more than 30 years ago. *T. solium* is the cause of 30% of epilepsy cases in many endemic areas where people and roaming pigs live in close proximity. More than 50 million of the world's populations are infected worldwide and 50000 die from Neurocysticercosis yearly. In Nepal, the prevalence ranges from 0.002-0.1% in general population. Accurate diagnosis required detailed post mortem examination involving slicing of affected tissue to determine the viability of cysts. Cysticerci occur most commonly in striated muscles tissues and brain of pigs. The application of vaccination with TSOL18 recombinant vaccine along with oral medication of oxfendazole can reduces the transmission by pigs whereas use of niclosamide is found effective in humans. Hence the use of medication eventually reduces the incidence of neurocysticercosis in human and porcine cysticercosis in pigs.

Keywords: Cysticercosis, Pigs, Prevalence, *Taenia solium*

INTRODUCTION

Taenia solium is a cestode parasite of zoonotic importance which causes cysticercosis in human and porcine cysticercosis in pigs. It is a food-borne infection which is emerging as a major health problem world wide (Sciutto *et.al.*, 2000) . The infective stage of *T. solium* develops in pig while that of *T. saginata* develops in cattle and buffalo. The infection is endemic in the area where pigs are reared under scavenging system with poor sanitation, open defecation and uncontrolled slaughter of infected pig. It is endemic in the developing countries of Latin America, Africa and Asia (Coral-Almeida *et.al.*, 2015). WHO has considered neurocysticercosis (NCC) as the most important neurologic disease in humans (Scheel *et.al.*, 2005). About 50 million people of world are infected and among them 50000 die from NCC yearly (Scheel *et al.*, 2005). Neurocysticercosis is one of the major causes of onset of symptomatic epilepsy in the areas where pork tapeworm *T. solium* is endemic. The cysticercosis caused by *T. solium* is considered as neglected tropical disease by WHO (2015). Various control measures are operated nowadays for successful in reducing the incidence of parasite. Vaccination using TSOL18 recombinant vaccine along with oral treatment of oxfendazole @ 30 mg per kg is found to be effective (Poudel *et.al.*, 2019). The main aim of this paper is to know the status of *T. solium* by

reviewing various articles through PubMed, Google scholars and research gate. The overall prevalence of *Taenia solium* in Nepal was determined along with its public health significance and proper suggestion was given to control its prevalence through rational use of vaccination and medication in both animals as well as in humans.

DISTRIBUTION AND EPIDEMIOLOGY

Taenia solium is distributed throughout the world and is particularly prevalent in the areas where small scale pig farming or pigs as household livestock is common (García *et.al.*, 2003). Therefore, the developing countries such as Latin America, Africa, and Asia are considered as endemic areas for tapeworm. Until recent years, there were only sporadic cases in the United States. However, due to increased immigration from Central America and travel to endemic areas, cysticercosis is on the rise in the United States, especially in California, Arizona, New Mexico, Texas, and Colorado. Different forms of the disease occur in different areas depending on type and location of infection, including autoinfection or by the fecal-oral pathway. The disease cysticercosis in pigs was first discovered by Aristophanes and Aristotle during 3rd century B.C. (Cook, 1988). Although accurate information on prevalence rates is rarely available, the disease is known to be endemic in all continents with the exception of Australasia. Exceptionally high prevalence rate occurs in Latin America from Mexico to Chile. In Mexico City 1.4-3.6% of autopsies in general population provide evidence of cysticercosis (Cook, 1988). The disease spreads sporadically throughout the Europe and it is also prevalent in India, Pakistan, Indonesia, Thailand and northern China.

In Nepal, *Taenia* cyst were first observed more than thirty years ago in the pork meat slaughtered in Kangeswari, Kathmandu (Joshi *et.al.*, 2007). Neurocysticercosis was reported in Nepal during December, 1997 along with its mode of infection (Joshi *et.al.*, 2007). Del Brutto *et.al.*, reported that neurocysticercosis is a common cause of epilepsy among the Nepalese soldiers stationed with British Army in Hong Kong. The available data suggest that the prevalence ranges from 0.002 -0.1 % in general population in Nepal (Bista, 2006).

LIFECYCLE

The completion for the lifecycle of *T. solium* requires two hosts, man as definitive host and pigs as intermediate host. Pigs harbour the infection due to consumption of vegetation contaminated by the parasite's eggs or gravid proglottids. The parasites then mature to oncospheres (embryos) in the pig's intestine, which break the intestinal wall and develop into infective cysticerci (larvae) in the muscles. Infection is spread to humans when they eat raw or undercooked, infected pork meat contacting infective cysticerci larva. The larva migrates to small intestine which then matures to adult tapeworm resulting to cause taeniasis. After the parasitic larval lodges in the brain, it causes neurocysticercosis. Completion of life cycle of the parasite has been prevented in many developed countries through improvements in sanitation and hygienic practices in pig rearing, which eventually prevent the animals from being exposed to human faeces. The prevention of completion of lifecycle can facilitate in reducing the prevalence as well as prevent human from exposure to disease. The presence of suitable environment for the completion of lifecycle can be one of the causes of prevalence of this parasite in the nation.

CURRENT SITUATION IN NEPAL

Various researches are carried out in Nepal regarding to the prevalence of *T. solium* Nepal and among them very few authentic research articles have been published till date. The published and unpublished data suggests that the disease has been established in the country, and is developing a serious public health problem.

Joshi (1973 and 1991) observed *Taenia* cysts in pig meat slaughtered in Kangeswari, Kathmandu for the first time more than 30 years ago. Likewise, (Bajaj, Satish K. 1997) reported about the cysticercosis and its mode of infection. Different intervention programmes are carried out in order to control its transmission. The applied intervention consists of intensive combination of mass human tapeworm deworming with niclosamide along with pig anthelmintic treatment with oxfendazole and pig immunization with the TSOL18 vaccine, in various rounds for approximately one year (Poudel *et.al.*, 2019). In Nepal, generally poor and marginalized people are prone to infection due to close contact with pigs, poor sanitation.

Table 1: Prevalence of *T. solium* in pigs of Nepal

District	No. of tested animals	Percentage of positive animals	Reference
Banke	110	32 (29%)	(Sah <i>et.al.</i> , 2017)
Syangja	180	77 (43%)	(Maharjan and Gaihre, 2010)
Tanahun	152	28 (18%)	(Joshi <i>et.al.</i> , 2004)
Kathmandu	196	28 (14%)	(Devleesschauwer <i>et.al.</i> , 2013)
Dharan	54	6 (11%)	(Joshi <i>et.al.</i> , 2004)

TAENIASIS IN HUMAN

T. solium causes two diseases when they infect the human: taeniasis and cysticercosis. Taeniasis is caused by infection of the adult tapeworm in the intestines of humans and is most often acquired by eating uncooked or poorly cooked infected pork meat. Cysticercosis causes a significant public health disorder when humans ingest parasite eggs, which liberate larvae that may settle in the nervous system, causing a serious condition called neurocysticercosis (NCC) (Carabin *et.al.*, 2017).

Cysticercosis (CC) and neurocysticercosis (NCC) are characterized by development of cyst in the eyes, skeletal muscle, heart, or brain caused due to the ingestion of *Taenia solium* eggs. Symptoms can vary from the clinically mild to severe, in which epileptic seizures, hydrocephalus, or localized neural deficits predate *T. solium* associated fatalities. Over 20 million people in the world are estimated to be infected with either the larvae or the adult tapeworm and its wide-ranging medical, economic and social effects are felt globally (Pawlowski *et.al.*, 2005). In 2015, the WHO Foodborne disease burden Epidemiology Reference Group identified *T. solium* as a leading cause of deaths from food-borne diseases, resulting in a considerable total of 2.8 million disability-adjusted life-years (DALYs) (Jansen *et.al.*, 2018). Neurocysticercosis is considered as one of the main causes of epilepsy in the developing countries of Asia, Latin America, and Africa (Pal *et.al.*, 2000). In Nepal, human

neurocysticercosis is a frequently diagnosed condition where it had been estimated to be responsible for the highest burden of disease caused by a parasitic zoonosis (Devleeschauwer *et.al.*, 2012). The cases of neurocysticercosis are reported from many hospitals of Kathmandu (Amatya and Kimula, 1999). Sixty two patients with cysticercosis were confirmed out of 23,402 biopsy cases detected at Patan hospital, Lalitpur. During the past five years, records from Bir Hospital, and Kanti Children's Hospital reported 4 and 11 cysticercosis cases in Kathmandu, respectively (Joshi *et.al.*, 2004). The main cause of epileptic seizure is now considered as neurocysticercosis which accounts for 50% of the patient with partial seizures (Rajshekhar *et.al.*, 2003). About 50 million people are infected worldwide and among them 50000 die from NCC yearly (Scheel *et.al.*, 2005).

DIAGNOSIS

For the proper diagnosis of *T. solium* cyst in pork meat, the pig should be slaughtered, viscera should be separated and heart, liver, brain as well as half the body skeletal musculature should be sliced using hand knives in order to determine the viability of cysts. If one or more viable or non-viable cysticerci were found in the muscle and or the brain, the pig should be confirmed of porcine cysticercosis (Poudel *et.al.*, 2019). The case of taeniasis can also be confirmed by findings eggs or gravid proglottids in direct fecal smear. Palpation of the whole body for the presence of subcutaneous and intramuscular nodules along with neuroimaging techniques such as CT scan and MRI can also be done for diagnostic approaches in humans (Scheel *et.al.*, 2005).

TREATMENT

Treatment of taeniasis is a good option due to direct causal link between taeniasis and human cysticercosis. For the proper treatment of taeniasis, pig should be vaccinated with 1ml Tsol18 vaccine (150µg TSOL18 recombinant protein in mineral oil adjuvant; Cysvax, Indian Immunological Limited, India) through intramuscular route (Flisser *et.al.*, 2004). Along with this intensive combination of mass human tapeworm deworming with niclosamide @ 2g as a single dose for adults along with pig anthelmintic treatment with oxfendazole @30 mg per kg body weight (Paranthic 10%, MCI, Morocco) is found to be effective (Poudel *et.al.*, 2019).

PREVENTION AND CONTROL

To improve *T. solium* control, we must train meat producers sellers in the safe handling of meat. Slaughtering of pigs in open space should be prohibited. The meat should be properly inspected by meat inspector before selling to market. Pig should be reared separately away from human settlement which reduces the contamination between human and pig. People must consume well-cooked as well as refrigerated meat. Sanitation and pig husbandry around the house should be properly maintained. The pigs should be immunized to control the transmission of disease. Regular and active surveillance should be done to know the prevalence and incidence of taeniasis and cysticercosis in both definitive and intermediate hosts.

DISCUSSION

The prevalence of *T.solium* in Nepal is found to be significantly different according to different research conducted at different region. The highest prevalence of procine cysticercosis was recorded in Syangja district where 77 out of 180 animals were found to have cysticercosis (Maharjan and Gaihre, 2010). The lowest prevalence was recorded from Dharan district where 6 out of 54 (11%) animals were found to have cysticercosis (Joshi *et.al.*, 2004). In Banke district 32 out of 110 (29%) animals were identified with *T.soliumcysticerci* (Sah *et.al.*, 2017) whereas in Kathmandu 28 out of 196 (14%) (Devleesschauwer *et.al.*, 2013) were found to be infected. Likewise, in Tanahun 28 out of 152 (18%) were found to harbour cysticercosis. These data are based on the detailed post mortem examination of carcass. These data suggest that the prevalence of cysticercosis is emerging day by day in Nepal. This is mainly due to poor sanitation, unhygienic management, close contact between pigs and animal, open defecation by humans, consumption of raw or poorly cooked pork meat. It is due to unhealthy rearing practices, where pigs are allowed to feed on human feces, lack of latrines, poor hygiene habits, and lack of meat inspection, has contributed to the prevalence of the disease. The people of terai are found rearing pig under scavenging system which initiates the cause of disease due to free roaming of animals; they transmit disease from one place to another.

The high prevalence in Syangja might be due to high number of pork consuming community such as magar, dalits etc. who rear pigs under free roaming system with poor sanitary methods (Maharjan and Gaihre, 2010). Moreover, lack of education, poor hygiene and low socio economic status may lead to its prevalence in high number.

The prevalence of porcine cysticercosis in pigs from the Banke District of Nepal indicates that there is a high rate of *T. solium* transmission in the region and, very likely to have a high rate of human cysticercosis (Poudel *et.al.*, 2019). The unhealthy pork rearing practices, open defecation, poor hygienic habits have contributed to the establishment and transmission of the disease in human as well. The high incidence in human may due to improper eating habits of pork meat in the form of barbeque.

The reduction of prevalence of procine cysticercosis can be done by combined use of vaccine Tsol18 and administration of oxfendazole @30mg/kg body weight (Poudel *et.al.*, 2019).The combined use provides both treatment as well as prevention from subsequent infection. The drug oxfendazole helps in reduction of cysticerci whereas vaccine protects from being exposure to cysticercosis.

CONCLUSION

The review of prevalence taeniasis and cysticercosis research in Nepal showed that parasitic infections have existed for many years. The zoonotic parasite is deeply rooted in the nation which is found unnoticed due to lack of sufficient research. If this condition goes one then it will develop hazardous problem in both human as well as pig. The available data and reported cases suggest that the disease is found to be endemic in the nation which is developing into serious public health problem. Many communities who rear pig for subsistence use are unaware of

zoonotic disease, proper sanitation and hygienic practices. Provision of public awareness should be conducted in those communities. Timely use of vaccination and medication will support for the reduction of incidence in both human as well as pigs.

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Author's Contribution

BKC reviewed the literature. KK provided guidance and logistic support to collect the articles. Those data were presented systematically and discussed about the reason behind the prevalence in Nepal.

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Antibiogram Pattern of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. Isolates from Broiler Chicken.

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ABSTRACT

This study was conducted on clinical cases of broiler chicken brought at National Avian Disease Investigation Laboratory (NADIL) and Veterinary Teaching Hospital, Agriculture and Forestry University during the period of December, 2018 to April, 2019. The study was aimed to find the antibiogram pattern of Escherichia coli, Salmonella species and Staphylococcus species. A total of 50 ill broiler liver samples were collected and inoculated in Nutrient Agar, XLD agar Mac-Conkey agar, EMB Agar and Mannitol Salt Agar and incubated for 24 hours at 37°C. During microbiological examination, prevalence of E.coli was 36 %, Salmonella species was 2% and Staphylococcus species was 8% where as mixed infection was 40%. Antibiogram profile for E. coli isolates were sensitive to Amikacin (88.89%) followed by Colistin (66.67%), Ciprofloxacin (50%), Levofloxacin (42.10%) and Gentamycin (27.78%) while Ceftriaxone (11.11%) and Tetracycline (11.11%) was recorded as least sensitive, for Salmonella species isolates were highly sensitive to Amikacin (100%) and other remaining antibiotics; Ceftriaxone, Gentamicin, Levofloxacin, Ciprofloxacin, Colistin and Tetracycline were observed to be resistant and for Staphylococcus spp. isolates were sensitive to Amikacin (75%) followed by Gentamicin (25%), Levofloxacin (25%), and Ciprofloxacin (25%) while Tetracycline and Colistin were resistant. In the conclusion, it is strongly recommended to decrease the unethical use of antibiotics to minimize the development of resistance strain of microbes in the future.

Keywords: Broiler, Antibiogram, *E. coli.*, *Salmonella*, *Staphylococcus*

INTRODUCTION

Poultry farming is flourishing in center of Nepal. Since last two decade poultry production had made tremendous difference from cottage industry to lively agribusiness. The GDP contribution of this sector stands at around 4 percent, and growing up rapidly. In fiscal year 2016/17, the share of the agriculture sector in GDP stood at 29.37 percent, while that of non-agriculture sector was 70.63 percent. The share of the agriculture sector in GDP has been in decreasing trend in the last 15 years. However, the number of commercial poultry farms, dairy cows and buffalo's farms and pigs has grown in recent period with growth in commercialization in the livestock sector (MOAC, 2017).

Chitwan district is the largest egg producer, and with approximately 11 percent of the total broiler population in the country, and has 1923 poultry farms (CBS, 2016). Though, many poultry farmers are facing the problem of poor production, disease and mortality (Conroy *et.al.*, 2005).

Escherichia coli, *Salmonella* and *Staphylococcus* species are the major bacteriological pathogens for the mortality of the chicken which causes great economic loss in the farm.

Escherichia coli is a Gram-negative, rod shaped, facultative anaerobic bacterium that belongs to the *Enterobacteriaceae* family, which may cause a great hazard to poultry industry causing high mortality, loss of weight and reduction in egg production (Abd El Tawab *et.al.*, 2015). Colibacillosis is one of the most common and serious problem that causes great economic loss to poultry enterprises. *E. coli* are mainly found as intestinal flora of human, mammals and birds and cause disease under the influence of poor managemental practices such as flawed ventilation, overcrowding of birds, extreme temperature and humidity, dehydration, hunger and in many immunosuppressive diseases. Coli septicemia is the most important disease caused by avian pathogenic *Escherichia coli* strains. This organism cause secondary bacterial infection in the upper respiratory tract of birds after primary viral infections such as the Newcastle disease, Infectious Bronchitis disease and *Mycoplasma* infection (Gross, 1991). The respiratory infection caused by avian pathogenic *E. coli* strains, further to the virus infection, is considered to be the initial step for colisepticemia development in birds (Gross, 1991). *E. coli* infection also called as aero sac disease; mostly it occurs among birds of two to twelve weeks of age with high mortality rates of about 20% (Dho-Moulin and Fairbrother, 1999). These bacterial strains are a potential reservoir for antimicrobial resistance genes and play an important role in the ecology of antimicrobial resistance of bacterial populations. The intestinal fecal flora from poultry and other meat producing animals can also transfer antimicrobial resistance to human pathogens via food chain (Rashid *et.al.*, 2017).

Salmonella are short bacilli, Gram-negative, aerobic, catalase positive, oxidase negative; they ferment sugars with gas production, produces H₂S, are non sporogenic and are normally motile with peritrichal flagella, except for *Salmonella pullorum* and *Salmonella gallinarum*, which are non-motile (Plym and Wierup, 2006). These organisms are Gram negative and rod shape which have been divided into over 2700 serotypes based on somatic, flagellar and capsular antigens (Gallegos-Robles *et.al.*, 2003). The pathogen can get transmitted by both horizontal and vertical routes. Avian salmonellosis is divided into two forms; Fowl typhoid and pullorum disease which both have a main economic significance worldwide. Fowl typhoid is attributed to *Salmonella enterica* subspecies. *Enterica* serovar *gallinarum* biovar *gallinarum* (*S. gallinarum*) can colonize and cause disease in various domestic and wild birds.

Staphylococci are spherical, ovoid gram positive cocci, non sporing, non motile, aerobic and normally facultatively arranged anaerobic cocci characteristically arranged in clusters. *Staphylococcus* spp. is an important opportunist that can cause superficial to life-threatening illnesses in a variety of animal species. In poultry, this organism has been implicated in osteomyelitis, synovitis, and cellulitis. The diseases associated with this organism vary in the severity from superficial skin infections to the more life threatening, systemic illnesses. It is not uncommon to isolate *S. aureus* from the yolk sac, liver, and skin of broiler.

In Nepal, Poultry farming has been growing tremendously over few decades. It has significant role to the national economy. It shares 8% of AGDP and 4% of GDP (Karki, 2005). Chitwan district has become the capital city in poultry production. *Salmonella* infection is one of the most significant causes of morbidity and mortality in the world and due to it is common between human

and animals, the human food chain is known as the main source of infection (Mkrtchyan *et.al.*, 2016)

The aim of this study is to estimate the prevalence of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. infection in broiler chickens and the antibiogram pattern against the isolated strains. Outbreaks of these bacterial infection remains as a serious economic problem in countries where control measures are not efficient or in those areas where climatic conditions, favor the environmental spread of the microbes. The economic losses are mainly due to morbidity, mortality, reduced growth rate and reduced feed conversion efficiency. Controlling *Escherichia coli*, *Salmonella* species and *Staphylococcus* species in poultry is problematic and it has relied historically on a combination of farm bio-security, water sanitization and the use of antibiotics.

The indiscriminate and injudicious use of antibiotics is an important factor in the emergence of antibiotic-resistant bacteria that subsequently can be transferred to humans through the food chain. In recent years, multidrug-resistant (MDR) phenotypes have been increasingly described among *Escherichia coli*, *Salmonella* species and *Staphylococcus* species all over the world.

