PREVALENCE OF BLOOD PROTOZOAN PARASITES OF CATTLE, BUFFALOES AND GOATS OF THREE VDCs OF SIRAHA DISTRICT, NEPAL

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Submitted to

Central Department of Zoology

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author (s) or institution (s).

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RECOMMENDATION

This is to recommend that the thesis entitled **"PREVALENCE OF BLOOD PROTOZOAN PARASITES OF CATTLE, BUFFALOES AND GOATS OF THREE VDCs OF SIRAHA DISTRICT, NEPAL"** has been carried out by Sujit Kumar Yadav for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor **"Dr. Mahendra Maharjan"** this thesis submitted by Sujit Kumar Yadav entitled **"PREVALENCE OF BLOOD PROTOZOAN PARASITES OF CATTLE, BUFFALOES AND GOATS OF THREE VDCs OF SIRAHA DISTRICT, NEPAL"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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This thesis work submitted by Sujit Kumar Yadav entitled **"PREVALENCE OF BLOOD PROTOZOAN PARASITES OF CATTLE, BUFFALOES AND GOATS OF THREE VDCs OF SIRAHA DISTRICT, NEPAL"** has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
BPDs	Blood Parasitic Diseases
BPP	Blood Protozoan Parasite
DLS	Department of Livestock Service
DLSO	District Livestock Service Office
GDP	Gross Domestic Product
LS	Livestock
TBD’s	Tick Borne Disease
VDC	Village Development Community

ABSTRACT

Among various diseases, parasitic diseases of domestic animals blood are important ones responsible for livestock health deterioration mainly in Terai belt. In order to assess the blood protozoan parasitic diseases in livestock of Siraha district, a total of 310 blood samples from 130 cattle, 115 buffaloes and 65 goats' were collected from three VDCs of Siraha district namely Marar, Khirauna and Malhaniya, during Mar 2013 – Feb 2014. Blood samples were collected by puncturing jugular vein of domestic animals. The blood samples collected were transported to DLSO, Siraha for thin blood smear preparation and Giemsa staining. Upon Microscopic examination of 310 blood samples revealed 56(18.06%) prevalence of blood protozoan parasites. Among them cattle and buffaloes (i.e. 19.23% & 19.13%) were found to be equally infected whereas goats were comparatively less infected i.e. 13.84%. Age wise analysis showed that 4-8 years domestic animals were found to be infected maximum by *Babesia* and *Theleria* compared to other age group. Prevalence of blood protozoan parasitic infection was slightly varied in three VDCs. Khirauna and Malhaniya VDCs were found to be equally infected i.e. 5.48% with BPP. While Marar VDC was found to be comparatively more (7.09%) infected. Out of 18.06% BPP infection in domestic animals high infection was found in cross bread (10.32%) than local breed (7.74%).

1. INTRODUCTION

1.1 Background

Nepal remains a predominantly agrarian economy. About 66% of its population is involved in agriculture, which accounts for 35% of the gross domestic product (GDP) (NSCoA 2013). The livestock (LS) subsector of agriculture contributes 31% of the total agricultural GDP (CPDD 2013), and Livestock farming prevails in all regions of the country, including the Mountain, Hill and Terai belts, with variations based on climate, topoFigurey, and socio-economic factors. Nepal has largely a smallholder livestock system under which farmers raise small numbers of livestock in small land holdings. A common stressor is livestock mortality and morbidity due to poor nutrition and disease. The LS has a strong gender dimension in that women contribute some 70% of the livestock farming work but usually have no significant role in livestock marketing and finance (ADS 2012). The LS contributes to human food security and nutrition, livelihood of farmers, employment and income generation, inputs for farm operation (such as draft and manure), industrial production, and rural transportation (MOAC 2012)