MATERIALS AND METHODS

Study was conducted from December, 2018 to April, 2019 in Chitwan District which is located in the Southwest part of Province 3. The samples were brought from National Avian Disease Investigation Laboratory (NADIL) and Agriculture and Forestry University (AFU), Bharatpur, Chitwan, where the dead birds from different areas of Chitwan were taken for postmortem and disease diagnosis.

A total of 50 swab were taken from liver sample of suspected birds was collected aseptically with the help of sterilized swab. Swab was kept in sterilized vial containing 1ml peptone for the pre-enrichment of the bacteria. The collected swab then it was transported on a cold chain box with ice packs immediately to the microbiology laboratory of AFU.

Further processing was carried out by keeping the peptone water containing sample in incubator for 24 hrs. Then after 24 hour streaking performed directly on MAC agar plate and then it was left for incubation at 37 °C aerobically for 24 hrs. A tentative case diagnosis of avian *Collibacillosis* was based on the appearance on the Mac Conkey Agar culture plates with colonies resembling *E. coli* (rose pink colonies due to Lactose fermentation). After primary culture a single isolated colony from Mac Conkey agar was sub-cultured into EMB agar plate and incubated at 37 °C aerobically for 24 hrs where it produce colonies with green metallic sheen. However, in *Salmonellosis* appearance on the Mac Conkey Agar culture plates was colonies resembling convex shape, Small, Circular, translucent, colourless colonies, none Lactose fermenter. After primary culture a single isolated colony from Mac Conkey agar was sub-cultured onto the XLD agar plate and incubated at 37 °C aerobically for 24 hrs where it produce black centered colonies.

Eventually, after enrichment of 24 hour, streaking performed directly on Mannitol salt agar plate also and then it was left for incubation at 37 °C aerobically for 24 hrs. A tentative case diagnosis of *Staphylococcus* species was based on the appearance on the Mannitol salt agar culture plates with colonies resembling *Staphylococcus* spp (phenol red of the media turns to yellow).

Suspected purified colonies was smeared, fixed and stained with Gram's staining for the identification and for further confirmation biochemical test should be done for the identification. Suspected isolates of *Escherichia coli*, *Salmonella* and *Staphylococcus* species was confirmed by performing different biochemical tests like motility, Indole, Methyl Red test, Voges-Proskauer test, Simmon citrate test, Triple sugar iron (TSI) test, Catalase and Oxidase test.

Antimicrobial susceptibility test was done by using the Kirby-Bauer disk diffusion method as described by (Bauer, Kirby, Sherris, and Turck, 1966) following the Clinical and Laboratory Standards Institute, CLSI (CLSI, 2014) guidelines was performed. Before AST, isolated *E. coli*, *Salmonella* and *Staphylococcus* species are again cultured on TSA media. All *E. coli*, *Salmonella* and *Staphylococcus* species isolates were tested for seven different antimicrobial agents on Muller Hinton Agar (HiMedia). Isolates were inoculated in nutrient broth and incubated at 37 °C for 6 hrs. Turbidity of the sample would be adjusted to a 0.5 McFarland standard by dilution. Immediately after dilution, a sterile swab was dipped into the inoculum and streaked over the entire surface of the Mueller Hinton agar three times. Seven suitable antibiotics were selected. They were Amikacin (30 mcg), Ceftriaxone (10 mcg), Gentamicin (10mcg), Levofloxacin (5 mcg), Ciprofloxacin (5 mcg), Colistin (10mcg) and Tetracycline (30 mcg).

RESULT AND DISCUSSION

A total of 18 samples were found positive of *E. coli*, 1 sample was *Salmonella* and 4 samples were positive for *Staphylococcus* and 20 isolates of mixed infection from 50 broiler liver samples. The overall prevalence of *E.coli* was 36 %, *Salmonella* spp. was 2% and *Staphylococcus* spp. was 8%, whereas mixed infection was found 40% (Table 1).

Table 1: Prevalence of different organism in broiler liver sample

Organism	No. of Isolates	Prevalence Percentage
<i>Escherichia coli</i>	18	36
<i>Salmonella</i> species	1	2
<i>Staphylococcus</i> species	4	8
Mixed infection	20	40
No growth	7	14

Prevalence of mixed infection was higher followed by *E.coli* similar result was recorded by Rashid *et.al.*, in 2017. This higher prevalence of mixed bacterial infection might be due to the poor management practices at poultry farms as *E. coli*, *Staphylococcus* species and *Salmonella* species is opportunistic pathogen. *E. coli* infection may occur as secondary infection when birds are immune suppressed due to other diseases or environmental stress. Poor management practices and general hygienic conditions contribute to higher infection of *E. coli* (Zanella *et.al.*, 2000).

Table 2. Sensitivity pattern of antibiotics against different isolates

Growth of organisms	Antibiotics						
	Amikacin	Colistin	Gentamicin	Levofloxacin	Ciprofloxacin	Ceftriaxone	Tetracycline
<i>Staphylococcus</i>	3	0	1	1	1	14	0
<i>Salmonella</i>	1	0	0	0	0	0	0
<i>E. coli</i>	16	12	5	7	9	2	2

Antibiogram profile for *E. coli* isolates was sensitive to Amikacin was higher i.e. 88.89% followed by Colistin 66.67% while Ceftriaxone and Tetracycline was recorded as least sensitive i.e. 11.11%. Sensitivity to other antibacterials were recorded as; Ciprofloxacin (50%), Levofloxacin (42.10%), Gentamycin (27.78%). Antibiogram profile for *Salmonella* species isolates was highly sensitive to Amikacin (100 %). Other remaining antibiotics; Ceftriaxone, Gentamicin, Levofloxacin, Ciprofloxacin, Colistin and Tetracycline were observed to be resistant for *Salmonella*.

Antibiogram pattern for *Staphylococcus* species was highly sensitive to Amikacin (75%) followed by Gentamicin 25%, Levofloxacin 25%, and Ciprofloxacin 25% Tetracycline and Colistin was resistant. This study showed 36% isolation rate of *E. coli* from clinical cases of broiler liver sample which is lower to 71.2 % isolation rate by P. Shikha (2018). In this study, Amikacin was highly sensitive to *E. coli* i.e. 88.89% which is comparable with the result of T. Khanal (2017) found no resistance to Amikacin. Similarly Subedi *et.al.*, (2018) found 16 % and P. Shikha (2018) found 6.89% resistance to Amikacin which is similar to this study.

CONCLUSION

This study showed high prevalence of mixed infection followed by *Escherichia coli*, *Staphylococcus* and *Salmonella* infection. Antibiogram profile for *E. coli* isolates were sensitive to Amikacin followed by Colistin, Ciprofloxacin, Levofloxacin and Gentamycin while Ceftriaxone and Tetracycline were recorded as least sensitive, for *Salmonella* spp. isolates were highly sensitive to Amikacin and other remaining antibiotics; Ceftriaxone, Gentamicin, Levofloxacin, Ciprofloxacin, Colistin and Tetracycline were observed to be resistant and for *Staphylococcus* spp. isolates were sensitive to Amikacin followed by Gentamicin, Levofloxacin, and Ciprofloxacin while Tetracycline and Colistin were resistant. Thus, it is strongly recommended to use the antibiotics after antibiotic sensitivity test to minimize the development of resistance strain of microbes in the future and to minimize the economic loss.

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Pregnancy Diagnosis with Progesterone ELISA Kit in Farm Animals, Its Accuracy and Application

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ABSTRACT

Pregnancy is a special condition where a female lodges one or more young ones within her uterus. It is maintained by various endocrine physiology and metabolic changes between maternal and fetal circumstances. Space dine secreted by corpus luteum increases extraordinarily throughout the pregnancy, estrogen increases rapidly during first trimester and prolonged lifespan of corpus luteum and small quantity of estrogen prevents prostaglandins pulsatic secretion. We reviewed available literature to evaluate the accuracy of progesterone ELISA test kit in pregnancy diagnosis in farm animals. We found varying accuracy in global and Nepalese context but found that this is an important tool for early pregnancy diagnosis and infertility monitoring with high accuracy which contributes to increase economic efficiency of a farm. This kit has been used for qualitative and quantitative study of progesterone to understand the reproductive status of animals. Therefore, its use is increasing in globally and in Nepal. This kit has been used at various breeding centers, and livestock service centers of Nepal. However, wider level use is still difficult due to lack of infrastructures and subsistence farming.

Keywords: ELISA Kit, Pregnancy diagnosis, Progesterone

INTRODUCTION

Pregnancy or gestation is the condition or time of a female while one or more young ones are developing within her uterus, maintained by various endocrines physiology and metabolic changes between mother and fetal boundary. Pregnancy diagnosis is a matter of curiosity for farmers since long time (Purohit, 2010). Early pregnancy diagnosis is associated with profitability of the farm due to factors like amount of milk or meat or fleece produced per day per herd, decisions of keeping or culling, feeding schedules and formulations (Dionysius, D.A., 1991) as well as the identification of reproductive diseases for their early treatment and rebreeding of the healthy non-pregnant ones (Engeland, Ropstad and Eik, 1997).

Per cow per day loss will be approximately US\$ 2 due to variable costs like feeding costs, breeding costs, labor costs, veterinary costs etc. (Ali, 2011); if the animal delays conception beyond 85 days postpartum which will increase the calving interval of the animal (Stevenson and Call, 1986). Pregnancy diagnosis is a key for shortening the calving interval by reducing the postpartum barren interval in farm animals (Balhara, Gupta, Singh, Mohanty, and Singh, 2013). So, the main target in a herd is not to allocate the pregnant but to identify the non pregnant animals and re-inseminating her as early as possible (Stevenson and Call, 1986)

Animal farm management will be vague and economically boredom without early diagnosis of pregnancy in farm animals. Accurate and early gestation diagnosis is essential which helps in proper management and identification and rebreeding of the non pregnant at the earliest. An early pregnancy diagnosis is essential in mares to tease them if non pregnant, and try to get them pregnant in the same season (Sah *et.al.*, 2017).

Progesterone (P4), an endogenous steroid is a progestogen sex hormone (Sah *et.al.*, 2017). It targets the uterine epithelium, mammary tissue, myometrium and hypothalamus causing endometrial secretion, reducing cervical mucus production, inhibition of GnRH release and promotes maintenance of pregnancy. Its main source is corpus luteum or yellow body of ovary (Stevenson and Call, 1986). But, during pregnancy of farm animals, there is wide species variation in source of progesterone. In case of sow and goat, corpus luteum is very essential source throughout the pregnancy. In sheep and mare, placental production of progesterone in sufficient quantity starts in about 50 and 70 days of pregnancy respectively. P4 gets secreted into blood and subsequently into milk. But its level is higher in milk than in blood due to having more affinity of steroids towards milk fat.

Progesterone at low level stimulates ovulation by promoting release of LH in coordination with estrogen, while at high level of progesterone; it hinders the secretion of FSH and LH by negative feedback mechanism. But, FSH is released sufficiently so that follicles may progress in luteal phase. In a normally cycling cow, levels of P4 are relatively low in both blood and milk around estrus. Then P4 increases in both fluids. On day 17 or 18 of the estrous cycle, it declines as a new follicle develops. If a cow is pregnant, P4 levels would not decrease on day 17 or 18, but would remain elevated (Petersson, 2007).

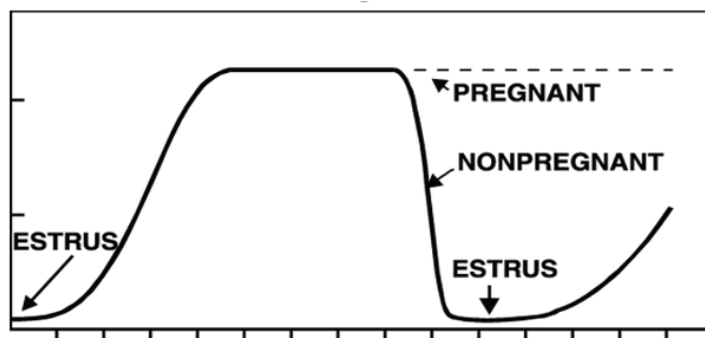


Figure 1. Changes in progesterone levels throughout the estrous cycle (adopted from Silvia and Heersche)

Progesterone quantification in blood and milk in laboratory can be obtained by Radioimmunoassay (RIA) or Enzyme-Linked Immuno-Sorbent Assay (ELISA) methods (Sah *et al.*, 2017). This quantification could help in pregnancy examination as early as 21 days after natural or artificial insemination (Fricke *et.al.*, 2016). This possibility that progesterone assay in plasma and milk could guide early pregnancy diagnosis in farm animals was proposed in 1971 by Laing and Heap (Rioux and Rajotte, 2004)

METHODS FOR PREGNANCY DIAGNOSIS

The ideal pregnancy diagnosis methods should have high sensitivity to detect pregnant animals and high specificity to detect non-pregnant ones. In addition, they need to be convenient to be used in field conditions and inexpensive for farmers to adopt it. The additional qualities such as embryonic or fetal viability, sex determination for the future benefit would add value to it (Fricke *et.al.*, 2016).

There are various direct and indirect methods for rapid and early gestation diagnosis in animals (Fricke *et.al.*, 2016). Repeat heat in 18/24 days post insemination, rectal palpation to find uterus and its contents (expert clinician can detect as early as in 35 days), ultrasonography and radioimmunoassay or enzyme linked assay of Progesterone hormone in blood serum, plasma and milk of lactating animals are some of them. There are mainly five chemical test kits available for detecting the hormone in milk and one for the blood serum in heifers (Stevenson and Call, 1986). And, the various ELISA kits available are Progesterone ELISA kit from the Abraxis LCC USA Company (used in A.K. Sah's experiment), Ovucheck product no.C006 purchased from the North American distributor Biovet, Saint-Hyacinthe, QC, Canada (in Stevenson and Call's experiment), Noctech Progesterone EIA (used in Dionysius' experiment), and Enzygnost Milk Progesterone Vet\$ (in Dionysius experiment).

Cattle has an estrous cycle of 21 days. Estrus duration is 18 hours on average and ovulation starts nearly 11 hours later. The size of corpus luteum increases significantly from day 3 to 12 and same does the level of progesterone in blood and milk (Rioux and Rajotte, 2004). Fluctuation in level of milk and blood progesterone implies the changes in the reproductive condition of farm animals (Romangolo and Nebel, 1993). These changes thus can monitor the manager to assess the optimal time of artificial insemination of the animals to obtain the better establishment of pregnancy. Significantly less than 10% of all herds are currently using milk progesterone as a diagnostic aid.

SCENARIO OF PREGNANCY DIAGNOSIS IN LIVESTOCK IN NEPAL

In the context of Nepal, farmers need to wait for more than a month to know the status of their cattle's pregnancy when cattle return to heat in about 21-22 days of estrous cycle. Only the trained and skilled expert can detect the pregnancy by rectal palpation in one month, the accuracy of which is highly variable (Sah *et.al.*, 2017). Annual report of NCRP, 2016 have highlighted that early pregnancy diagnosis to maintain the reproductive efficiency of farm animals have been a challenge in Nepal due to the lack of infrastructures such as the Ultrasonography (USG) machine, trained personnel to use it and poor economic status of farmers in rural areas (Sah *et.al.*, 2017).

RESULT

We reviewed available scientific articles to identify the accuracy of progesterone kit in Nepal for pregnancy diagnosis in different farm animals such as sheep, goat, cattle of various breeds. We found varying percentage for accuracy of progesterone kits ranging from 60 to 100% for pregnant and 80 to 100% for non-pregnant with varying degree of sensitivity and specificity. In a

research of Chitwan, Nepal, 80% accuracy with 92% sensitivity and 57% specificity was found (Sah *et.al.*, 2017). The results of progesterone kit in pregnancy diagnosis in different farm animals are presented in Table 1.

Table 1. Results of progesterone kit accuracy in the pregnancy diagnosis in different farm animals

SN	Farm animals	Number of animals	Types of samples	Days of sampling (Post-insemination)	Accuracy percentage		Remarks	Reference
					Positive	Negative		
1	Crossbred Jersey and Holstein dairy cows	20	Blood (serum)	37-40	80		92% sensitivity 57% specificity	Sah, <i>et.al.</i> , 2017
2	Lactating holstein + Guernsey cows	200	Milk	21-22	77	100		Zaied, <i>et.al.</i> , 1979
			Milk	27-28	95	100		
3	Lactating Friesian cow	25	Blood (Peripheral plasma)	21-22	85			(Skemesh, <i>et.al.</i> , 1973)
4	Lactating ewes of local breed	62	Blood (Peripheral plasma)	16-21	93	100		(Skemesh, <i>et.al.</i> , 1973)
				31-35	100			
5	Lactating Holstein cows	91	Milk	20	67.3	87.2		(Cox <i>et.al.</i> , 2010)
6	Cows	476		21-24	88.6	93.9		(Reimers <i>et.al.</i> , 2010)
7	Zebu cattle	64	Milk	20	84	100		Alam, and Ghosh, 1994
			Plasma		84			
			Milk	24	90			
					92			
8	Holstein Friesian cows (Multi-parous + Heifers)	10+8	Blood (plasma)	22	71.4	100		Muhammd, <i>et.al.</i> , 2000
				25	83.3			
9	Italian Friesian cows	82		19-23	92.3	68.2		Romangolo, and Nebel, 1992
10	Dairy goats	73	Milk+ Blood	20	82	88		Engeland, <i>et.al.</i> , 1996
11	3 commercial dairy goats	28	Milk	21-24	96.4	100		Dionysius, 1991
12	Cows of 4 commercial dairies	42	Milk	22	65.5	90.9	Ovuch-eck EIA was used	
					68.2	90	Enzygn-ost vet was used	
13	Dairy cattle		Milk	21-24	84	90-100		Stevenson and Call
14	Cows of 14 dairy farms	752	Milk				97% heat detection accuracy ; 90% sensitivity 98.7% specificity	Ingenhoff, <i>et.al.</i> , 2013
15	Zebu cattles	46	Plasma	20	84	100	RIA was used with ¹²⁵ I isotope.	Alamand Ghosh, 1994
				24	90			
		58	Milk	20	84			
				24	92			

DISCUSSION

The ELISA kit has not gained popularity due to its low specificity and less accuracy. Progesterone kits are not used in NLBC, Pokhara and NCRP, Rampur, Chitwan currently for pregnancy diagnosis. Ultrasonography is the gold standard test for pregnancy diagnosis in Nepal (Personal communication Dr. A.K Sah). Various pregnancy specific proteins kits like BIOPRYN, with 100% accuracy, have also been used for rapid test (Karki *et.al.*, 2018). Even in

IAAS farm, no provision of early pregnancy diagnosis is practiced using USG or progesterone assays. Rectal palpation only after 3 months is being practised, which is surely to increase the farm cost if the animal is detected to be open cow.

Accuracy of pregnancy positive is repeatedly lower than that of negative (non-pregnant), mostly due to various causes like embryonic mortality, inaccurate sampling, ovarian luteal cysts, prolonged luteal lifespan and breeding during luteal phase. Presence of some animals having long estrus intervals, or short estrous cycles increases the false positive results of this test. Cost of P4 testing and the labor involved in obtaining multiple samples also incur loss. Early pregnancy loss will also limit the accuracy of many direct and indirect methods of pregnancy diagnosis.

Potential uses of progesterone testing in a reproductive management program according to Nebel, R.L. are in identifying errors in detection of estrus. Animals with questionable signs of estrus can be verified by on-farm milk progesterone tests. Low P4 concentration of milk alone cannot be a single positive indicator of estrus. But, high P4 level confirms the non-estrus state of animals, even if they have any behavioral heat symptoms. Likewise, it may be used to predict time of estrus. Level of progesterone will be very low at estrus and then will go increasing. If the animal gets fertilized, the level will remain elevated throughout the gestation but if not, it will be reduced rapidly in 17/18 days of estrous cycle (2-4 days before estrus) due to the decrease in the size of the corpus luteum in the absence of fertilization and again will come in heat. Likewise, the possibility of establishing a rapid gestation diagnosis in domestic animals by means of a progesterone assay in plasma and milk was introduced from 1971 by Laing and Heap (Rioux and Rajotte, 2004). This is also helpful in differentiating types of ovarian cysts. Ovarian cysts mainly follicular and luteal cysts can be differentiated by the quantification of progesterone where follicular cyst is characterized by low progesterone level whereas luteal cyst is characterized by very high level of progesterone, sometimes up to ml in milk or blood. In addition, this test has been used in evaluating response to endocrine therapy. Any endocrine treatment affecting the ovarian status can be evaluated by the progesterone test. Example: for follicular cyst for leutinization, if GnRH is administered, Progesterone testing 10 days after GnRH administration could guide accurately if retreatment is needed or not.