1.2 Livestock population and production in Nepal

The growth rate of livestock is less than that of fishery and cash crop, but the contribution of livestock farming in the overall agricultural GDP is more than fishery and cash crops agriculture, the overall growth rate of livestock is around 5.3% per annum (MOAC statistics 2012). Cattle are the main source for animal traction and manure in terms of animal mass units; it is the largest livestock in Nepal. The population of cattle was about 72,44,944 with the annual growth rate of 0.42% (DLS statistics 2012). Buffalo is the main source for milk and meat production and also it is useful as manure and draft power for soil fertility. There were about 51,33,139 buffalo and the annual growth was 3.36% per annum, the production of buffalo milk was around 11,53,838 metric tons with the annual growth rate of 3.8% and the production of meat was around 1,72,414 metric tons with 3.2% annual growth (MOAC statistics 2012). Cow is just used for the production of milk in Nepal. As cow is our national animal, it is not used as meat production livestock, the population of milking cow was about 9,98,960 with the annual growth rate of 1.8% the production of cow milk was about 4,68,913 metric tons with the annual growth rate of 3%(MOAC statistics 2012). There were about 8,07,267 sheep and the annual growth was 0.25% per annum, the meat production was around 2,720 metric tons with the negative growth annual growth rate of -2.58% around 5,87,017 metric tons of wool was produce

(MOAC statistics 2012). Primarily goat is the second most popular source of meat there were about 9512958 goats annual growth was 4% per annum around 53,956 metric tons of meat was produced with the growth rate of 3.6% per annum (DLS statistics 2012). The population of pigs was around 11,37,489 and the annual growth rate was 2.2% per annum the total production of pig meat was around 18,277 metric tons with the annual growth rate of 1.7% per annum (DLS statistics 2012).

1.3 Livestock Diseases

Livestock diseases are classified into four group zoonoses, food borne, endemic and epidemic whereas endemic are ticks and tick-borne diseases, trypanosomasis, gastro intestinal parasites, zoonoses and food borne are hydatdosis, cysticerosis, brucellosis and tuberculosis, epidemic are classical swine fever, African swine fever, foot and mouth disease (Perry et al. 2001).

The livestock sector is suffering from a number of disease problems caused by bacteria, viruses, fungi, and parasites. Among the parasitological problems, the damage caused by TTBDs is considered very high (Ghosh et al. 2007). Of various factors attributing low productivity of livestock, prevalence of infectious and parasitic diseases form one of the major constraints in reducing production and productivity of livestock causing substantial economic loss to livestock industries. This loss has been exacerbated by the incursion of emerging and re-emerging animal diseases. Majority of the Nepalese LS population suffers from one or other type of parasitic problems and they have tremendous negative effect on their population. Among them Blood parasitic disease (BPD's) of LS are widely spread in the Terai region and occasionally seen in mid hills region due to the ticks' vector *Boophilus, Hyaloma, Rhiphicephalus* and *Ixodes*. Six regional veterinary laboratory investigation report shows BPD's present in Biratnagr and Jankpur region tick borne haemoprotozoan disease are endemic in Eastern Terai region of Nepal (VEC 2012).

1.4 Tick and ticks borne diseases (TBD's)

Ticks are small, blood sucking creatures which feed on many different types of birds and animals, including humans. During feeding they can spread infections and cause disease. Globally ticks transmit a greater variety of pathogenic microorganisms, protozoa, rickettsiae, spirochaets, and viruses than any other arthropods and are among the most important vectors of diseases affecting livestock, humans, and companion animals. The lack of available estimates of the prevalence and incidence of each tick-borne disease

makes it difficult to determine their impact. However, since TBDs are each vectored by particular tick species, the potential distribution of each disease can be estimated from the distribution of its vectors. This potential distribution, however, is not always an accurate reflection of present disease incidence. Ticks and tick-borne diseases (TBDs) affect 80% of the world cattle population and are widely distributed throughout the world, particularly in tropical and subtropical countries including India, Pakistan, and Bangladesh(Ghosh et al. 2007).

Ticks transmit a greater variety of pathogenic microorganisms, than any other arthropod vector group, and are among the most important vectors of diseases affecting livestock. In general, tick-borne protozoan diseases (e.g Theileriosis and Babesiosis) and rickettsial diseases (e.g. Anaplasmosis and Heartwater or Cowdriosis) are preeminent health and management problems of cattle, small ruminants and buffaloes, affecting the livelihood of farming communities in Africa, Asia and Latin America (Minjauw and McLeod 2003).