CONCLUSION AND RECOMMENDATION

Progesterone (P4) is a very crucial component of pregnancy and uterine biology, as well as for mammogenesis and lactogenesis. Due to having less accuracy and specificity, progesterone ELISA cannot be used for confirmatory diagnosis, but can be used for screening purpose after 21 days post insemination. In agro-veterinary sciences, one can demonstrate the usefulness and efficiency of this test to predict estrus (heat), gestation, and to evaluate pathological conditions and responses to different hormone-based medicines. However, further research is needed to determine the relative milk progesterone concentrations to improve the reproductive efficiency and economy of dairy farms.

We recommend that due to less accuracy and specificity of progesterone assay, it should not be used for confirmatory pregnancy but may be used as a part of the reproductive management program.

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Factors Leading to Early Embryonic Death

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Early embryonic death is defined as the loss of embryo or conceptus which occurs between fertilization and 15 days post insemination. Early embryonic death leads to reproductive failure in animals resulting in reduced pregnancy rates, slower genetic improvement and substantial impact on farm profitability. Early embryonic death is an eloquent limiting factor for the completion of pregnancy in animals (Inskeep and Dailey, 2005). Incidence of embryonic losses is usually higher than perinatal losses. Notably, fetal development may or may not have affected by maternal infections during pregnancy (Givens and Marley, 2008). Early embryonic death occurs before the fetal calcification. Complete resorption of the embryo is usually seen during early embryonic death (Wrathall, 1975). Embryonic or fetal death leads to resorption, mummification, maceration or abortion. Gestational age, cause of death, and source of progesterone for pregnancy maintenance are the factors that impacts the outcome of the embryonic or fetal death (Givens and Marley, 2008). Most often, early embryonic death, post implantation is due to the uterine environment rather than fetal development (Clark et al., 1986). Dead embryos, between the time of implantation and calcification, are also resorbed. If the entire litter is lost, the dam will return to service at an irregular interval, usually 5 to 10 days post pregnancy loss (Wrathall, 1975) and pregnancy is continued if even a single number of embryo remains (Christianson, 1992). In a farm or within a particular litter, more than one type of embryo or fetal death can be found, but accurate classification is important for further better investigation (Christianson, 1992). Diagnosis is usually very difficult; as the agent or cause of embryonic death is no longer present while investigation is taking place. So, it is important to be aware of variety of causes of embryonic death that helps to rule out the particular problems that can be diagnosed and treated (Christianson, 1992). Proper history such as vaccination status, feed changes, housing, environmental temperature and so forth should be taken, that helps to narrow down the potential list of problems to consider and root cause can be found.

Keywords: Early embryonic death, Embryonic, Fetal death, Gestation, Pregnancy

INTRODUCTION

Early embryonic death has a disastrous impact on the fertility of domestic animals. It mostly occurs during days of fertilization and during the process of implantation. Causes of early embryonic death can be divided into infectious and non-infectious categories. Major attention has often been given to the infectious causes, but non-infectious causes probably accounts for more than 70% of the cases of early embryonic death (Vanroose *dt.al*, 2000). Embryonic environment can be infected by specific and non-specific uterine pathogens. Specific uterine infections are caused by various bacteria, virus, protozoa and possibly mycoplasma, which enter the uterus through haematogenous route or via the vagina. Non-specific pathogens are mostly bacteria that enter the uterus by ascending infection. Uterine pathogens may cause early

embryonic death directly by contaminating the uterine environment leading to endometritis or by direct cytolytic effect on the embryo and directly by systemic effects via septicemias, viremias or toxemias on the dam (Blackwell *et.al.* 1987). Non-infectious causes of early embryonic death are chromosomal aberrations, external factors e.g. high ambient temperature, nutritional factors and maternal factors. Causes of early embryonic death are multifactorial and very difficult to diagnose (Vanroose *et.al.*, 2000). A useful procedure in differentiating infectious and non-infectious causes of early embryonic death is to test the presence of immunoglobulins in uterine contents by immunodiffusion (Chaniago *et.al.*, 1978). If immunoglobulin is present, further tests for antibodies to specific agents are performed (Christianson, 1992). The overall objective of this study is to identify the factors leading to early embryonic death in animals. Specific objectives to study the infectious and non-infectious causes of early embryonic death in animals and to study the nutritional, maternal and other factors leading to early embryonic death in animals.

MATERIALS AND METHODS

Comprehensive review of various articles, journals, literatures and websites were done to find out the major factors leading to early embryonic death in animals as follows:

Infectious Causes

Infection of the embryonic environment (oviduct and uterus) can be caused by specific and non-specific pathogens such as *Toxoplasma gondii*, *Campylobacter* spp. Bovine viral diarrhea virus (BVDV), etc. These infection results in inflammatory products which must be eliminated before the embryo descends into the uterus. Particularly in older animals (e.g., the mare), this uterine clearance can be impaired. Acute endometritis, after mating or artificial insemination, has direct effect on the embryonic environment and in severe cases is accompanied by the production of luteolytic substances such as prostaglandins (De Winter *et.al.*, 1995, van). Uterine infection due to bacteria mostly causes diffused and severe purulent inflammation while viral infection, in most cases is characterized by a necrotizing endometritis leading diffused and total lymphocytic and plasmacytic changes in the endometrium. *Chronic endometritis* involves morphological and functional changes in the uterus besides inflammation. The layers of fibrous tissue are deposited around endometrial glands, which results in a deficiency of functional glands, which deprive the embryo of the protein-rich exocrine secretion. Infections of the embryo can take place at two important phases in the embryonic development; before hatching and after hatching.

Before Hatching (Zona Pellucida-Intact Embryos)

Of all pathogens, viruses are the most insidious and dangerous type of infection for the early-stage embryo. Viral infection of the zona pellucida-intact embryo can already have taken place before fertilization during maturation of the oocyte. Viruses, e.g. bovine herpes virus 1 BHV-1 and BVDV, might be present in follicular fluid or granulosa cells of bovine oocytes (Bielanski *et.al.*, 1993), and can also contaminate the embryos by sticking to the glycoprotein layer, which surrounds the oocyte, the so-called zona pellucida. In persistently-infected cattle, BVDV antigen has even been detected inside the oocyte (Brownlie *et.al.* 1997). Virus adhering to the zona pellucida or to the fertilizing spermatozoon might be introduced into the oocyte by the sperm track in the zona pellucida created at the time of fertilization (Bowen, 1979). Passive migration

of virus through the meshes in the zona pellucida is highly unlikely to occur, since particles with a diameter of 40 and 200 nm comparable size as BVDV and BHV1 remain stuck in the peripheral part of the zona pellucida (Vanroose, 1999). One of the smallest viruses, the porcine parvovirus, could pass the zona pellucida in pigs is seen in only one report ever (Bolin *et.al.* 1983). After fertilization, the zona pellucida can be considered as an effective barrier for virus penetration (Stringfellow *et.al.*, 1991; Vanroose *et.al.*, 1999a). However, death of a zona pellucida-intact embryo can occur because of a hostile uterine environment, at the early embryonic stages.

After Hatching

Embryos hatch from the zona pellucida at 8–9 days in cattle, 7–8 days in sheep and 6–7 days in pigs. In the horse, a non-cellular membrane envelops the early equine conceptus which resembles the zona pellucida is gone by day 20. After hatching or after removal of the zona pellucida, the embryonic cells are susceptible to some infectious agents (Wrathall and Suttmoller, 1998). For example, zona pellucida-free bovine morula and blastocysts are susceptible to bovine herpesvirus-1 (Bowen *et.al.*, 1985; Bielanski *et.al.*, 1987; Vanroose *et.al.*, 1997) and BVDV (Brock and Stringfellow, 1993; Vanroose *et.al.*, 1998), but not to bovine parvovirus (Bowen, 1979). Recent research on embryo–pathogen interactions has mainly been performed in cattle, by exposing in vivo derived or in vitro produced embryos to specific pathogens in vitro (Wrathall and Suttmoller, 1998; Vanroose, 1999).

Besides these in vitro studies, earlier in vivo studies have shown that viral uterine infections can result in extensive viral replication in embryonic cells after hatching and implantation. In addition, cells undergoing quick division, as occurs in embryos, are particularly susceptible to the replication of certain viruses (Bowen, 1979). The outcome of such an infection can either be cytolytic or non-cytolytic. Both can result in early embryonic death, but a non-cytolytic infection can also cause chromosomal damage and induce embryonic cells to divide more slowly. This retardation in cell division occurs during the critical phase of organogenesis. Consequently, viral infection of embryos may result in the development of congenital malformations.

After implantation, the haematogenous route of uterine infection becomes more important, since both endometritis and a direct cytolytic effect on the embryo are possible.

Non-infectious Causes

Chromosomal aberrations are a major cause of early pregnancy failure in animals (King, 1990). A range of misalliances can occur during the pairing of the haploid parental chromosome sets at the time of fertilization, which are subsequently lethal to the embryo. Chromosomal abnormalities may also originate by penetration of more than one sperm cell (polyspermia). Mixoploidy, polyploidy and haploidy are all aberrations that are encountered frequently in in vitro produced embryos (Kawarsky *et.al.*, 1996; Viuff *et.al.*, 1999) but it has not been investigated yet whether this could be a cause for the higher embryonic death rates, which are observed after the transfer of in vitro-produced bovine embryos (Van Soom *et.al.*, 1994). King (1990) reported that chromosomal abnormalities may account for approximately 20% of the total embryonic and fetal loss. Sometimes an enzyme deficiency results in early embryonic death, e.g.,

deficiency of uridine monophosphate synthetase (DUMPS) in cattle, which is an autosomal recessive disorder (Schwenger *et.al.*, 1993).

External Factors

High Environmental Temperatures

High environmental temperatures during the first months of gestation can also have inimical effects on the embryo (DelaSota *et.al.*, 1998). The mechanism for this effect has been explained by a direct damaging effect of a high uterine temperature on the embryo and by the shunting of blood away from the uterus to the periphery in an attempt to maintain body temperature, resulting in a reduced nutrient load (Dziuk, 1992).

Specific Nutritional Deficiencies or Malnutrition

Specific nutrient deficiencies or malnutrition can have a negative effect on the embryo. Especially a severe deficiency of vitamins (vitamin A) or other nutrients (Cu, Zn, I) that serve as regulators of metabolism can cause early embryonic death (Graham *et.al.*, 1995). Malnutrition or a severe negative energy balance may affect the follicular development, the quality of the oocyte, and the secretory and motile activity of the oviduct which is the place of the fertilization process. These findings demonstrate that nutrition affects the very early stages of conceptus (Butler and Smith, 1989; Foxcroft, 1997).

Stress

Stress has a harmful effect on reproductive efficiency in animals (Dobson and Smith, 1995). Stressors (e.g. transport, mechanical injury, isolation, pain, changes in blood pressure, etc) affect the reproductive function via actions at the hypothalamic level (GnRH) or at the ovarian level (progesterone).

Environmental Toxicants, Teratogenic Compounds and Mycotoxins

Environmental toxicants, teratogenic compounds and mycotoxins can have drastic adverse effects on the survival of embryos when ingested at crucial early stages of gestation (Christianson, 1992; Brendemuehl *et.al.*, 1994).

Maternal Factors

Hormonal Imbalance

Progesterone is necessary for the maintenance of pregnancy. A deficiency of progesterone caused by primary luteal insufficiency has been reported as a cause of early embryonic death, but is probably not of frequent occurrence (Wathes, 1992; Mann *et.al.*, 1998).

Disturbance of the Embryo-maternal Interactions

Disturbance of the embryo–maternal interactions can result in embryonic loss. Before implantation, embryonic signaling is necessary for maternal recognition of pregnancy. This commences the hormonal changes, which are needed to evoke the transformations in uterus necessary for implantation (Gandolfi *et.al.*, 1992; Geisert *et.al.*, 1992; Hansen, 1997).

Insufficient Uterine Space

Embryonic loss is resulted from insufficient embryonic loss (Allen, 1992; Dziuk, 1992). The presence of extra embryo, more than one, in cattle and horses and more than 20–25 embryos in swine causes a competition between the embryos for access to the endometrium. For example, in the mare, natural embryo reduction of twin embryos (10–20% of the pregnancies) occurs in a majority of the cases, especially between the second and sixth week of pregnancy (Ginther, 1989).

Age of Dam

Aged animals have minimal follicular activity and oocyte quality resulting in a reduction of the developmental competence of embryos. Furthermore, the quality of the endometrium is deteriorating with increasing age.

Inbreeding

Inbreeding has been reported as a cause of early embryonic death (Hanzen *et.al.*, 1999). It has also been proven that the early embryonic death differs from breed to breed.

CONCLUSION

The death of embryo between 1-15 days post insemination, before fetal calcification, is called early embryonic death. It has high impact on farm profitability. Early embryonic death leads to resorption, mummification, maceration or abortion. The causes of early embryonic death may be infectious or non-infectious. Infectious causes includes specific and non-specific uterine pathogens while non-infectious causes includes chromosomal aberrations, maternal factors; hormonal imbalance, age of dam, insufficient uterine space, embryo-maternal interaction disturbances and inbreeding, and other factors; environmental temperature, nutritional deficiency, stress and toxicants. Although it is difficult to diagnose the cause of early embryonic death, it is necessary for proper diagnosis and elimination of early embryonic death as it is limiting factor for the successful pregnancy, and disastrously affecting the animal farms and overall animal industry economy.

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Bacterial Assessment of Ready to Eat Sweet (*Burfi*) Sold in Different Sweet Houses in Narayangadh, Chitwan: A Pilot Study

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ABSTRACT

As a sweet product, burfi is highly esteemed by Nepalese consumer and kept in high priority from the time immemorial. However, the milk and milk products borne outbreaks account for 2 to 6 % of bacterial foodborne outbreaks in several countries. Hence, with an objective to determine the bacterial load of the commercial sweet (burfi) sold in Bharatpur metropolitan city 1, Narayangadh, Chitwan, a cross-sectional study was conducted. Ten sample of ready to eat sweet (Burfi) along with the packaging material were collected purposively from different sweet shops of Bharatpur metropolitan on January 2019. The preparation of samples was done as per Food Microbiology Protocols (2001) and the aerobic colony count (ACC) was determined by spread plate technique using plate count agar. It was found that the aerobic plate count of burfi sample ranges from 9.1×10^9 to 1.5×10^{10} CFU/g with an average of 1.2×10^{10} CFU/g, which is higher than the permissible Standard Plate Count according to Indian Standards Institution (ISI) specification. In conclusion, the high bacterial count in Burfi samples from different sweet shops indicates insufficient hygienic condition duration handling and unhygienic conditions of storage. This may give rise to public health hazard.

Keywords: Bacterial load, Bharatpur

INTRODUCTION

Milk based traditional sweet products such as lalmohan, gundpak, khoa, pedha, burfi, tar, sikarni, pustakari, and kalakand contribute the dominant share in the food market of Nepal (Acharya, Kharel, and Chetana, 2015). Khoa is used for different types of sweet making like, pedha, burfi, gulab jam and other sweets (Vaidya, Dr. Ghugare, and Dr. Kutty, 2015). Khoa can serve as a favorable medium for the growth of a variety of microorganisms due to its high moisture content and good nutritive value (Microbiological Quality and Safety Aspects of Traditional Dairy Products, 2012). Burfi is a dense milk based sweet from the Indian subcontinent, a type of mithai (Wikipedia, 2019). Burfi is prepared by the addition of khoa, sugar and other additives like aromatic spices, nuts, chocolate, shredded coconut, etc. which are mixed well and heated over low fire until it assumes a consistency capable of forming a hard flat surface on spreading, the hard mass is collected in a tray and after setting, cut down into desired shape (Yadav, 2002). As a sweet product, burfi is highly esteemed by Nepalese consumer and kept in high priority from the time immemorial. The milk borne and milk products borne outbreaks account for 2 to 6 % of bacterial foodborne outbreaks in several countries (Pal and Jadhav, 2013). An outbreak of food poisoning due to the consumption of pedha and burfi has been reported by Mandokhot and Chandiramani (1983).

The objective of the present study is to find out the microbiological quality of the commercial burfi sold in Bharatpur metropolitan city 1, Narayangadh, Chitwan.

MATERIALS AND METHODS

Sample Collection

A total of ten purposive samples of ready to eat burfi were collected in sterilized glass petri plates from the different sweet houses located at Bharatpur metropolitan city 1, Narayangard, Chitwan during January 2019. Samples were collected in same packaging material which is normally given to customers.

Transportation

Collected samples were transported to Veterinary Microbiology Laboratory of Agriculture and Forestry University, Rampur, within an hour of collection in thermo cool box by placing ice packs in between and placed in refrigerator at 4 °C until further preparation was done.

Sample Preparation

Ten gram of the sample was homogenized with 90 ml sterilized distilled water by mincing in sterilized mortar and pestle for 2 minutes. Tenfold serial dilution of the homogenate was done in sterilized distilled water up to 10^{-7} folds.

Inoculation of Sample

Immediately after homogenization, 0.1 ml of diluted sample from each dilution were plated at plate count agar plates prepared 24 hours before inoculation. Samples were inoculated evenly over the surface of the plates using spread plate method with the help of sterile glass spreader. Each dilution was plated in duplicate. All the plates were incubated at 32 °C for 48 hours.

Counting of Colony

Two plates corresponding to one dilution and showing between 30 to 300 colonies per plate were computed. The average of two count was recorded as final colony forming unit (CFU) count.

CFU per gram was calculated by using formula,

$$\text{CFU/g} = \text{CFU/plate} \times \text{dilution factor} \times 1/\text{aliquot}$$

RESULT

The result of microbiological count of burfi is shown in the Table 1. The aerobic plate count of burfi sample ranges from 9.1×10^9 to 1.5×10^{10} CFU/g with an average of 1.2×10^{10} CFU/g. This is higher than the permissible Standard Plate Count according to Indian Standards Institution (ISI) specification which is 30,000 CFU/g (ISI 1970) (Bandekar, Kamat, and Thomas, 1998). Food Safety and Standard Act, 2006 India (FSSAI) (Government of India, Ministry of Health and Family Welfare., 2011) has given microbial standard for khoa where the acceptable level of Total Plate Count is set at 50,000 CFU/g.

Table 1: Microbial Quality of Burfi

Sample Id	CFU/g	Sample Id	CFU/g
A	1.05×10^{10}	F	1.2×10^{10}
B	1.2×10^{10}	G	1.3×10^{10}
C	1.0×10^{10}	H	1.5×10^{10}
D	1.4×10^{10}	I	1.1×10^{10}
E	1.3×10^{10}	J	9.1×10^9

DISCUSSION

The average aerobic plate count of burfi sample is found to be 1.2×10^{10} CFU/g which is higher than the findings by Motina and Shekllar (2007) in India where the mean bacterial count in burfi was 4.5×10^5 CFU/g. Suryawanshi (1991) found the average total bacterial counts of burfi samples in Parbhani, India to be 4,580,000 CFU/g and the range of bacterial count was 126,000 and 26,700,000 CFU/g. Standard plate count (SPC) in market samples of burfi ranged from 2×10^3 to 6×10^5 CFU/g (Ghodekar *et.al.*, 1974), 2.16×10^6 /g (Singh *et.al.*, 1975), from 4×10^2 to 5.2×10^2 CFU/g (Dwarkanath and Srikanta, 1977), from 5.3×10^2 to 7.4×10^4 CFU/g (Gary, S.R., 1981) and from 5.0×10^2 to 4.4×10^5 CFU/g (Misra and Kuila, 1988).

CONCLUSION

The high standard plate counts as observed in the present investigation clearly indicated that the conditions of product preparation were unsatisfactory. Since, quite high temperature of preparation is normally employed, the high bacterial counts are suggestive of post preparation contamination of the products and unhygienic conditions of storage. This may give rise to public health hazard.

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Prevalence of *Dirofilaria* and Hemato-biochemical Effect in Street Dogs of Kathmandu Valley and Siddharthanagar Municipality, Bhairahawa, Nepal

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ABSTRACT

A cross sectional study was conducted to determine the prevalence of dirofilariosis in street dogs of Kathmandu valley and Siddharthanagar, Rupandehi from January 2019 to March 2019. Blood sample from 155 dogs of Kathmandu Valley and 150 from Siddharthanagar municipality were collected and examined by Wet Smear, Modified Knott Technique and Buffy Coat methods. PCV value and biochemical parameters were analyzed. Data were analyzed to determine the Prevalence of *Dirofilaria* and correlation of infections with age, sex and biochemical parameters. The prevalence in Kathmandu valley was 0% (0/156) while the prevalence in Siddharthanagar was 19.33%, while the prevalence in direct smear, buffy coat and modified Knott technique were found to be 16%, 16% and 19.33% respectively. The prevalence were significantly ($p < 0.05$) higher in old age and higher in 3-5 years of age i.e. 51.70%. Prevalence was higher in female (21.70%) than male (17.3%). AST and ALT were significantly ($p < 0.05$) increased in infected dogs. Total protein, ALP, BUN, Creatinine level were elevated in the infected dogs.

Keywords: *Dirofilaria*, Dog, Biochemical

INTRODUCTION

Dirofilaria is an intravascular nematode parasite of the superfamily Filarioidea that consists of mostly two species *Dirofilaria immitis* and *Dirofilaria repens* affecting canine soulsby, 2012. Dirofilariosis is prevalent in tropical, subtropical and temperate region worldwide.

Transmission occurs through blood sucking mosquitoes of *Aedes*, *Culex* and *Anopheles* species (Soulsby, 2012). Over 60 mosquito species have been shown to be able to *Dirofilaria* under experimental or natural conditions (Norma *et.al.*, 1998). The mosquito vectors that can transmit *Dirofilaria* commonly present in Kathmandu valley and Rupandehi are *Aedes albopictus*, *Culex quinquefasciatus*, *Aedes aegypti* (Darsie *et.al.*, 1990). Development of *D. immitis* to L3 larval stage in mosquitoes occurs at threshold temperature 14 °C (Genchi *et.al.*, 2010). Pathological changes in *Dirofilaria immitis* has been reported in reductions of RBC, haemoglobin and PCV along with alterations of BUN, Creatinine, ALP, AST and GGT (Bahadori *et.al.*, 2010).

The prevalence rate of heart worm in Assam and Mizoram area of Northeastern state of India is 11.38% out of 413 sample of stray dog (Borthakur *et.al.*, 2015). The prevalence of *Dirofilaria immitis* in Busan, Korea in 77 stray dogs was 6.5% (Byein *et.al.*, 2007). Wang *et.al.*, reported prevalence of *Dirofilaria* in Henan province of central China from March 2015 to Feb 2016 with

the overall recorded seroprevalence of *D. immitis* in dogs in 13.18%. In case of Nepal, Singh *et. al.*, (2018) has reported a case study of heartworm with chylothorax at Veterinary Teaching hospital, Bhairahawa. Pradhan *et.al.*, (2018) reported the case of *Dirofilaria* in anterior chamber of eye in human in Biratnagar Eye Hospital.

Dilofilaria immitis is routinely treated in canines under the guidelines prescribed by the American Heartworm Evaluation of Growth Society using the adulticidal therapy, Melarsomine. The three-dose protocol is the treatment of choice due to its increased safety and efficacy (Humpreys, 2015). *Dirofilaria* is also a zoonotic parasite and humans are dead end hosts. *D. repens* infection in human is associated with the subcutaneous lump in the affected area i.e. face and conjunctiva of the eye while human *D. immitis* infection has been associated with pulmonary dirofilariosis and is usually asymptomatic (Reddy, 2013).

In case of Nepal, there are no research published on prevalence of *Dirofilaria*. Climatic factors, Vectors and geographical location supports the prevalence of *Dirofilaria* in Nepal. Its presence have been observed in necropsy findings in 4 street dogs in Veterinary Teaching Hospital, Paklihawa, Siddharthanagar. As the disease when prevailed is complex to treat and expensive too, similarly it is a burden for public health as it is zoonotic. No vaccination practice against *Dirofilaria* have been seen in Nepal. So this research was mainly conducted to know the overall prevalence.

- of *Dirofilaria* and hemato-biochemical effect in street dogs of Kathmandu Valley and Siddharthanagar Municipality, Bhairahawa, Nepal.

MATERIALS AND METHODS

Study Area: This study was conducted in Siddharthanagar municipality, Rupandehi and Kathmandu valley. 160 samples from Kathmandu valley and 150 samples from Siddharthanagar were collected.

Collection of Blood: About 10 ml of blood was withdrawn in sterile blood collecting vial containing EDTA. The blood was immediately transferred to a sterile vacutainer. The tube was kept under refrigeration at -4 degree celsius until further laboratory works were performed.

Techniques Used: Three methods were used as a diagnostic method for the detection of *Dirofilaria* as described by Taylor *et.al.*, (2016):

Wet Smear Method: one drop of venous blood was kept on a clean microscope slide coverslip was placed. The coverslip area was examined under low magnification (10×) of the microscope. Undulating movements of larvae can be observed.

Haematocrit Test: Fresh whole blood was drawn into a Microhematocrit tube and centrifuged for 3 minutes at 1500 rpm. The plasma portion of the separated blood was examined under low magnification (10×). Moving microfilariae was observed in the plasma above the buffy coat.

Modified Knott's Test: Sample of blood was drawn into a syringe containing EDTA. 1mL of the blood was mixed with 9mL of a 2% formalin solution and the mixture was centrifuged the mixture at 1500 rpm for 5 minutes. Discarding the supernatant, one drop of 0.1% methylene blue was added to the sediment and mixed well. The stained sediment was transferred to a microscope slide using a Pasteur pipette to collect the entire sample and examined using the 10× microscope objective. Microfilariae was observed in an extended position with nuclei stained blue.

Packed Cell Volume Determination: PCV was determined by microhematocrit tube to separate cells from plasma.

Biochemical Test: Serum biochemical values were determined by using Autozyme reagent of Accurex Biomedical PVT. LTD. and Advanced Microprocessor Based Colorimeter.

Data Analysis: Statistical analysis was conducted using Statistical package on social science (SPSS) v.25 with chi square test, one sample T test and compare mean and MS excel was also used for data presentation.

RESULT

Overall Prevalence of *Dirofilaria*:

Overall prevalence in Kathmandu valley was found to be zero while 19.33% was found to be positive among 150 samples in Siddharthanagar.

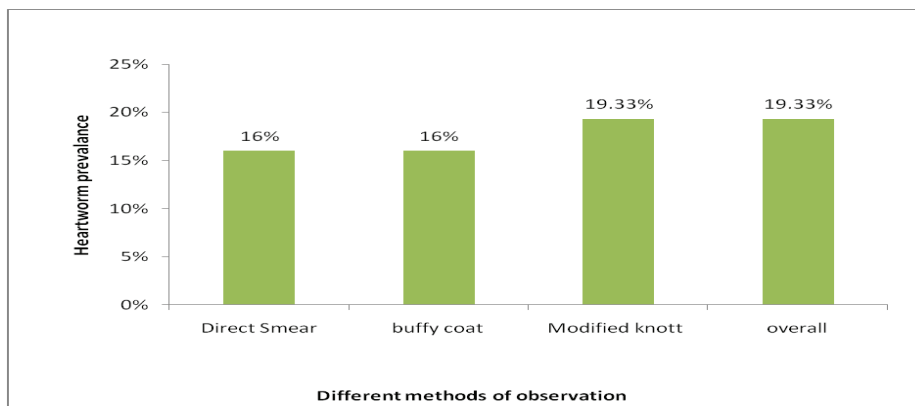


Fig. 1 Prevalence of heartworm infection in street dog of Bhairahawa in 2019

Sex-wise Prevalence of *Dirofilaria*:

This study revealed the prevalence in male 17.3% and in female was 21.70% among the infected dogs out of 150 samples of Siddharthanagar.

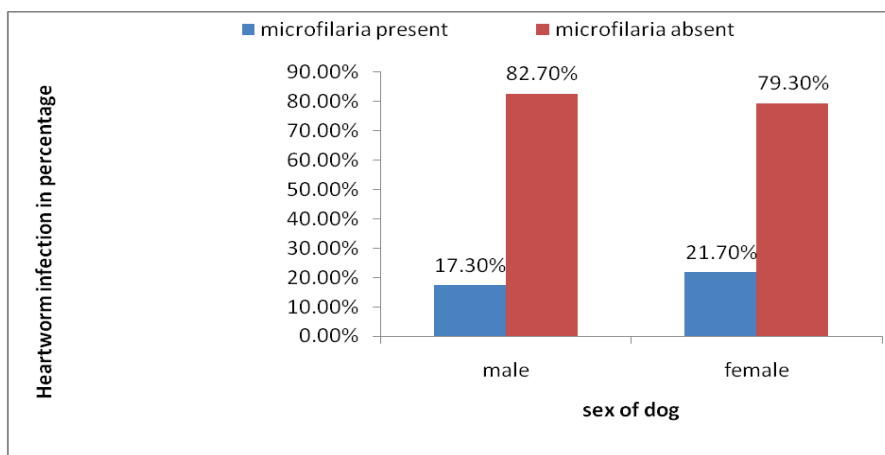


Fig. 2 Heartworm infection in male and female dog on Bhairahawa in 2019

Age-wise Prevalence of *Dirofilaria*:

Out of 150 samples of siddharthanagar 3.50% were from age below 1 year, 10.30% from 1-5 year, 51.70% between 3-5 years and 34.50% greater than 5 year.

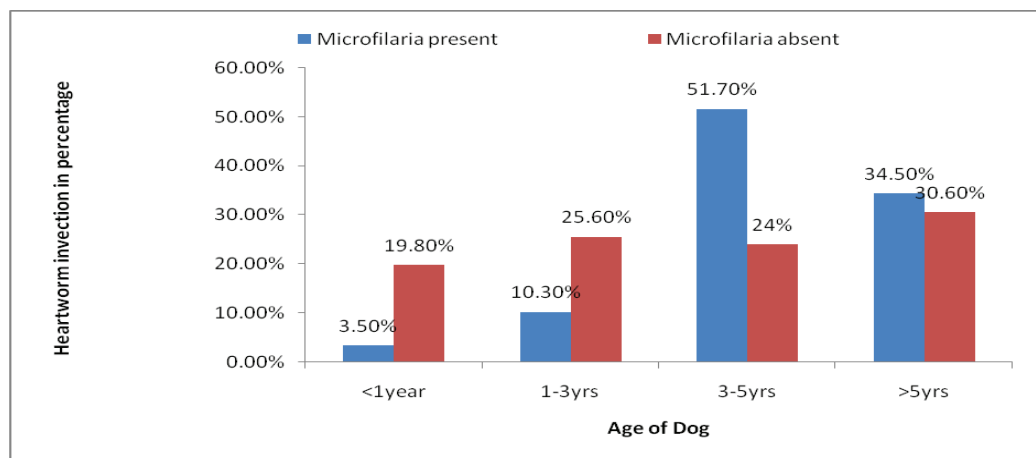


Fig. 3 Heartworm infection against different age of dog on Bhairahawa in 2019

Variation of Prevalence with the Tests:

The prevalence of heartworm microfilaria on direct smear, buffy coat method and modified Knott smear were 16%, 16% and 19.33% respectively on Siddharthanagar municipality, Bhairahawa.

Temperature Variation:

Of the total infected dog, 13.80% have temperature greater than 102.5 degree F while 86.20% have the range between 99.5-102.5 degree F.

Haematological and Biochemical Variations:

Chi square test shows PCV, Total protein, ALP, Urea, BUN and creatinine value insignificant with infection while AST (IU/L) and ALT (IU/L) value were found to be significant with mean value 26.73 and 27.25 in infected dogs and 17.98 and 19.91 in non infected dogs respectively. There was overall increase in AST, ALT, BUN, creatinine and Total protein level in infected dogs.

S.N.	Parameter	Infected dog	Non infected dog	P value	T value
1	Total protein (mg/dl)	7.37 ± 0.36	6.56 ± 0.29	p> 0.05	1.166
2	AST (IU/L)	26.73 ± 3.54	17.98± 2.52	P<0.05	2.013
3	ALP (IU/L)	376.11 ± 39.60	337.35± 53.71	p> 0.05	0.581
4	ALT (IU/L)	27.25 ± 2.65	19.91± 2.47	P<0.05	0.049
5	Urea (mg/dl)	22.19.12 ± 1.91	25.24± 2.90	P>0.05	-0.876
6	BUN (mg/dl)	11.39 ± 01.01	12.21 ± 1.49	P>0.05	-0.509
7	Creatinine (mg/dl)	1.63 ± 0.132	1.46± 0.12	p>0.05	0.934

DISCUSSION

The prevalence of *Dirofilaria* in our study in Siddharthanagar is lower than the findings of Retnasbapathy, 1976 i.e. 25.8% in Malaysia and Vieira *et.al.*, 2014 in Portugal with prevalence 27.3%. However the prevalence is higher than the findings of Borthukar *et.al.*, 2014 i.e. 11.38% in Assam, India and Wang *et.al.*, 2015 with prevalence of 13.18% in Henan, Central China. This may be due to the fact that transmission of dirofilariosis is dependent upon the presence of sufficient numbers of infected, microfilaraemic dogs, susceptible mosquitoes, and a suitable climate to permit extrinsic incubation of *Dirofilaria* in the mosquito intermediate host (Medlock *et.al.*, 2007, Genchi *et.al.*, 2009).

Biochemical variation in infected dogs is similar to Zalvaris *et.al.*, 2008 i.e Increase in AST (54.84%), ALT (51.61%), Creatinine (54.84%), Total protein (25.81%), Urea (32.26%) out of 31 infected dogs in Greece. Our findings also coincides with findings of Hashem *et.al.*, 2007 in Egypt who found among 14% infected dogs there was increase in AST, ALT, BUN, Creatinine and Total protein level. Increase in AST and ALT level may be due to loss of renal function while increase in BUN and creatinine level may be due to renal damage result of glomerulonephritis (Zalvaris *et.al.*, 2008 , Hashem *et.al.*, 2007).

In this study among the positive sample of Siddharthanagar, prevalence of male was 17.3% and female was 21.70%. This finding is similar to the findings of Adanir *et al.* where prevalence rate was 22.8% in male and 21.2% in female. It was higher in the study of Yaman *et.al.*, 2009 in Turkey where prevalence rate in male was 24.5% and female 33.3%. The difference in male and female infection is not statistically significant, difference may be due to the sample size taken from both population (Zarei *et.al.*, 2016).

In this study, prevalence was higher in age group 3-5 years (51.70%). Prevalence was also higher in old age dogs. Adanir et al findings shows higher prevalence of age greater than 7 years i.e 53.3%. The findings of Boonyapakan where the prevalence was higher in 3-6 years of age 33.3% while in old age greater than 10 years was 41.5%. The greater infection rate in older dogs (more than two years old) than younger dogs was possibly due to the longer exposure of older dogs to the mosquito bite (Zarei *et.al.*, 2016).

Prevalence of *Dirofilaria* was found to be negative in Kathmandu Valley despite being favourable temperature for the parasite, presence of vector and large population of dog. However it cannot be said it is 100 % negative. Further research is to be carried out taking large population size and using antigen test kit with high sensitivity and specificity.

CONCLUSION

Our study revealed the prevalence of *Dirofilaria* for the first time in Nepal. Prevalence of *Dirofilaria* in Siddharthanagar i.e. 19.33% is an alarming situation as in Nepal there is no vaccination practice against *Dirofilaria*. Although there was no prevalence in Kathmandu valley, a more detail study is to be carried out. Transmission of disease can take place to the major cities of Nepal as they have favourable environment, favourable temperature and presence of vectors. A holistic approach is required for the prevention in early stage and this will require the active involvement and cooperation of veterinary, allied professionals and government at all levels.

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Evaluation of Growth and Carcass Characteristics of Broiler Chickens (Cobb 500) Feed on Different Level of Organic Acids Inclusion in Diet at Parwanipur

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ABSTRACT

An experiment was carried out to evaluate the effect of different level of organic acids on productive traits, carcass yields and body parts (Thigh, Back, Neck, wings and Breast) of broiler chicken. This experiment was conducted at Regional Agricultural Research Station, Parwanipur, Bara for 41 days to test the effect of organic acids inclusion in broiler feed and its effect on growth performance and carcass study. Altogether 225 day old Cobb-500 broiler birds were procured from Shivam Hatchery, Birgung and divided into 5 treatments with 3 replications (15 birds in each replication) by using completely randomized design. Concentrate feed was purchased from Posak Feed industry, Birgung. Control group (T1) was feed without organic acid inclusion and whereas T2, T3, T4 and T5 groups were fed concentrate mixture mixed with different combination of organic acids @ 400ml/ per 100 kg feed, respectively. Experimental birds were provided adlib amount of grower feed (B1) for 21 days and that after finisher feed (B3) for 20 days and had easily access to drinking water. Feed intake was recorded daily and body weight gain was measured in 7 days interval. Experiment revealed that highest weight gain was observed in T2 (2.6 kg) followed by T3 (2.5) where combination of organic acids were formic acid 65% + propionic acid 35% and formic acid 65% and citric acid 35%, respectively, however, it was not significant among diet groups. From every treatment group each birds were selected for carcass and body parts study. The study showed significant difference ($P<0.5$) in carcass quantity and body parts of the birds between the treatment groups. The dressing percentage of T1, T2, T3, T4 and T5 were 68.96%, 67.87%, 70.38%, 69.88% and 69.67% respectively. Experiment suggested that further study should be carried out to precise the appropriate level of organic acids inclusion and higher cost benefit ratio.

Keywords: Broiler, Organic acids feeding, Growth performance

INTRODUCTION

Broiler chickens (*Gallus gallus domesticus*) are specifically raised for meat. They are a hybrid of the egg-laying chicken, both being a subspecies of red jungle fowl (*Gallus gallus*). Typical broilers have white feathers and yellowish skin. Most commercial broilers reach slaughter-weight at between five and seven weeks of age, although slower growing breeds reach slaughter-weight at approximately 14 weeks of age (Kruchten; 2002).

Since last few years in Nepal, there are so many farmers engaged in poultry farming. The poultry population of Nepal is estimated to be 50,195,285 (MoAD, 2016). Certain antibiotics even as residues can cause allergic or hypersensitive reactions in consumers. Tiwari *et.al.*, (2013) reported that utilization of organic acids has been increased as growth promoters in animal which could help in providing protection from adverse human health implications. Organic acid (1% sorbic acid and 0.2% citric acid) supplementation significantly increased the villus width, height and area of the duodenum, jejunum and ileum of broiler chicks at 14 days of age (Kum *et.al.*, 2010). Garcia *et.al.*, (2007) reported that broilers fed diets containing formic acid had the longest villi (1273 and 1250 μm for 0.5 and 1% formic acid, respectively) compared with control (1088 μm). Similarly, crypts of jejunum were deeper in birds fed the formic acid diet (1%) than birds fed the antibiotic diets (266 vs. 186 μm , respectively) in the same experiment. In some studies, organic acid salt also significantly improved villus height in the duodenum, jejunum and ileum. Pelicano *et.al.*, (2005) reported higher villus height in the ileum with the diet based on organic acid salts compared with diet fed without mannan oligosaccharide + Organic acid salt.

Organic acid treatments composed of individual acids and blends of several acids have been found to perform antimicrobial activities similar to those of antibiotics (Wang *et.al.*, 2009). The cost of using antibiotics in poultry farm could be reduced by the addition of organic acids in broilers diet. The mixture of organic acid in diet also enhances the growth of poultry with reduced mortality rates. The quality of meat flavor seems to be improved with the addition of organic acid in poultry diets. This type of work has not been carried out in Nepal. Therefore, this study was carried out to evaluate the effect of addition of various levels of organic acids a natural growth promoter on the performance of the broilers.

The marketing of poultry has been greatly diversified with a significant increase in cut-up (parts) and processed products (Le Bihan Duval *et.al.*, 2001). Demand for high quality cut-up (parts) and further processed convenience foods have driven the poultry industry to change its marketing practices (Watts and Kennett, 1995). Breeders of meat-type chickens have become interested in adult body weight and other growth traits, aimed at big-bodied weight at early age in order to attract better price at marketing (Malik *et.al.*, 1997) since the age at which broiler chicken are sold is greatly influenced by consumer preferences (Omaga *et.al.*, 2006).

The main goal of broiler rearing is production of quality broiler carcasses that will be acceptable from the consumers. Acceptability depends on the quality and quantity of edible parts of carcasses, and the amount of muscle mass in carcass. Broilers carcasses are evaluated mainly through yield edible parts of which are expressed by dressing percentage (slaughter yields) and the quality of edible parts of carcass. All quality characteristics of carcass are inherent in the hybrids (genotype) and are therefore conditionally hereditary characteristics with precisely defined heritability proposed values (Chen *et.al.*, 1987).