1.5 Blood protozoan parasite (BPP)

Anaplasmosis in cattle caused by obligate intra erythrocytic parasite of the genus *Anaplasma*. Symptoms that occur are fever that last for 4 to 10 days, anorexia, weight loss, lethargy, cough and increased rate and pulse rate, abortion and decreased milk production. *Haemobartonella* and *Eperythrozoon* differ from *Anaplasma* in that they are wall less attach to the surface of red cells and do not invade erthtocytes (Neimark et al. 2001). Babesiosis is a disease caused by parasitic protozoa of the genus *Babesia*.. Symptoms in animals are anorexia, fever, anemia, increased respiratory rate and pulse rate, jaundice, diarrhea, constipation and respiratory problems. Fever that occurs can lead to abortion and decreased sperm fertility. Theileriosis is a disease caused by protozoa of the genus *Theileria*. It is an obligate intracellular parasite. Clinical symptoms that The blood smear was observed by using microscope with occur are fever, anorexia, decreased milk production and weight, respiratory problems, poor growth and production, jaundice, anemia and abortion

Among the blood parasites in mammals are *Eperythrozoon*, *Haemobartonella* and *Trypanosoma* species. Their effects on the susceptible hosts vary from mild effect to death (Robson and Kemp 2007). *Haemobartonella* and *Eperythrozoon* species in different animals have been shown to be transmitted by various bloods feeding arthropods, including tick, lice, fleas, flies and mosquitoes (Urquhart et al. 2003). Recently,

Eperythrozoon and *Haemobartonella* have been reported in cattle, sheep, goat, pigs, dogs and cats as well as humans in several parts of the world (Zhibiao et al. 2007).

Objective of the study

1.6.1 General Objective

To find out prevalence of the blood protozoan parasite in cattle, buffaloes and goats of Marar, Khirauna and Malhaniya VDCs of Siraha district.

1.6.2 Specific Objectives

i. To determine the blood protozoan parasite in cattle, buffaloes and goats.
ii. To compare prevalence of blood protozoan parasite in three different VDCs of Siraha district.
iii. To compare BPP in between local and cross breed species of cattle, buffaloes and goats.

2. LITERATURE REVIEW

2.1 Blood protozoan parasites in global context

2.1.1 Ticks and tick borne disease

On global basic ticks transmit a greater variety of pathogenic microorganism protozoa, rickettsia, and spirochetes. Ticks and tick borne disease affect 80% of the world cattle population and widely distributed throughout the world (Ghosh et al. 2007). Ticks and tick born disease affect animal and human health worldwide and are the cause of significant of economic losses. Approximately 10% of the currently known 867 ticks' species acts as vectors (Jongejan et al. 2004).

Ticks fever or cattle fever is economically the most important arthropod borne disease of cattle worldwide. Ticks fever was the first disease which transmission by an arthropod (Bock et al. 2004). The diseases of cattle are transmitted by *Rhiphicephallus* (Boophilis) ticks which can affect the immune systems of vertebrate host (Suarez et al. 2007). Clinically examination revealed enlargement of superficial lymph nodes, fever congested mucous membranes corneal opacity emaciation were found in case of theileriosis, while fever paleness of mucous membranes and brown coffee urine were common clinical finding in case of babesiosis. Hematological finding revealed that cattle suffered from theileriosis showed normocytic hypochromic anemia, while those suffered from babesiosis showed normocytic normochromic anemia (Hussein et al. 2007). Due to the ticks born parasite in southern Panjab out of 107 samples 40 sheep and 67 goats collected where 20 sheep and 16 goats were positive appearance (Iqbal et al. 2011)

2.1.2 Babesiosis

Babesiosis is a tick-borne disease of cattle caused by the *Babesia* species. The *Babesia* parasites are transmitted by ticks such as *Boophilus* and *Dermacentor*. The parasites are taken up by ticks while feeding on the definitive host. In the tick, the gametocytes obtained from erythrocytes form zygotes, which differentiate into ookinetes. There are over 100 species of *Babesia* identified however only a handful of species have been documented as pathogenic in humans. In the United States, *Babesia microti* is the most common strain associated with humans with other species infecting cattle, livestock and occasionally domestic animals (Despommier and Dickson 1995).