MATERIALS AND METHODS

Experimental Birds: The experiment was carried out on Cobb 500 broiler chickens at Regional Agricultural Research Station, Parwanipur, Bara from 12 February 2018 to 3 April 2018 (074/11/9 to 074/12/20 BS) for 41 days. Two hundred twenty five experimental day old birds were procured from Shivam hatchery, Birgung, Parsa and were allotted into five treatments with five replications having 15 birds in each replication by using Complete Randomized Design (CRD). All experimental birds were vaccinated with F1 vaccine @ one drop /bird against Ranikhet at the first week.

Diet Composition: The Control group (T1) was feed without organic acid inclusion and whereas T2, T3, T4 and T5 groups were fed concentrate mixture mixed with different combination of organic acids @ 500 ml/g per 100 kg feed, respectively.

Chemical Analysis: Representative samples were analyzed for Dry Matter (DM), crude Protein (CP), crude Fibre (CF), organic matter (OM) and ash contents (TA). The DM was determined by oven drying at 100°C for 24 hrs. Crude protein of the samples was determined using the Kjeldahl method. Ash content was determined by ashing at 550°C in a muffle furnace for 16 hrs (AOAC, 1980). Crude Ether of the samples was determined using the Van Soest method (Goering, H.K. and Van Soest, 1970).

Experimental Diet: The following experimental diet was provided to the birds (Table 1).

Table 1: Experimental diet

Treatment	Diet
1	Adlib concentrate mixture (without inclusion of organic acid)
2	Adlib concentrate mixture treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)
3	Adlib concentrate mixture treated with 0.5% mixture (formic acid 65%+ citric acid 35%)
4	Adlib concentrate mixture treated with 0.5% mixture (formic acid 65%+ sorbic acid 35%)
5	Adlib concentrate mixture treated with 0.5% mixture (formic acid 65%+ lactic acid 35%)

Feeding Regime: Concentrate mixture was given on group basis and was provided to the experimental birds once a day (morning) in *adlib* amount for both periods (starter–21 days and finisher – 20 days) of the experiment. Drinking water was provided in adequate amount.

Carcass and Body Parts Study: At 41 days of age, all birds were transported to a slaughterhouse. Feed was removed 12 hour before processing. Experimental procedures followed

the principles for care of animals in experimentation [13]. Measurements of carcass, body parts like breast yield and dressing percentage (% of birds) etc were performed.

Data Measurement: The trial period consisted for 41 days (21 days starter and 20 days finisher). Quantity of concentrate mixture given daily to the birds in groups weighed daily and refusal was weighed in the next morning. The body weight gain was measured in group basis (replication-wise) in seven days interval in the morning before feeding. The carcass and body parts were also measured after slaughtering the birds.

Data Analysis: Data of feed intake and body weight gain were analyzed by “One way Anova” test for every measurement using statistical package SPSS 2006, released versions 15.0.

RESULT AND DISCUSSION

Chemical Composition of Concentrate Mixture: The chemical composition of treated and non-treated concentrate mixture is given in Table 2.

Table 2: Chemical composition of the compound feed (on DM basis)

Concentrate mixture	DM	TA	OM	CP	CF
Feed (Starter)					
Control feed	90.47	6.15	93.85	22.16	3.69
Feed treated with treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	88.29	4.72	96.28	21.84	4.33
Feed treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	88.44	6.08	93.92	22.05	4.45
Feed treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	88.49	5.90	94.10	22.15	4.35
Feed treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	88.32	5.82	94.18	20.98	4.21
Feed (Finisher)					
Control feed	88.60	4.58	95.42	19.37	4.28
Feed treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	88.59	6.09	93.91	17.70	3.46
Feed treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	89.38	5.98	94.02	18.44	4.14
Feed treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	88.43	4.33	95.67	20.20	3.70
Feed treated with 0.5% mixture (formic acid 65%+ propionic acid 35%)	88.26	4.99	95.01	19.12	4.65

Body Weight Gain: The body weight gain trend of experimental birds is given in Table 3.

Table 3: Body weight gain trend of experimental birds, g (Mean± SD)

TRT	Days							Total weight gain (g)	Daily weight gain (g)	FCR, Kg
	0	7	14	21	28	35	41			
1	48.55±1.5	184.33±5.8	476.0±2.2	968.7±5.6	1436±9.7	1824.6±2.5	2498.0±1.4	2449.45±0.1	59.74±0.1	1.58
2	48.2±0.19	185.89±6.5	488.22±1.5	1005.7±2.2	1476.3±5.9	1904.6±1.4	2658.0±5.5	2609.8±3.6	63.65±0.3	1.48
3	48.8±1.3	175.67±1.7	456.33±6.8	928.22±1.3	1366.6±1.3	1835.4±3.6	2608.7±2.7	2559.9±1.4	62.4±0.1	1.50
4	48.3±0.5	181.78±1.0	512.00±1.5	988.00±3.8	1463.4±8.4	1866.7±1.9	2485 ±1.1	2436.7±0.6	59.04±0.2	1.57
5	48.9±0.5	183.56±6.8	484.11±2.2	993.67±4.7	1458.7±4.3	2031.1±5.2	2569±1.4	2520.1±0.9	61.4±0.1	1.52
Mean	48.5±0.79	182.24±4.36	483.33±3.5	976.87±4.8	1440.2±5.92	1892.5±3.32	2563.74±4.94	2515.24±1.32	58.42±0.5	1.53
P-value	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Average mean body weight of experimental birds was 48.5 gm at the beginning which reached 2563.74 gm by the end of experiment. At the 7 days of experiment, highest body weight gain was noted for T2 (185.89 gm) followed by T1 (184.33 gm). During 14 days of experiment, higher body weight gain was found in T4 (512 gm) followed by T2 (488.22gm). Similarly, at 21 days of experiment, higher body weight gain was found in T2 (1005.7gm) followed by T5 (993.67 gm). At the 28 days of experiment, higher body weight gain was observed in T2 group (1476.3 gm) followed by T4 (1463.4 gm), T5 (1458.7 gm), T1 (1436 gm) and T3 (1366.6 gm). During 35 days of experiment, higher body weight gain was observed in T5 group (2031.1 gm) followed by T2 (1904.6 gm), T4 (1866.7 gm), T3 (1835.4 gm) and T1 (1824.6 gm). Finally at 41 days of experiment, higher body weight gain was observed in T2 group (2658 gm) followed by T3 (2608.7 gm), T5 (2569 gm), T1 (2498 gm) and T4 (2485 gm). Experiment revealed that highest weight gain was observed in T2 (2.60 kg) followed by T3 (2.55 kg) where combination of organic acids were formic acid 65% + propionic acid 35% and formic acid 65% + citric acid 35% respectively. Similarly, highest FCR was recorded for T1 group (1.58) followed by T4 (1.57), T5 (1.52), T3 (1.50 kg) and T2 (1.48) respectively. There was non significant effect of different level of organic acid inclusion in diet on body weight gain and FCR.

The non-edible carcass weight of experimental birds is given in Table 4.

Table 4: Non-edible carcass weight of experimental birds, g (Mean± SD)

Treatment	Head wt	Feet Wt	Giblet* Wt	Croup, Intestines Wt	Live Wt	Skin wt	Abdominal Fat	Blood
1	50	102	132	126	2120	136	28	85
2	46	78	115	152	2310	172	44	80
3	50	88	122	168	2600	204	52	90
4	65	103	140	198	2650	210	52	85
5	52	126	150	162	2770	206	71	75
Mean	54.6	99.2	131.8	161.2	2490	185.6	43.2	83
Standard Deviation	8.3	18.0	13.9	26.0	0.26	31.6	9.9	5.7
P-value	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

* Giblet includes heart, gizzard, liver etc.

Average mean head weight of experimental birds was 54.6 gm, highest head weight was noted for T4 (65 g) followed by T3 and T1 (50 gm). The average feet weight of broiler was 99.2 gm, highest feet weight gain was noted for T5 (126 gm) followed by T4 (103 gm). Similarly, the average giblet weight was 131.8 gm, highest giblet weight gain was noted for T5 (150 gm) followed by T4 (140) gm. The average croup, intestines weight was 161.2 gm, highest croup, intestine was noted for T4 (198 gm) followed by T3 (168 gm). The average skin weight of experimental birds were 185.6 gm, highest skin weight was noted for T4 (210 followed by T5 (206 gm). The average abdominal fat of broiler was 43.2 gm, highest abdominal fat weight gain was noted for T5 (71 gm) followed by T3, T4 (52 gm). Finally, the average blood collected during slaughtering of broiler was 83 gm, highest blood collection was noted for T3 (90 gm) followed by T1, T4 (85 gm). A Non- significant difference was observed in carcass among the treatment groups.

Table 5: Cut parts weight of experimental birds, g (Mean± SD)

Treatment	Thigh wt	Back Wt	Neck Wt	Wings Wt	Breast Wt
1	372	200	60	110	472
2	412	256	52	140	648
3	466	362	72	156	650
4	436	336	74	152	714
5	480	380	66	164	692
Mean	433.2	306.8	64.8	144.4	635.2
Standard Deviation	43.2	76.23	9.0	21.0	95.4
P-value	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Average mean thigh weight of experimental birds was 433.2 gm, highest thigh weight was noted for T5 (480 gm) followed by T3 (466 gm) (Table 5). Average mean back weight of experimental birds was 306.8 gm, highest back weight was noted for T5 (380 gm) followed by T3 (362 gm). Average mean neck weight of experimental birds was 64.8 gm, highest neck weight was noted for T4 (74 gm) followed by T3 (72 gm). Average mean wings weight of experimental birds was 144.4 gm, highest wing weight was noted for T5 (164 gm) followed by T3 (156 gm). Average mean breast weight of experimental birds was 635.2 gm, highest breast weight was noted for T4 (714 gm) followed by T5 (692 gm). A significant difference was observed in cut parts of birds among the treatment groups.

Table 6: Dressing % of experimental birds, g (Mean $\pm$ SD)

Treatment	Dressing %
1	68.96
2	67.87
3	70.38
4	69.88
5	69.67
Mean	69.35

The mean dressing percentage of experimental birds was noted 69.35 gm, highest dressing % was noted in T3 (70.38 gm) followed by T4 (69.88 gm). The dressing percentage of T1, T2, T3, T4 and T5 were 68.96%, 67.87%, 70.38%, 69.88% and 69.67 % respectively (Table 6).

DISCUSSION

This experiment was initiated with aim to increase productivity of broiler with the reduction of their mortality rates. The mean FCR was noted 1.53 and was highest in T1 (1.58 kg) lowest in T2 (1.48). Likewise, mean total weight gain was 2515.24 g and it was higher for T2 (26,098 gm) and lowest in T4 (2,436.7 gm). The body weight gain was non-significant ($P>0.05$) in 7 days that after it was non-significant up to 41 days of experiment. Dietary organic acids did not significantly affect weight gain.

Islam *et al.* (2008) reported 3118.6 g and 3101.9 g cumulative feed intake of bird at 5 weeks of age fed with 0.5% citric acid and 0.5% citric + 0.5% acetic acid included group, respectively which was non-significant between groups. Moreover, they noted that better FCR was in 0.5% citric acid included group and lower in 0.5% acetic acid included group during the 5 weeks of experiment. Furthermore, significant ($P<0.05$) difference in body weight of birds among the

groups were observed at all ages. Adil *et al.* (2012) and Boiling *et al.* (2000) also reported improved FCR with supplementation of organic acids. Birds on treatment (0.5% acetic acid) showed lower ($P<0.05$) weight gain than control group and treatment (0.5% citric acid) showed the highest ($P<0.05$) weight gain. Improved weight gain was observed when administration of both citric acid in diets and acetic acid in water was done together. Highest weight gain on 0.5% citric acid agreed with previous findings of Fang *et al.* (2005); Denli *et al.* (2003) and Stipkovits *et al.* (1992) where improved weight gain was observed with administration of citric acid in diets at 0.3, 0.5 and 0.7%, respectively.

In a study, Celik *et al.* (2003) observed better FCR of turkey chicks supplemented with organic acids (propionic acid, formic acid, acetic acid) as compared to un-supplemented group. Propionic acid increases feed intake by improving palatability of feed and may increase permeability of mucosal cell of the intestine which increases the rate of utilization of nutrients and results better feed conversion.

Average mean thigh, back, neck, wing and breast weights were 433.2 gm, 306.8 gm, 64.8 gm, 144.4 gm and 635.2 g respectively. Average mean head, feet, giblet, and croup, intestines weights were 54.6 gm, 99.2 gm, 131.8 gm and 161.2 g respectively. The average abdominal fat of broiler was 43.2 gm, highest abdominal fat weight gain was noted for T5 (71 gm) followed by T3, T4 (52 gm). Finally, the average blood collected during slaughtering of broiler was 83 gm, highest blood collection was noted for T3 (90 gm) followed by T1, T4 (85 gm). At the 6th week of carcass assessment, live weight, carcass weight, thigh weight and back weight values obtained were not agreed with the work of Makram *et al.*, (2010) and Olawumi *et al.*, (2010). These authors reported lower values for live weight, carcass weight and thigh weight for three different strains of broilers respectively. However, dressing, breast and back weight values in this study were consistent with the range of values reported by Makram *et al.*, (2010) for four commercial broiler strain chickens under summer season in Egypt. Meanwhile, the superior carcass and dressing weight values noted were disagreed with the reports of Olawumi and Fagbenro, (2010); Ekeocha and Afolabi (2012). These authors documented lower values for Marshall, Hubbard and Arbor Acre respectively. The breast weight and back weight values obtained in this present study were not compatible with the results of Olawumi *et al.*, (2012) who noted lower values for these variables for Marshall, Hubbard and Arbor Acre birds.

CONCLUSION

Organic acids can be used in poultry diet as a growth promoter. It has resulted better body parts and growth performance with reduced bird mortality. However, further study should be conducted to ascertain the optimum level of inclusion and duration of experiment to get more benefit from broiler production. The expensive antibiotics cost could be reduced by the addition of organic acids in the poultry diets.

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Inclusion of Different Level of Khesari for Promotion in Broiler

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ABSTRACT

An experiment was conducted at Regional Agricultural Research Station, Parwanipur, Bara for 38 days to test the effect of Khesari leaves inclusion in broiler feed and its effect on growth performance. Altogether 135 day-olds Cobb-500 broiler birds were procured from Shivam Hatchery, Birgung and divided into 3 treatments with 3 replications (15 birds in each replication) by using completely randomized design. Concentrate feed was purchased from Posak Feed industry, Birgung. Control group (T1) was fed without inclusion of Khesari leaves and whereas T2 and T3 groups were fed 5% and 10 % Khesari leaves added feed respectively. Diet replaced with 5 % and 10 % Khesari leaves were offered from 14th day of trial to T2 and T3 broilers group respectively. Experimental birds were provided adlib amount of grower feed (B1) for 21 days and that after finisher feed (B3) for 21 days and had easily access to drinking water. Feed intake was recorded daily and body weight gain was measured in 7 days interval. Experiment revealed that highest weight gain was observed in T3 (2.55 kg) followed by T1 (2.48) where inclusion of 10 % Khesari leaves and normal feed respectively, which was also significant among diet groups. Similarly, highest cumulative feed intake was also observed for T3 (4.23 kg) followed by T1 (3.90 kg) which was also significant among diet groups. Experiment suggested that further study should be carried out to precise the appropriate level of organic acids inclusion and higher cost benefit ratio.

Keywords: Broiler, Growth performance, *Lathyrus sativus*

INTRODUCTION

Agriculture is the major sector of Nepalese economy. Therefore, the development of agriculture sector is key for the development of national economy. Livestock, including poultry is an integral part of the agricultural part of the agricultural production system in Nepal, providing manure and high value animal protein such as meat and eggs for the human consumption. Agricultural production is the main product and more than 65% of the population depends upon agriculture. Contribution of agricultural sector in Gross Domestic product (GDP) of Nepal is 33%. Among them contribution of livestock sector in total GDP is 26.8% and poultry sub sector within livestock contributes 8% of Agriculture Gross Domestic product (AGDP) and Indigenous poultry is widely prevalent which contributes 55% of total poultry population (MOAD,2015). A Nepali eats 4.1 kg per chicken and 44 eggs annually, well below the global average, according to a recent survey report titled “Nepal Commercial Poultry Survey 2014-15” (CBS, 2016). The global average chicken meat consumption is 12 kg per person, while average per capita egg

consumption is 153 units annually, according to the Food and Agriculture Organization of the United Nations.

The high price of feed cost in poultry could be controlled by replacing with forage diets like Khesari leaves. Broiler diet replaced with forage might reduce the feed cost of broiler that definitely enhances the productivity of the poultry farming. Feed cost occupies 70 % of broiler farming. Therefore, forage inclusion in feed reduces its cost. In many areas, feed savings are typically greatest in the late spring and early fall, when lush pasture provides plenty of high-quality forages to offset a significant amount of the cost of feeding poultry. Additionally, insect and other invertebrate populations (poultry favourites) in the pasture are booming at the same time. The use of different forage materials for like Khesari (*Lathyrus sativus*) organic poultry could support the principle of increasing utilisation of local resources which could have positive effects on the cycling of nutrients in the system. Grass pea (*Lathyrus sativus*) is a dual- purpose annual legume grown for its seeds for human consumption, and fodder for livestock feeding. Grass pea is one of the preferred legume seeds in low fertility soils and arid areas because of its outstanding tolerance of dry or flooding conditions Forage materials grown on farm utilise nitrogen (N) and phosphorus (P) from the soil. Therefore, foraging behavior can reduce the accumulation of N and P in the soil and hence ensure a desirable balance of N and P in a natural ecosystem which subsequently will protect the environment from acidification and global warming (Dekker *et. al.*, 2012). Forage materials have welfare benefits in terms of reduced feather pecking and other undesirable behavior (Van Krimpen *et. al.*, 2005). However, little attention has been paid to consider the nutrients of forage materials to facilitate the transition to 100% organic feed supply within the stipulated time.

Lathyrus sativus (Khesari), also known as grass pea, blue sweet pea, chickling pea, chickling vetch, Indian pea, white pea and white vetch, is a legume (family Fabaceae). It is a particularly important crop in areas that are prone to drought and famine, and is thought of as an 'insurance crop' as it produces reliable yields when all other crops fail. The seeds contain a neurotoxin that causes a neuro-degenerative disease when the seeds are consumed as a primary protein source for a prolonged period. *Lathyrus sativus* grows best where the average temperature is 10–25 °C and average rainfall is 400–650 mm (16–26 in) per year. Like other legumes, it improves the nitrogen content of soil. The crop can survive drought or floods but grows best in moist soils. It tolerates a range of soil types from light sandy through loamy to heavy clay, and acid, neutral, or alkaline soils. It does not tolerate shade.

MATERIALS AND METHODS

Experimental Birds: The experiment was carried out on Cobb 500 broiler chickens at Regional Agricultural Research Station, Parwanipur, Bara from 24 December 2019 to 7 February for 38 days. 135 days-old birds were procured from Shivam hatchery, Birgung, Parsa and were allotted into three treatments with three replications having 15 birds in each replication by using Complete Randomized Design (CRD). All experimental birds were vaccinated with F1 vaccine @ one drop /bird against Ranikhet at the first week. The vaccination schedule was followed as referred by broiler vaccination protocol.

Diet Composition: The control group (T1) was normal diet without inclusion of Khesari leaves whereas T2 and T3 groups diet were prepared by inclusion 5% and 10% Khesari leaves, respectively.

Chemical Analysis: Representative samples were analyzed for Dry Matter (DM), crude Protein (CP), crude Fibre (CF), organic matter (OM) and ash contents (TA). The DM was determined by oven drying at 100°C for 24 hrs. Crude protein of the samples was determined using the Kjeldahl method. Ash content was determined by ashing at 550°C in a muffle furnace for 16 hrs (AOAC, 1980). Crude Ether of the samples was determined using the Van Soest method (Goering, H.K. and Van Soest 1970).

Experimental Diet: The following experimental diet was provided to the birds (Table 1).

Table 1: Experimental diet

Treatment	Diet
1	Adlib concentrate mixture (without inclusion of Khesari leaves)
2	Adlib concentrate mixture (inclusion 5 % Khesari leaves in diet)
3	Adlib concentrate mixture (inclusion 10 % Khesari leaves in diet)

Feeding Regime: Concentrate mixture was given on group basis and was provided to the experimental birds twice a day (morning and evening) in *adlib* amount for both periods (starter–21 days and finisher – 21 days) of the experiment. Drinking water was provided in adequate amount. Diet replaced with 5% and 10% Khesari leaves were offered from 14th day of trial to T2 and T3 broilers group respectively.

Data Measurement: The trial period consisted for 38 days (21 days starter and 17 days finisher). Quantity of concentrate mixture given daily to the birds in groups weighed daily and refusal was weighed in the next morning. The body weight gain was measured in group basis (replication-wise) in seven days interval in the morning before feeding.

RESULT AND DISCUSSION

Chemical Composition of Concentrate Mixture

The chemical composition of treated and non-treated concentrate mixture is given in Table 2.

Table 2: Chemical composition of the compound feed (on DM basis)

Concentrate mixture	DM	TA	OM	CP	CF
Feed (Starter)	90.47	6.15	93.85	22.16	3.69
Feed (Finisher)	88.60	4.58	95.42	19.37	4.28
Khesari Leaves	21.7	10.5	69.4	24.2	25.8

Data Analysis

Data of feed intake and body weight gain were analyzed by “One-way ANOVA” test for every measurement using statistical package Minitab 2003, versions 13.20.

Cumulative Feed Intake

The average feed intake of experimental bird is presented in Table (3). Mean feed intake of experimental birds was recorded 40.82g/min 7 days which reached 184.04 gm at the end of experiment (38 days which was significant among diet groups. At the 7 days of experiment, feed intake of T1, T2 and T3 was noted (42, 38.88 and 41.60 gm, respectively). By the 28 days of experiment, highest feed intake was recorded for T3 (170.80 gm) followed by T1 (153.78 gm). Similarly, at the 35 days of experiment, feed intake was higher in T3 group (186.40 gm) followed by T1 (174.49 gm). Finally, at the 38 days of experiment, feed intake was higher in T3 group (198.40 gm) followed by T1 (185.24 gm). The cumulative feed intake of the experiment (2019) highest cumulative feed intake was for T3 (4232.711 gm) followed by T1 (3909.46 gm) and T2 (3793.68 gm) respectively. There was non-significant effect in feed intake for 7 days and 14 days whereas there was significant effect in feed intake during 21, 28, 35 and 38 days during entire experimental period between treatment groups.

Table 3: Feed intake of the experimental bird /day, g (Mean $\pm$ SD)

TRT	Days						Cumulative
	7	14	21	28	35	38	Feed intake
1	42.0 $\pm$ 3.05	77.51 $\pm$ 1.88	119.73 $\pm$ 6.1	153.78 $\pm$ 6.6	174.49 $\pm$ 3.0	185.24 $\pm$ 4.2	3909.47
2	38.88 $\pm$ 1.01	77.60 $\pm$ 2.55	121.07 $\pm$ 1.66	152.04 $\pm$ 4.5	169.69 $\pm$ 2.16	168.89 $\pm$ 1.8	3793.69
3	41.60 $\pm$ 2.27	78.53 $\pm$ 1.33	134.71 $\pm$ 5.09	170.80 $\pm$ 1.4	186.40 $\pm$ 8.9	198.00 $\pm$ 0.66	4232.71
Mean	40.82 $\pm$ 2.45	77.88 $\pm$ 1.66	125.17 $\pm$ 8.25	158.87 $\pm$ 9.8	176.86 $\pm$ 8.89	184.04 $\pm$ 12.8	3978.62
P value	P>0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
*P<0.05 significant							

Body Weight Gain: The body weight gain trend of experimental birds is given in Table 4 and Figure 1.

Table 4: Body weight gain trend of experimental birds, g (Mean $\pm$ SD)

TRT	0 days	7 days	14 days	21 days	28 days	35 days	38 days	Total weight gain (gm)	Average wt gain (gm)	C R
T1	45.37 $\pm$ 2.87	155.2 $\pm$ 13.29	364.8 $\pm$ 8.3	829.3 $\pm$ 31.41	1446.4 $\pm$ 59.2	2203.1 $\pm$ 49.9	2532 $\pm$ 45.1	2486.63	65.43	1.57
T2	43.28 $\pm$ 0.33	151.0 $\pm$ 2.1	364.4 $\pm$ 8.4	801.4 $\pm$ 23.8	1382.1 $\pm$ 35.6	2126.2 $\pm$ 58.0	2468.5 $\pm$ 47.8	2425.22	63.82	1.56
T3	46.0 $\pm$ 2.69	164.6 $\pm$ 4.05	380.6 $\pm$ 12.6	841.3 $\pm$ 19.1	1459.8 $\pm$ 11.4	2243.4 $\pm$ 65.4	2605.4 $\pm$ 20.6	2559.4	67.35	1.65
Mean	44.53 $\pm$ 2.21	156.9 $\pm$ 9.28	370.0 $\pm$ 11.81	824.0 $\pm$ 28.20	1429.5 $\pm$ 50.2	2190.9 $\pm$ 72.09	2535.3 $\pm$ 68.6	2490.77	65.52	1.59
P value	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05

Average mean body weight of experimental birds was 44.53 gm at the beginning which reached 2490.77g by the end of experiment. At the 7 days of experiment, highest body weight gain was noted for T3 (164.6 gm) followed by T1 (155.22 gm). During 14 days of experiment, higher body weight gain was found in T3 (380.6 gm) followed by T1 (364.89 gm). Similarly, at 21 days of experiment, higher body weight gain was found in T3 (841.3 gm) followed by T1 (829.33 gm). At the 28 days of experiment, higher body weight gain was observed in T3 group (1459.8

gm) followed by T1 (1446.4 gm). During 35 days of experiment, higher body weight gain was observed in T3 group (2243.4 gm) followed by T1 (2203.1 gm). Finally, at 38 days of experiment, higher body weight gain was observed in T3 group (2605.4 gm) followed by T1 (2532.0 gm). Experiment revealed that highest weight gain was observed in T3 (2.5 kg) followed by T1 (2.4) where diets were replaced by 10 % Khesari leaves and normal diet without inclusion of Khesari leaves respectively. There was significant effect at total body weight gain. Similarly, least FCR was recorded for T3 group (1.65) followed by T1 (1.57) and T2 (1.56) respectively. There was significant effect in diet on body weight gain and FCR.

Table 5: Non-edible carcass weight of experimental birds, g (mean $\pm$ SD)

Treatment	Head Wt	Feet Wt (2)	Giblet Wt (heart, gizzard, liver etc.)	Croup, Intestines Wt	Live Wt	Skin wt	Abdominal Fat	Blood
1	63	74	132	126	2532	145	39	72
2	60	68	138	139	2468	158	42	77
3	68	90	158	188	2605	170	43	75
Mean	63.5 $\pm$ 3.67	77.3 $\pm$ 9.8	136.67 $\pm$ 3.80	151.0 $\pm$ 28.3	2535.0 $\pm$ 59.3	157.67 $\pm$ 10.8	41.11 $\pm$ 2.0	74.66 $\pm$ 2.34
P-value	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Average mean head weight of experimental birds was 63.5 gm, highest head weight was noted for T3 (68 gm) followed by T1 (63 gm). The average feet weight of broiler was 77.3 gm, highest feet weight gain was noted for T3 (90 gm) followed by T1 (74 gm). Similarly, the average giblet weight was 136.67 gm, highest giblet weight gain was noted for T3 (158 gm) followed by T2 (138) g. The average croup, intestines weight was 151.0 gm, highest croup, intestine was noted for T3 (188 gm) followed by T2 (139 gm). The average skin weight of experimental birds were 157.67 gm, highest skin weight was noted for T3 (170 gm) followed by T2 (158 gm). The average abdominal fat of broiler was 41.11 gm, highest abdominal fat weight gain was noted for T3 (43 gm) followed by T2 (42 gm). Finally, the average blood collected during slaughtering of broiler was 84.66 gm, highest blood collection was noted for T2 (87 gm) followed by T3 (85gm). A significant difference was observed between non-edible carcasses between the treatment groups.

Table 6: Cut parts weight of experimental birds, g (mean $\pm$ SD)

Treatment	Thigh wt	Back Wt	Neck Wt	Wings Wt	Breast Wt
1	321	280	53	130	610
2	314	286	57	126	632
3	500	380	59	164	712
Mean	373.00 $\pm$ 95.33	320.00 $\pm$ 45.14	56.33 $\pm$ 2.91	141.67 $\pm$ 17.2	564.33 $\pm$ 110.8
P-value	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Average mean thigh weight of experimental birds was 373 gm, highest thigh weight was noted for T3 (500 gm) followed by T1 (321 gm). Average mean back weight of experimental birds was 320 gm, highest back weight was noted for T3 (380 gm) followed by T2 (286 gm). Average mean neck weight of experimental birds was 56.33 gm, highest neck weight was noted for T3 (59 gm) followed by T2 (59 gm). Average mean wings weight of experimental birds was 141.67 gm, highest wing weight was noted for T3 (164 gm) followed by T1 (130 gm). Average mean breast weight of experimental birds was 564.33 gm, highest breast weight was noted for T3 (712 gm) followed by T2 (632 gm). A significant difference was observed in cut parts of birds among the treatment groups.

Table 7: Dressing % of experimental birds, g (Mean $\pm$ SD)

Treatment	Dressing %
1	73.14
2	77.22
3	69.5
Mean	73.28

The mean dressing percentage of experimental birds was noted 73.28%, highest dressing % was noted in T2 (77.22%) followed by T1 (73.14%). The dressing percentage of T1, T2 and T3 were 68.96%, 73.14%, 77.22% and 69.5 % respectively.

DISCUSSION

This experiment was initiated with aim to increase productivity of broiler with the reduction of their feed cost. The mean cumulative feed intake of bird per day was noted 3978.62 gm and it was highest (4232.71 gm) for T3 lowest (3909.46 gm) in T1. There was significant effect in feed intake during entire experimental period between 21, 28, 35 and 38 days between treatment groups. Similarly, mean FCR was noted 1.59 and was highest in T3 (1.65) lowest in T2 (1.56 kg). Likewise, mean total weight gain was 2535.3 gm and it was higher for T3 (2605.4 gm) and lowest in T2 (2468.8 gm). There was significantly difference ($P<0.05$) in total weight gain and average weight gain of experiment. The total weight gain and average weight gain was lower in T2 due to low intake of diet in comparison to other treatment groups. Average mean head weight of experimental birds was 63.5 gm, highest head weight was noted for T3 (68 gm) followed by T1 (63 gm). The average feet weight of broiler was 77.3 gm, highest feet weight gain was noted for T3 (90 gm) followed by T1 (74 gm). The dressing percentage of T1, T2 and T3 were 68.96%, 73.14%, 77.22% and 69.5 % respectively.

Latif *et al.* (1975) conducted a trial where he reared for 14 day in battery brooders, that were given diets containing 100, 222 or 367 g/kg of Khesari meal (*Lathyrus sativus*) in three different experiments. When Khesari was either autoclaved or heated in a commercial “micronisation” process and then incorporated at 367 g/kg in a balanced chick diet, growth and efficiency of food utilisation were at least equal to those obtained with a maize-soya diet. Chicken provide a readily available, high-quality and inexpensive source of proteins, vitamins, and micronutrients accepted by all ethnic groups. Village also increases food security for vulnerable families. Economically,

village can provide a ready source of cash. Feedstuff is an aspect of high economic importance in the rearing of commercial poultry not only because it is primarily responsible for the growth response of birds, but mainly because it represents the largest cost in the production cycle (Avila *et.al.*, 1992). For instance, the broilers' energy requirements are responsible for 70% of the cost of the ration (Skinner *et.al.*, 1992) and, besides, the processing method and the grain type interfere differently on the economic viability and animal performance.

Forages can provide a significant amount of poultry nutrition, reducing the amount of feed that a poultry farmer feeds a flock. Although poultry are not ruminants - they're monogastric - a good pasture is still a valuable resource for the flock. In many areas, feed savings are typically greatest in the late spring and early fall, when lush pasture provides plenty of high-quality forages to offset a significant amount of the cost of feeding poultry. Khesari plants are available in all region of Nepal. They could be cultivated easily in every regions of Nepal. Therefore, plant leaves are used in this poultry trial being low toxic comparison to its seed. Although the general opinion of poultry producers and feed manufacturers is that the fibre content of the rations should be kept below 7%. The fibre content in Khesari plant is found to be 25% approximately.

Therefore, in this experiment Khesari leaves are included in small amount (5% and 10%) for the treatment groups. The feed cost of broiler could be reduced by the inclusion of feed by Khesari leaves. Farmers are not aware about the various types of improved management practices, the development of new technologies. In other words, the farmers themselves are practising traditional farming system. This might lead to production but only it meets the farmers' basic needs. There is lack of technical knowledge on production, processing and slaughtering of poultry.

CONCLUSION

Khesari (*Lathyrus sativus*) leaves can be included in poultry diet for reducing feed cost and as a growth promoter. It has resulted better growth performance. However, further study should be conducted to ascertain the optimum level of inclusion and duration of experiment to get more benefit from broiler production. The expensive feed cost could be reduced by the addition of Khesari leaves in the poultry diets.

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Monitoring of Serological Status in Response to PPR Vaccination in the Goat Population of Parbat, Baglung and Myagdi District of Nepal

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ABSTRACT

Peste des Petits ruminants (PPR) is a highly contagious trans-boundary animal disease associated with 100% morbidity and 80-90% mortality in goat herds. The study was carried out to assess the immune status against PPRV in unvaccinated and post vaccinated flock as well as immune response as it relates to age, sex and breed using a homologous tissue culture PPR vaccine produced by National Vaccine Production Laboratory, Kathmandu, Nepal. A total 276 blood samples were randomly collected from different regions of Parbat, Baglung and Myagdi districts with a population of 0.0189 million goats. Out of these, 214 goats were vaccinated one month earlier and 62 were unvaccinated. PPR antibody was detected by using competitive enzyme linked immunosorbent assay (c-ELISA) (ID Screen, PPR competition test kit, IDvet France). Of the unvaccinated goats, 25.8% (16/62) were sero-positive suggesting prior exposure to PPRV. In the vaccinated goats, 75.2% (161/214) were sero-positive and 2.8% (6/214) animals were doubtful, suggesting a significant proportion failed to respond to vaccination. The competition percentage (S/N%±SD) of post-immunization (49.19±32.19) were significantly ($p<0.01$) lower in comparison to the respective unvaccinated mean titre (75.76±35.51). There is no significant effect of age, sex or breed on the antibody titre value in PPR vaccinated animals. The sero-prevalence of PPR in unvaccinated animals suggested that these flocks are in high susceptibility to PPR outbreak and needs an implementation of control measures to reduce economic losses. Vaccination will increase the proportion of animals with a protective antibody level, however more investigation needs to be done to determine why almost one-quarter of animals failed to seroconvert to vaccination. Further detailed study is needed to find out the risk factors responsible for low prevalence of antibody against PPRV in the immunized flock.

Keywords: Sero-monitoring; PPR; Vaccination; Goats;

INTRODUCTION

Peste des petits ruminants (PPR) or "goat plague" is an acute or sub-acute, highly contagious viral disease of small ruminants with high morbidity and mortality that threatens the food security and sustainable livelihood of farmers (Gari *et al.*, 2017). The causative agent of PPR is Peste des petits ruminants virus (PPRV) belonging to the genus morbillivirus and family Paramyxoviridae. It is characterized by fever, mucopurulent ocular-nasal discharges, necrotizing and erosive stomatitis, severe enteritis, and pneumonia finally leading to death.

PPR first outbreak was reported in terai (Dhanusha, Mahottari, Bara, Sarlahi, Rautahat) and Gorkha districts of Nepal in 1994 as epidemic disease of goats and officially recognized as PPR in 1995 (GF-TADs, 2017). PPR has affected more than 68 districts and established as endemic disease in many districts (VEC, 2015). It was the major TADs recorded in small ruminants during the year 2015 as there were 71 outbreaks with 18,261 and 7,118 animals affected and died respectively (VEC, 2015). This disease is still prevailing as endemic in many regions of country, causing huge economic losses every year.

Acharya *et al.* (2018) reported the sero-prevalence of PPR 82.60% in unvaccinated goat population in a study conducted in the Syangja and Kaski districts which are the neighboring districts of Parbat, Baglung and Myagdi and lies in the same eco-zone. Due to the endemicity of the disease, the Govt. of Nepal, National Vaccine Production Laboratory had started production of the PPR homologous vaccine using tissue culture technology since 2000 (<http://www.cbpl.gov.np/content.php?id=2>) with a aim to vaccinate all susceptible goats and sheep. The sero-prevalence found is unexpectedly high though mass vaccination is practiced every year and the government is investing huge amount of budget. Sero-monitoring of the antibody titre could be insightful indicator of national herd immunity. Therefore, the present study was aimed to find out the vaccination coverage, its success rate and associated risk factors contributing for the development of the safe titre level in the immunized herd.

METHODOLOGY

Site of Study: A cross sectional study was conducted in Parbat, Baglung and Myagdi districts. Parbat district covers an area of 494 km², which lies 28°14'0"N latitude and 83°31'0"E longitude with the goat population of 0.0058 million. Baglung district covers an area of 1,784 km², which lies latitude 28°16'N and 83°36'E longitude with the goat population of 0.0094 million. Myagdi district covers an area of 2,297 km², which lies latitude 28.3417°N and 83.5666°E longitude with the goat population of 0.0037 million. These districts are located same eco-zone in the central part of Nepal with typical weather.

Sample Collection: All essential epidemiological information were collected on individual animal using a well-designed questionnaire. The data of PPR outbreaks, vaccination, animal census, and animal movement were collected from the veterinary hospital of aforementioned districts. A total of 276 blood sample was collected from the randomly selected goat. Among them, 214 goats were vaccinated one month earlier against PPR whereas 62 goats were unvaccinated. In particular, 92 blood samples from each district were collected. The collected sample was kept overnight at room temperature for separation of serum and the separated serum was stored in a serum vial and placed inside the cool box until it was transferred to at -20°C in the laboratory.

Laboratory Techniques: Serum samples were analyzed at the Veterinary Laboratory, Pokhara, Nepal. The preserved serum samples were thawed and tested for the presence of PPR antibody by using c-ELISA kit according to the instruction of manufacturer company (ID Vet France) and the optical density (OD) readings were taken using ELISA micro plate reader (MultiSkan Company) with an inference filter of 450 nm. This ELISA kit was 94.5% and 99.4% sensitivity and specificity respectively.

$$\frac{S}{P} \% = \left(\frac{OD_s}{OD_{nc}} \right) * 100$$

OD_s-optical density of sample, OD_{nc}- optical density of negative control

The test serum samples showing less than 50 percent competition percentage (CP) of mean OD values of monoclonal control wells were considered as positive for PPR antibodies whereas samples having OD values more than 50 were considered negative (Table-1).

Statistical Analysis: The data entry and analysis were performed using MS-Excel 2013. The univariate analysis by using 2-tailed Chi-square test was conducted to find out the significant association between potential risk factors. Furthermore, logistic regression model was used to assess the association between the risks factors and the PPR disease. Association in the logistic regression model were considered significant when $p < 0.05$.

ELISA Result

Interpretation of ELISA result described by Manufacture Company

Positive	if $S/P\% \leq 50\%$
Doubtful	if $50 < S/N \leq 60\%$
Negative	if $S/P\% > 60\%$

RESULT AND DISCUSSION

The overall sero-positive value of 73.74%, 76.86% and 75.13% were found in goat population of Parbat, Baglung and Myagdi districts, respectively with the vaccination coverage of 77.54% (Table-1).

Table 1. Overall percentage of sero- positive result in the goat population of three districts

Result	Parbat		Baglung		Myagdi	
Positive	67	73.74%	71	76.86%	69	75.13%
Negative	24	25.24%	18	13.04%	21	22.7%
Doubtful	1	1.02%	3	3.26%	2	2.17%

In our study, the male and female ratio was found 0.14 (Table-2)

Table 2 Competition Percentage value for different variables

Particulars	Sero-positive percentage competition of vaccinated herds (S/N%±SD)				
	Sex		Breed		
Result	Female	Male	Khari	Boar	Chyangra
Mean± SD	45.03±29.59	43.48±29.42	43.56± 29.35	45.98± 24.31	47.75±35.51

Table 3. Herd structure and sex wise sero-positive result

Sex	Herd Structure		Sero- positive Result (%)		
			Positive	Negative	Doubtful
Male	33	12%	76.19%	23.81%	0
Female	243	88%	76.86%	21.71%	1.43%

The above finding suggests that the farmers only rear buck for breeding purpose for longer duration. In the vaccinated goat population (n=214), 75.2% (161/214) were found sero-positive to PPR vaccines with the CP 49.19 ± 32.19 , this means that the vaccinated herds are protected against PPRV, while 2.8% (6/214) were found doubtful (Table-3).

Table 4. Competition Percentage (CP) of vaccinated and unvaccinated herds, showing vaccination coverage of 77.54%

Particulars	Vaccination status					
	Vaccinated (n=214)			Unvaccinated (n=62)		
	Sero-positive	Sero-negative	Doubtful	Seropositive	Sero-negative	Doubtful
Result	75.2% (161/214)	21.74 % (47/214)	2.8% (6/214)	25.8% (16/62)	74.19% (46/62)	0
(S/N%) ±SD	49.19±32.19			75.76±35.51		

The findings of present study is consistent with the study of Kabir *et.al.*, (2016) where they found sero-prevalence and sero-negative as 75% and 25%, respectively in goats of Bangladesh. However, Luka *et.al.*, (2011) had reported PPRV specific antibody sero-conversion of 55.26% (84/152) (among Vaccinated) in Uganda which is much lesser than the finding of present study.

Similarly, out of 62 unvaccinated goats, 25.8% (16/62) were found sero-positive with the CP value of 75.76 ± 35.51 (>60), this means that these herds are very prone to PPR disease (Table-3). Rahman *et.al.*, (2016) found the sero-prevalence of 38.77% in the non-immunized herd which is higher than the sero-prevalence recorded in this study (25.8%). Acharya *et al.* (2018) recorded sero-prevalence of 82.60% in a study conducted in the unvaccinated goat population of Syangja and Kaski districts which is higher than the finding of this study. Detection of PPRV antibodies can confirm the diagnosis of PPR disease in the unvaccinated herds, however the presence of maternal antibodies may yield false picture of the prevalence of infection. Thus, the isolation of PPR virus in clinical samples becomes essential in such condition because it is a gold standard test for confirmation of PPR virus (Mehmood *et.al.*, 2009).

The CP for unvaccinated herds is significantly higher ($P < 0.01$) than the immunized herds which means that the antibody titre value required to protect the animal from PPRV is sufficiently developed in the vaccinated animals. But the sero-positivity of only 75.2% suggests that the 25% vaccinated population failed to develop immunity. It was estimated that minimum of 75-80%

herd immunity is essential for controlling the rinderpest, a related disease (Rossiter and James, 1989). If we consider this fact, the herd immunity in the vaccinated flock in the present study is somewhat satisfactory. There may be several factors contributing for successful outcome of the vaccination campaign like cold chain maintenance, duration of reconstituted vaccine, skill of vaccinators, area coverage and the health condition of animals during the immunization time. In this study, there was no any association of individual factors (sex, breed) to develop immunity (Ab-titre) after PPR vaccination. The CP for male and female was 43.48 ± 29.42 and 45.03 ± 29.59 respectively. Similarly, the sero-positivity was found 76.19% and 76.86% for male and female respectively (Table- 4).

CONCLUSION

The sero-positivity of PPR in unvaccinated population suggests that these animals are highly prone to PPR disease and there is a need to implement the PPR disease control measures to reduce the economic losses. Vaccination has markedly increased the proportion of animals with a protective antibody level. The further study is needed to probe the risk factors that are responsible for only 75% protection against PPRV in the immunized flock and to look for the reason why one-quarter of animals failed to seroconvert to vaccination.

RECOMMENDATION

The effectiveness of PPR vaccination also depends on the level of vaccine coverage. High herd immunity levels prevent virus transmission and low herd immunity levels encourage epidemic transmission as well as intermediate levels of immunity assist the establishment of endemicity (Rossiter and James, 1989). Therefore, it is essential to increase the coverage of immunization by implementing different vaccination strategies.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Helminth Parasites of Pigs and Development of Suitable Strategy for Its Control

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ABSTRACT

*The study aimed to formulate the suitable development strategies to control helminthiasis in pig by understanding the nature of the epidemiological pattern that is yet to exploit to further enhance its productivity. A longitudinal study was done to find the general prevalence pattern of helminth parasites from June/ July 2017 to May/June 2018 in the research farm of Swine and Avian Research Program, Khumaltar. A total of 50 animals of different age groups were considered for the study and they were deprived of antihelminthic treatment throughout the study period. The fecal samples were collected at 3-4 months interval and EPG was done as per the standard protocol. Three different groups of antihelminthic were used including a control group as different treatments and they were assigned as T1- Pyrantel pamoate, T2- Benzimidazoles, T-3 Imidazothiazole, and T-4 Control. The EPG was done for at least 3-week post-treatment. The result of this study showed the significant effect of the season ($p < 0.05$) as a high prevalence of roundworms especially *Ascaris* (60%) and *Strongylus* (40%) during June/ July with more than 30 cases. However, a slight increase in parasitic prevalence was seen in September/October and Jan/Feb too. Similarly, all the antihelminthics used were 100% effective ($p > 0.05$) against these roundworms. The female and young groups of animals showed remarkable symptoms of parasitic infections compared to the male ones. In conclusion, the different groups of drugs demonstrated very satisfactory performances in terms of the removal of parasites for good health of pigs and these should be given at 3-4 months interval as routine deworming schedule.*

Keywords: Antihelminthics, Helminth parasites, Pig, Productivity

INTRODUCTION

Pig and pork productions are an important component of the subsistence agriculture system in Nepal and are raised by different communities of Nepalese society for their diverse use. The population of pig has increased gradually from 0.76 million to 1.2 million whereas pork production has increased from 0.13 metric tons to 0.24 metric tons in 15 years of period (FAO, 2014). The increasing trend in the number indicates that pig and pork have played an important role in Nepalese agriculture for many years. Simultaneously, many constraints like the trend of parasites have been increasing as well. Loss of piglets during the parturition process and the pre-weaning period is one of the

serious causes of the reduced production efficiency in the pig breeding herd due to diseases caused by different helminth parasites (Zanga *et. al.*, 2003).

The major diseases affecting pig production are internal helminth parasites (Zanga *et.al.*, 2003; Mul, 2007). Although endo parasitism has been frequently reported, limited study has been carried out in the country. The role of parasitism is important in village reared pigs throughout the territory and contributes substantially to high-mortality and retarded growth. There is still a lack of well-managed study on such a sensitive issue in the country. The exact loss due to these causes is yet to exploit. Swine endoparasitism is heterogeneous regarding the species involved and pathogenicity of the parasites (Roepstorff *et.al.*, 1998; Urquhart *et.al.*, 1996).

The effects of endoparasites depend on the parasite load and the individual resistance of the host, which are greatly influenced by environmental and nutritional factors (Banerjee, 1980). Endoparasitism can occur with or without the presence of clinical symptoms. In the first case, it can be lethal, especially in the initial growth phase. The absence of clinical symptoms is important for production because if it goes unnoticed, it can cause economic loss due to the reduction in pig performance (Saeed and Roepstorff, 2001). Endoparasitism does not manifest itself as a relevant problem in current pig farming. This is due to new technology, and above all, those technologies related to the interruption of the parasitic cycles, such as confinement facilities when used with specific prophylactic techniques. However, not all preventive techniques can be practiced in outdoor free-range or deep litter systems.

Parasites act as a stressor stimulus to animal metabolism, altering the nutritional demands of the host (Petkevicius *et. al.*, 1999). However, little is known about the implications of the interaction of endoparasitism with nutritional components and animal performance. Given, the study aimed to formulate the suitable development strategies by understanding the nature of the epidemiological pattern that is yet to exploit and enhance productivity.

MATERIALS AND METHODS

Site Selection

The experiment was conducted in the Farm of Swine and Avian Research Program, Khumaltar, Lalitpur District located in the Central Development Region of the country. The geographical diversity ranges from 1400 masl.

Study on Management Practices of Pig Production.

The pigs managed in Swine and Avian Research Program, Khumaltar were provided 2-4 kgs of greens and plenty of fresh water per day and vaccination against swine fever, Foot and Mouth Disease was done as per routine. The female pigs attending heat were made to conceive with pure breeds of its own, however avoiding inbreeding. The pregnant and gestated pigs were handled as per recommendations. The general practices of hygiene and bio-security maintenance were followed.

Epidemiological Sstudy on Parasitic Occurrence

The routine farm visit, fecal collection from each pig and piglets were done (two/three month's interval) with suitable labeling in zip lock bag and brought in the laboratory of Animal Health Research Division, Khumaltar. Egg per gram described by (Soulsby *et.al.*, 2008) was carried out to explore general prevalence.

RESULT AND DISCUSSION

The epidemiological pattern of the parasitic infection obtained revealed the following results which have been given through Figure 1 enlisted below

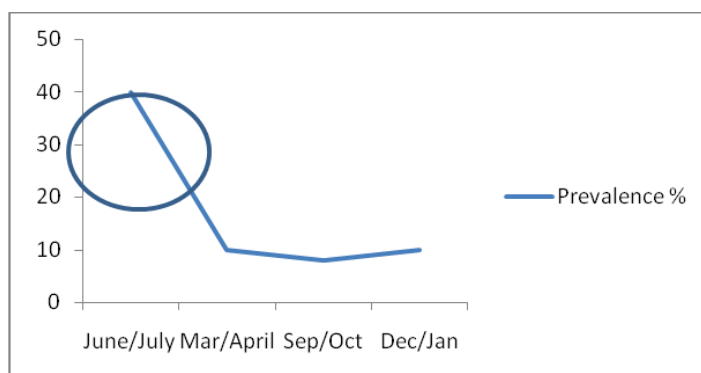


Figure 1: Parasitic occurrence in different months.

The above figure indicate that the parasitic incidence was higher in June/July representing 40% (40 out of 100) of prevalence. These patterns were found to decrease in the remaining months ranging from 10-15% (15 positive cases) in March/April, August/September. The difference in temperature, relative humidity, and the rainfall pattern might be associated with the outcome. The temperature and rainfall of the June/ July ranged between 28-32⁰c and 1000mm while the same of the remaining months were less. This result resembles that of Martin *et.al.*, 1988 advocating about warm and wet conditions providing a very suitable environment for the survival and multiplications of insects/parasites as a vector of disease. In this study, the month of June/July revealed the warmest and wet season compared to others.

Types of Parasites : The *Ascaris suum*, *Strongylus*, and mixed infection were the principle parasites as per the findings. Both *Ascaris Suum* and *Strongylus* were highest in June/July and their incidence decreased in the remaining months. No other species were reported (Figure 2).

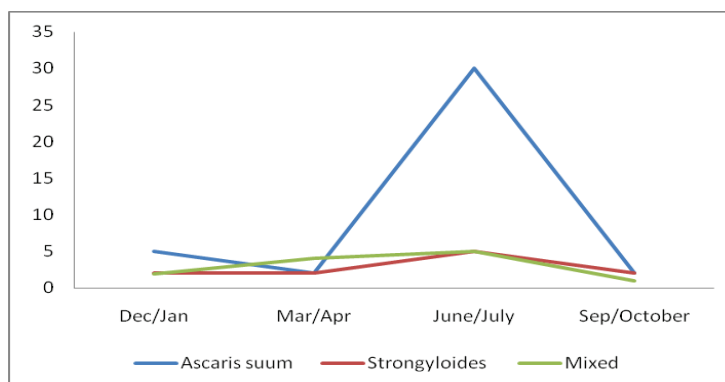


Figure 2: Different types of parasites infecting the animals have been presented by the graph

Thus, these findings contradict that of Thakur, 1998 reporting more than six to seven species in the eastern hills of Nepal. Similarly, in the study carried out by Tablot, 1970 in Papua New Guinea showed more than 25 different types of parasites. The difference in geography, management pattern and the sample size might be the causes of such variation.

Age-wise Prevalence Pattern

In the different groups of piglets, animals below 4 months showed a high prevalence of *Ascaris* and the animals above 9 months showed a prevalence of both *Ascaris* and *Strongylous*. However, there was a mixed infection for the animals between 4-9 months of age. Our results were similar to the findings by Thakur, 1997 and stated that the age of pigs has an effect on the level of infection especially for *Ascaris*, unlike others. The exact reason could not be explored, though it is thought to be due to the development of age-related immunity in piglets.

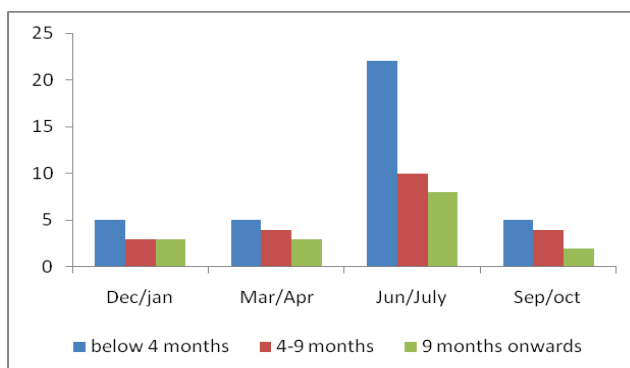


Figure 3: Age-wise prevalence pattern of the parasites

Conduction of Trial Using Several Groups of Anthelmintics

Different groups of anthelmintics available in the market were used for the conduction of trial at their recommended doses. Pyrantel pamoate was provided to the younger ones from one week to 2 months of age and the rest of the animals aging one month onwards was provided with benzimidazoles, imidazothiazoles, and control group. The initial and later EPG count was done according to the standard protocol provided by (Soulsby *et. al.*, 2008). Egg reduction count was done at the interval of one week until three weeks and the data were recorded as well. The result is given below.

Table 1: Group of drugs and their effectiveness

Group of Drugs	Initial Count	1 st week	2 nd week	3 rd week
T1- Pyrantel pamoate	15	2	1	0
T2- Benzimidazoles	16	1	0	0
T-3 Imidazothiazoles	15	1	0	0
T-4 Control	15	18	20	22

Results obtained from the trial revealed a significant effect of treatment over the control. All the groups of drugs were 100 % effective against the helminth parasites that existed till 28 days post-experiment.

Table 2: Average egg count per gram from the samples of trial goats during the pre- and post-treatment periods

Day	T ₁ (mean)	Mean % reduction	T ₂ (mean)	Mean % reduction	T ₃ (mean)	Mean % reduction	T ₃ (mean)	Mean % increased	P- value	Level of significance
0	584.5	0	436.2	0	436.2	0	281.6	0	0.04	***
7	8.7	98.5	24.2	94.5	24.2	94.5	296.6	5.4	0.02	***
14	0	100	12.0	97.3	12.0	97.3	455.9	61.95	0.00	***
21	0	100	8.7	98.0	8.7	98.0	586.4	108.2	0.00	***
28	0	100	5.3	98.8	5.3	98.8	755.9	168.5	0.00	***

Note***= Level of significance at 0.01%

The finding is under that of different researchers. The efficacy of ivermectin against both ecto- and endoparasites in the present study corresponded to the findings of earlier studies by Ratnaparhki *et.al.* 1996; Mahbub *et.al.*, 1992. The studies reported 89.5%-100% efficacy against endo- and ectoparasitic infection in goats or other small ruminants reared in a backyard system in Bangladesh and overseas. A slight variation of the efficacy of ivermectin in those previous studies might be due to the degree of ectoparasitic infection, the sample size used for different experiments, dose and preparation, and the route used for administering the drugs. However, ivermectin proved its efficacy in the present study without any adverse effect.

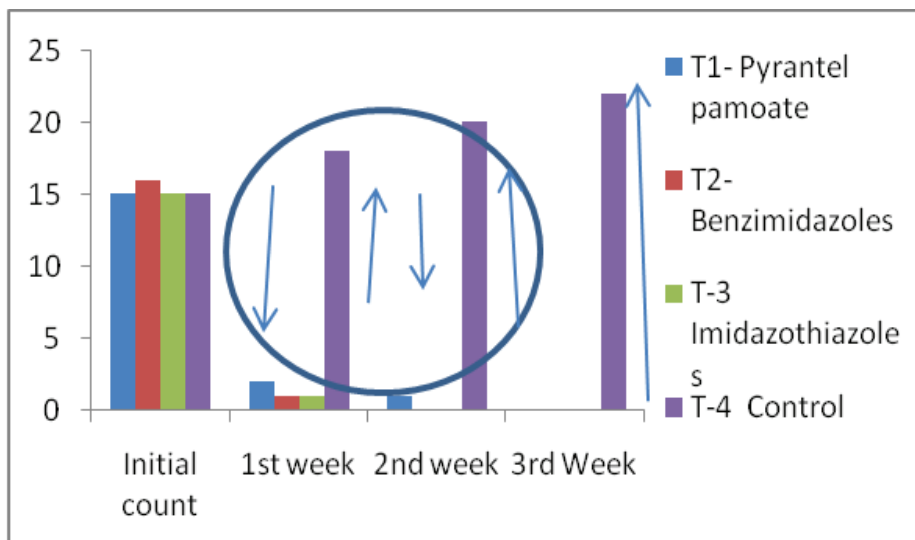


Figure 4: Result of the trial using several groups of anthelmintics

The effective performance of triclabendazole along with levamisole against endoparasites in the current study also coincided with the performance of triclabendazole alone reported earlier in backyard goats and sheep (Mahbub, 1996 and Zanga *et.al*, 2003). Moreover, using the levamisole (with triclabendazole) in this study could have modulated the body's immune system to give necessary protection against different infectious organisms. However, this study has not studied immune status with the administration of levamisole.

CONCLUSION

The present study revealed that the helminth parasite is the major cause of helminthiasis in pig in Nepal which affects the pig productivity and further shows the detrimental effect on the pig industry. This study was conducted to formulate different strategies to control helminthiasis in pig and from this conducted research, the prevalence pattern of parasites was found to be high in June/July (Rainy season). Similarly, different groups of antihelminthics used in this trial were found to be 100% effective. However, due to the sporadic occurrence of parasites throughout the year, deworming should be done at the interval of 3-4 months interval.

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Dog: A Friendly Pathway to Zoonoses

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ABSTRACT

Canis lupus familiaris (Dog); a companion animal or even considered man's best friend is believed to have been living in harmony with humans since thousands of years. Recent discovery in Chauvet cave in France: foot print of 8-year-old boy alongside the paw print is believed to have been dated back to Paleolithic period. Reports even suggest that pet owners are less prone to high cholesterol and high blood pressure than the non-pet owners. However, dogs are also the major reservoir of various zoonotic infections. Several bacterial, viral or protozoal diseases that occur in human are transmitted from the dogs. Such diseases are known as zoonotic diseases. These diseases can be transmitted by simple contact with the infected dogs or by infected urine or feces, saliva or aerosols. Viral infections such as rabies and noro virus, bacterial infection such as pasteurellosis, salmonellosis, campylobacteriosis, leptospirosis and Methicillin-Resistant Staphylococcus aureus (MRSA) infections and protozoal infections like ancylostomiasis, toxocariasis and Dipylidium caninum infection are the most common zoonotic infections. This paper focuses on these diseases including information on signs and symptoms, mode of transmission and prevention of these diseases. As much as these infections are concerned, knowledge boosting of the pet owners regarding zoonotic disease along with proper hygiene and good sanitation practices could considerably decline the rate of zoonoses transmission and consequences.

Keywords: Canine, Infections, Zoonoses

INTRODUCTION

The bond between humans and dog is believed to have begun since ten thousand years (World Health Organization, 1990). However, the recent discovery of 8-year-old boy and paw print alongside him in the Chauvet cave of France, which is believed to be from the Paleolithic period; suggests that the relationship between the two species existed for thousand years back and these two species have been living together on harmony mutually benefitting each other's existence. Pet animals such as dogs and cats have become a fundamental part of families residing on every corner of the world. Furthermore, it has been reported that, pet owners are less prone to high cholesterol and high blood pressure than the non-pet owners (Headey and Krause, 1999).

Along with the pet animals that stay with the humans in their homes, the population of free roaming dogs, commonly known as stray dogs is also increasing in an alarming rate. Street dogs appear to aggregate near slaughter house and garbage piles which forms a hub for breeding and transmitting diseases (Nasution, 2015). With the ever increasing population density in the developing countries, uncontrolled population of stray dogs along with lack of veterinary

knowledge and zoonotic awareness elevates the risks of disease transmission (Traub, Robertson, Irwin, Mencke, and Thompson, 2005).

Although these animals provide pivotal advantages to our society, adopting a pet may manifest several health hazards to the owners. Many bacterial, viral or parasitic infections that may occur in dogs have a high zoonotic significance and may transmit to the humans (Plaut, 1996).

ZOONOTIC DISEASES IN DOGS.

Viral Zoonotic Diseases.

Rabies

Rabies is caused by the single stranded DNA virus which belongs to Rhabdoviridae family. It is an ancient disease which causes high mortality rate both in human and animal population (Ghasemzadeh and Namazi, 2015). World Health Organization has reported that 30000 to 70000 deaths annually throughout the world because of rabies infection (Krebs, Mandel, Swerdlow, and Rupprecht, 2004). The major reservoir of rabies infection are dogs. Besides dogs the animals like racoons, bats and foxes also harbor the virus and are major cause of infection in developed countries (Tang, Luo, and Zhang, 2005). Depending on the site of the wound and amount of virus induced, incubation period of the disease ranges from 4 days to several years. Infected ones may manifest signs such as confusion, hydrophobia, hallucination, agitation and anxiety. If bitten by the suspected animals, post exposure prophylaxis with frequent dose of human rabies immunoglobulin (HRIG) within 14 days can prevent the disease (Lucas et.al., 2008).

Noro Virus Infection

This infection occurs due to heterogenous single stranded RNA virus which belongs to Caliciviridae family. The previous studies have suggested that, it is one of the leading cause of diarrheal diseases in humans of all age groups (Patel, Hall, Vinjé, and Parashar, 2009). Intestinal canal of dogs is primary affecting site of the virus thus are consequently seen in the feces of the infected dogs. Contaminated food or water is the main route of transmission of the virus to humans and can be controlled by strict hygiene maintenance and reduction of secondary outbreak propagation from human-to-human.

Bacterial Zoonotic Diseases

Pasteurellosis

Pasteurella are the gram-negative coccobacilli, which add up to the normal body microflora of upper respiratory tract of dogs and cats. Pasteurellosis can be transmitted directly and indirectly to humans both via dog or cat bites and sometimes even from their licks or scratches (Ghasemzadeh and Namazi, 2015). Primary infection occurs in soft tissues while secondary infection may affect bones, joints, brain and respiratory tract (Kristinsson and York, 2015). The infection can be treated by second and third generation cephalosporin, macrolides, fluoroquinolones, cotrimoxazole, and penicillin (Lloret et.al., 2013).

Salmonellosis

Salmonella are anaerobic, gram-negative motile bacilli that develop in distal part of colon and mesenteric lymph nodes of the canine species and are responsible for causing Salmonellosis. Transmission in humans occurs through the fecal transmission route and manifests clinical signs like gastroenteritis, enteric fever, bacteremia and osteomyelitis. The most common signs of this disease in both the species viz dogs and humans are gastrointestinal signs; however, majority of these infection go unnoticed. Infected animal may shed the pathogen through feces for a period of 6 weeks and transmit them to other animals or individuals (Finley *et.al.*, 2007). For the treatment of the infected animals and individuals, antibiotics including fluroquinolones, beta-lactams and macrolides are preferred (Leonard *et.al.*, 2011).

Campylobacteriosis

Campylobacter, a gram-negative bacterium, causes campylobacteriosis. The most prevalent species include *Campylobacter jejuni* and *Campylobacter coli*. This microorganism resides in the gastrointestinal tract of many animals and hence acts as a prominent zoonotic agent. Incubation period varies from one day to a week at max. Common clinical signs include fever, vomiting, diarrhea and abdominal pain. Apart from this, bloody diarrhea may also be evident. There has been report of convulsion and seizures in some patients (Gazaigne *et.al.*, 2008). Normally, campylobacter infection doesn't need any antimicrobial therapy. However, hydration should be considered for correction of electrolyte imbalance. In case of severe infections, antibiotics like fluoroquinolones, macrolides, or aminoglycosides should be considered (Ternhag, Asikainen, Giesecke, and Ekdahl, 2007).

Leptospirosis

Leptospirosis is caused by a spirochete bacteria *Leptospira*. Leptospirosis in humans is mostly transmitted by environmental factors including contaminated soil, water, urine or infected animal's tissue. Rodents play the role of major reservoirs for Leptospirosis; however, domestic animals including dogs also transmit leptospirosis in endemic regions (Moore *et.al.*, 2006). The major pathway for leptospirosis transmission is direct contact of contaminated urine with the mucosal surfaces of eye, vagina, nose, mouth, or erosive lesions on the human body. Clinical signs are seen in the infected humans within 10 days of disease transmission [ranges from 2 to 26 days] (Sehgal, 2006).

Leptospirosis sometimes is asymptomatic whereas sometime is seen with variety of symptoms including nonproductive cough, headache, musculoskeletal pain, diarrhea, nausea, vomiting, alveolar hemorrhage and sometimes meningitis (World Health Organization, 2003). Usually antibiotics such as doxycycline, cefotaxime, penicillin, amoxicillin and ampicillin is used for Leptospirosis treatment (Kobayashi, 2001).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection

Staphylococcus aureus is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, habitually found on the skin

and upper respiratory tract. Otherwise harmless, they can sometime cause serious infections leading to sepsis resulting in death. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a cause of staph infection which is burdensome during treatment as it is resistant to some antibiotics. It has been reported that the pathogen has been isolated from farm animals such as pigs, horses, cattle, cats and dogs. Among these animals, some believed that dogs and cats were the main reservoirs for transmission of MRSA by direct contact with their owners (Ghasemzadeh and Namazi, 2015). The infection probability exceeds more in immuno-compromised patients than the healthy ones. However, some evidences revealed that bacteria attack the healthy owners with infected pets (Iii, Rue, Wilckens, and Frassica, 2004; Va, Sahi, Suominen, and Va, 2005). With the advancement in the bacteria, traditional antibiotics are quite not efficient in treating the disease. Newer drugs including linezolid, daptomycin and vancomycin are effective in the treatment of MRSA infection (Morgan *et.al.*, 2008).

Parasitic Zoonotic Diseases

Ancylostomiasis

Ancylostomiasis is caused by the *Ancylostoma* spp. mainly *Ancylostomacanis*. It is one of the most common hookworms of dogs. This parasite reach adulthood in humans and cause eosinophilic enteritis (Landmann and Prociw, 2003). After contact with the human host, the parasite enters the skin and reaches heart and lungs. Then by penetrating through the pulmonary alveoli, reach pharynx via bronchial tree and are swallowed. The parasite then reach the small intestine where they gets attached to the intestinal wall and cause huge blood loss in the hosts (Centers for Disease Control and Prevention, n.d.). The clinical signs of the disease include abdominal pain, colic or cramps, nausea, pyrexia, bloody stool, loss of appetite and itchy rash (Brooker, Bethony, and Hotez, 2004).

Toxocariasis

Toxocariasis is a zoonotic infection caused by the parasitic roundworms commonly found in the intestines of dogs. (*Toxocara canis*) Infection in humans are commonly mediated by the accidental ingestion of embryonated eggs present in the soil or contaminated food/water (Oliart-Guzmañ *et.al.*, 2014) or less frequently by meat ingestion from intermediate hosts (Choi *et.al.*, 2012). Once the parasite enters the human host, the parasite reach circulation and gets distributed throughout the body. Majority of these cases go unnoticed: but in severe cases manifested by extensive larval migration:- signs like fever, eosinophilia, hepatomegaly, ocular symptoms, cardiac or pulmonary symptoms and sometimes even cerebral lesions are seen (Overgaaauw, 1997).

Dipylidium caninum infection

Dipylidium caninum, occasionally found in humans, is a common tapeworm of dogs and cat. It's also known as "flea tapeworm", "cucumber tapeworm" and double pored tapeworm" (Centers for Disease Control and Prevention, 2019). The transmission of this parasite in dogs is mainly mediated by fleas, *Ctenocephalides canis*, *C. felis* along with dog biting louse, *Trichodectes canis*. Dogs accidently gets infected by ingesting the flea or lice (Soulsby, 1982). The infected

dogs release the proglottids along with their feces in the environment and these eggs with proglottids gets consumed by flea larvae (Yasuda, Nishikawa, Ujihashi, and Watanabe, 1970). Though the parasite is not quite harmful in adult dogs, there may be some hemorrhages at the site of attachments resulting in enteritis and diarrhea (Soulsby, 1982). Accidental infection in humans occurs when the dog flea with parasite are swallowed. The infections of *Dipylidium caninum* is mostly reported in children (Narasimham *et.al.*, 2013; Ramana, Rao, Rao, Mohanty, and Wilson, 2011). When the gravid proglottids pass through the anus, pruritus is seen in the infected host. Treatment of *Dipylidium caninum* can be successfully carried out on both cases of humans and animals (Saini, Gupta, Kasondra, Rakesh, and Latchumikanthan, 2016).

Tackling Zoonotic Diseases in Dogs

With the advancement of the medical industries different commercial vaccines are available in the pet owners and veterinarian's disposal for prevention of the diseases. However, to ensure disease prevention, strict protocol should be followed in dogs. The vaccination protocol is given below.

S.N.	Vaccine	Type of Vaccine	Age	Booster	Repeat
1.	Canine Distemper and Measles virus	Modified live virus	6-8 weeks	12-16 weeks	Annual
2.	Infectious Canine Hepatitis Virus	Modified live virus (CAV-1 or CAV-2) or inactivated (CAV-1) vaccine	6-8 weeks	12-16 weeks	Annual
3.	Rabies Virus	Modified Live vaccine Inactivated virus	3-4 months 3-4 months	1 year 3-4 weeks	3 years Annual
4.	Canine Leptospirosis	Killed bacteria (bacterin)	9 weeks	12-16 weeks	Annual
5.	Parvo Virus	Inactivated	6-8 weeks	12 weeks	Annual

(Source: Chakrabarti, 1993)

Infection can also be prevented by sanitation, hygiene and vaccination. Along with this cautious use of antibiotics in the farm animals should also be utilized. For the prevention of zoonotic disease and betterment of public health, veterinarians, physicians and public health agencies have varieties roles to play. Efficient communication between the veterinarians and physicians should be established to help estimate the risk of different zoonotic disease, properly educate pet owners and promptly diagnose zoonotic disease in animals and people (Lipton, Hopkins, Koehler, and Ronald, 2008).

CONCLUSION

Zoonotic diseases are those disease affecting both animals and humans and can be transferred either by pets or wildlife. The ever-increasing trend of pet rearing and huge number of stray dogs that coexist with human dwellings in the city area of developing nations bring about great quantity of dogs in and around our surroundings. With a dramatic number of canines around

human population, the risk of dog-borne zoonoses are also increasing in an alarming rate. Dogs harbor different virus, bacterial and protozoal disease that have a very high zoonotic significance and in worst case scenario can be fatal. Moreover, dog transmitted parasitic infections causing different infections and affecting lives of billions of people often goes unnoticed (Macpherson, 2005).

Thus, to reduce these dog-borne zoonoses, the pet owners should be well informed. To tackle such infections several prophylactic and chemotherapeutic measures can be applied. It is recommended that the dog owners follow the vaccination protocol for the prevention of diseases. Along with this, strict sanitation and proper hygiene is to be maintained by the pet owners. As most of these pathogens gain entry in the dogs via feco-oral route, food hygiene must be addressed too. Raw meats should be completely avoided. And to avoid feco-oral transmission in humans, vegetables and food items should be properly washed before being cooked or eaten. If the knowledge level of pet owners about zoonotic disease and their treatment can be increased alongside the pet owning trend, then it will be safe to say that it would greatly reduce the prevalence of zoonoses in dog owners and common people.

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Impact of Climate Change on Livestock Production

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ABSTRACT

Climate change is one of the global challenges of this century. There is increase in large number of climate related events. Livestock sector has also been affected by changing climate due to which there is increased loss of livestock assets and several other indirect losses. Some of the effects of climate change in livestock include thermal and cold stress, increased diseases incidences and decrease in the feed, fodder and water availability. This results in decreased animal production and productivity. The paper mainly reviews the impacts of climate change on livestock production.

Keywords: Climate change, Livestock, Livestock production

INTRODUCTION

A long-term shift in temperature, radiation, wind and rainfall of a place in a long term of time is climate. It is the change in climatic conditions over time due to either anthropogenic or nature induced causes, which remains for decades or longer and shows distinct variations in its mean (IPCC 2007). The issue of events caused by climate change is seen frequently nowadays. It is causing huge loss of lives and economy of various countries. As greenhouse gas is increasing in the atmosphere leading to increase in the temperature of earth, there is a prediction that there will be an increase in temperature by 1.5°C to 2°C in future may cause loss of 20 to 30 percentages of animal and plant species (Thronton, 2010). This shows that to survive in future changing climate, plant and animal species need to alter their behavior and distribution patterns and get acclimatized to changing climate. Direct and indirect effects of temperature, humidity, rainfall and other climate factors influence animal performance: growth, milk production, wool production and reproduction (Houghton *et.al.*, 2001).

Livestock system is one of the major sources of nutrition to the increasing population of the world. Human populations largely depend on animals and animal products like milk, meat, eggs, fibers, wool, and feather. Animals are also used for transport, draft and their manure. Livestock production is adversely affected by various events of extreme climatic conditions. This paper focuses in the effect of climate change on livestock production.

Climate Change

Climate change is a phenomenon due to emissions of greenhouse gases from fuel combustion, deforestation, urbanization and industrialization (Upreti, 1999) resulting variations in solar energy, temperature and precipitation. It is a real threat to the lives in the world that largely

affects water resources, agriculture, livestock, coastal regions, freshwater habitats, vegetation, forests, melting of snow-covered mountains and increase in climatic events such as landslide, desertification and flood. Around the world the three major components of climate change already evident and escalating in magnitude and significance are; Warming, altered patterns of precipitation and increased incidence of extreme climatic events.

Effect of Climate Change on Livestock

The effect of climate change is found to be real and is affecting livestock population in many ways. Climate change is expected to cause increase in weather-related disasters and extreme weather events, such as droughts, heat waves, storms, desertification, and increases in insect infestations (Khanal, 2010). Long-term changes in climate will affect the future of all animals including those in oceans, on farms, in forests, in wilderness areas, and in our homes (Khanal, 2010). Warmer and wetter weather (particularly warmer winters due to climate change) will increase the risk and occurrence of animal diseases, as certain species who serve as disease vectors, such as biting flies and ticks, are more likely to survive year-round. At higher temperature, numerous diseases display greater virulence. As a result of climate change the environment becomes favorable for the disease agent (bacteria, virus, etc.) and the host will become susceptible easily. The epidemiological triad between agent, host and environment becomes imbalanced and - different diseases which were not present in -an ecological region may be seen, and certain existing parasitic diseases may also become more prevalent, or their geographical range may spread, if rainfall increases.

Increased temperature may cause thermal stress in terrestrial and aquatic animals, leading to reduced growth, sub optimal behaviors, decrease productivity and reduced immune competence of the animals. Higher temperatures tend to reduce animal feed intake and lower feed conversion rates (Rowlinson, 2008) and extra investment cost to keep animal warm or cool during climatic extremes is forecasted to increase. Unusual climatic changes and variability such as rising temperature, irregular monsoon, precipitation and erratic rainfall patterns have led to loss of large number livestock species ultimately affecting the income and food security of marginalized people. Climate change can be expected to have several impacts on forage, feed crops, grazing system, and emergence of unpalatable forage species in the rangeland and decrease the byproducts of agriculture and forage causing scarcity of fodder and forage for livestock.

Climate Influence on Livestock Productivity

The influence of climate change on livestock productivity is categorized on following headings.

Milk Production

High production animals are highly influenced by heat stress (Martello *et.al.*, 2010). The threshold level of temperature humidity index (THI) for the high milk yielding cow is around THI 72 in tropical and subtropical climates. However, recent studies on THI in temperate climate emphasized that the THI lower than 68 is suitable for cattle performance (Gauly *et.al.*, 2013). Early heat stress signs in dairy cows include panting and sweating and longer standing. These factors lead to cows failing to eat as much as usual.

Heat stress decreases the quality and composition of milk. This can result in a rapid decline in milk yield up to 40%. Heat stress increases body temperature and affects the fat synthesis in mammary gland and components like fat (%), solid-non-fat, protein, casein and lactose content is altered. Somatic cell count is also increased causing reduction in the quality of milk. Further, heat stress can also cause imbalance in the levels of prolactin, thyroid hormones, glucocorticoid, growth hormone, estrogen, progesterone and oxytocin which ultimately affects the milk production (Prathap *et.al.*, 2017).

Heat stress increases udder temperature and can cause mastitis in dairy cows. In addition, heat stress during dry period might trigger mammary gland involution accompanied with apoptosis and autophagy and decreased amount of mammary epithelial cells can ultimately cause decline in milk yield (Prathap *et.al.*, 2017).

As temperature rises above 35°C stress response systems are activated and there is reduced feed intake declining the milk synthesis (Wheelock *et.al.*, 2010). Moreover, maintenance energy requirement is also increased by 30% in heat stressed dairy animal. Energy intake will not be enough to cover the daily requirements and milk production. This results in negative energy balance is created deteriorate health condition of the animal (West *et.al.*, 2003). A reduction in dry matter intake by 0.85 kg with every 1°C rise in air temperature decreases milk production approximately by 36% (Rhoads *et.al.*, 2009).

Growth Performances

Exposure of animals to hot or cold stress reduces growth and feed efficiency (Ames *et.al.*, 1980). Cold climate during winter causes metabolic acclimatization, resulting in decreased animal performance and production efficiency (Young, 1981; Birkelo *et.al.*, 1991). Cold stress increases concentrations of plasma corticosteroids (Alvarez and Johnson, 1973) and circulating nonesterified fatty acid (NEFA) concentrations (Broucek *et.al.*, 1987; Nonnecke *et.al.*, 2009). Heat stress has an effect on the uterine environment reducing the total embryo cell number and placental size and small sized calf are born. Temperatures ranging between 15°C and 29°C seem to have no effect on growth performance. High ambient temperature decreases the anabolic activity and increase in tissue catabolism (Marai *et.al.*, 2007). Reduced anabolism activity decreases voluntary feed intake and increase tissue catabolism causes fat depots resulting in adverse effect in growth performance.

Reproductive Performance

Reproductive performances of livestock are vulnerable to climate change. It has adverse effect in both female and male reproductive system. Heat stress reduces the length and intensity of oestrous cycle and besides increases incidence of anestrus and silent heat in animals (Kadokawa *et.al.*, 2012). There is increase in adrenocorticotrophic hormone and cortisol secretion (Singh *et.al.*, 2013), resulting in blockage of estradiol-induced sexual behavior (Hein, K.G. and Allrich, R.D. 1992). When the body temperature exceeds 40°C, follicles suffer damage and become non-viable (Roth *et.al.*, 2000). When a female goat is exposed to 36.8°C temperature and 70% relative humidity for 48 hours, follicular growth up to ovulation is suppressed, accompanied by decreased LH receptor level and follicular estradiol synthesis activity. Infertility is seen in high-temperature as there is increase secretion of endometrial PGF-2 α (Bilby *et.al.*, 2008). Conception

rates decrease and reaches only 10-20% compared to 40% to 60% in cooler months (Cavestany *et.al.*, 1985).

Heat stress causes embryonic death by interfering with protein synthesis, oxidative cell damage reducing interferon- tau production for signaling pregnancy recognition and expression of stress-related genes associated with apoptosis (Hansen, P.J. 2009). Embryos subjected to high temperatures in vitro or in vivo until day 7 of development phase as blastocyst lowers pregnancy at day 30 and increases embryonic loss on 42 days of gestation (Cardozo *et.al.*, 2006).

Increased testicular temperature in bull from thermal stress could change the quality of the semen and alter the biochemical composition leading to infertility problems. There is a change in testicular volume, hormonal profiles, sexual behavior and semen quality that affect the reproductive performance of males (Balic *et.al.*, 2012).

CONCLUSION

The global demand of animal and animal products is increasing with the increasing human population. However, climate change is causing negative effects in the animal production and productivity. Climate extreme hazards are increasing causing huge loss of livestock assets and there is decrease in the feed, fodder and water availability for the animals, and increase in thermal and cold stress, diseases resulting in the decrease in animal production. Proper breeding strategies should be identified so that animal become tolerant to heat and cold and survive (Hoffmann, 2008). Identifying and strengthening local breeds adapted to local climatic stress and feed sources would be one of the options to mitigate the negative effects of climate change in livestock. Likewise, early warning systems and forecasting system for livestock diseases should be developed.

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