In the China nine species of *Babesia* have been recognized in livestock *B. bigemina, B. bovis, B. major, B. motasi, B. ovis, B. perroncitoi, B. equi, B.trautmanni B. cablli*. Which are manily caused by tick vectors *Boophilus microplus*, *Rhipicephals haemaphysaloides*, *Haemaphysalis* punctate and *Haemaphysalis longicronis* (Hong et al. 1997). The Bovine babesiosis disease is widely spread in north eastern region of Thailand which is tick transmitted hemoprotozoan disease caused by *Babesia bovis* and *Babesia bigemina*. Prevalence of *B. bovis* and *B. bigemina* was 11.2% and 3.6% by PCR, 14.7% and 5.9% by ELISA and 16.8% and 5.6% by IFAT respectively (Terkawi et al. 2011). In the case of cattle of Taiwan *B. bovis* 1.9% and *B. bigemina* 0.6% detected from dairy cows (Tasi et al. 2011). Other species of *Babesia canis* is most frequently agent of canine babesiosis from three geoFigureically isolate lowland are of southern Slovakia prevalence of *B. canis* was found in eastern Slovakia 14.4%, southwest 2.3% (Kubelova et al. 2008). In the east black sea region of turkey distribution of tick borne haemoprotozoan parasite. The mostly found species was *T. buffelilorientalis* 11.56%, *T. annulate* 1.28%, *B. bigemina* 0.77%, *B. major* 0.51% (Kursat et al. 2008).

2.1.3 Theileriosis

Theileriosis is an important haemoparasitic disease of animals inducing a variety of clinical manifestations ranging from a subclinical presentation to a fatal disease depending, in part, on the animal species, host, age and the species of the microorganism.

In the west and North West Iran infection rate of ticks for bovine theileiosis reveales that 39.9% of *Hyalomma anatolicum anatolicum*, 3.5% *H. asiaticum asiaticum* and 18.2% *H. anatolicum excavatum*, were infected with *T. annulata*. *H. a. anatolicum* may play major role in transmission of *T. annulata* infection (Tavassoli et al. 2011). In western half of Iran microscopic examination revealed that 9. 2% sheep were infected by *Theileria* spp. piroplasmas where 54.8% *Theleria lestoquaridi* and 40.2% *Theleria ovis* (zaeemi et al. 2011).

Theileria uilenbergi is one of the causative agents of theleriosis in small runinants in china. By the ELISA for the detection of circulating antibodies in sera of *T. uilenbergi* infected sheep (abdo et al. 2010). Babesia caballia and Theileria equi is widespred in Asia which caused piroplasmosis in camels, horse and dogs microscopic examine revealed that 67% *T. equi* presence in horse whereas negative appearance in camel and dog (sloboda et al. 2011).

Abdomen area of hard ticks *Hyalomma a. anatolicum* revealed high rate of infection with *Theleria* sp. 43% and *Babesia* sp. 15.2%. *Babesia* was recorded for the first time in Iraq from Ticks (Hadi et al. 2012). Similarly in Sudan *Hyalomma a. anatolicum* nymphs were revealed that 49.6% infected with *Theleria* (salih et al. 2005).

Cross breed cattle of Dehradun district revealed that 27.2% overall prevalence of Theleriosis. Whereas high prevalence was found in rainy season 45.4% (Kohli et al. 2014). Reverse line blot hybridization with polymerase chain reaction detected 48.6% positive samples in cattle. Where high infection was found in *T. buffeli* 39.2% and *T. annulata* 17.3% but less infection found in *B. bovis* 6.8% and *B. bigemina* 4.3% (Ghirbi et al. 2008).

2.1.4 Anaplasmosis

Anaplasmosis is an arthropod-borne rickettsial disease of cattle, sheep and goats, and has a wide distribution. The disease in cattle is caused by *Anaplasma marginale* and *A. centrale.* Infection caused by *A. marginale* is characterized by severe anaemia, cachexia, abortion and death (Alderink and Dietrich 1981). In the Norteast germans prevalence of *anaplasma phagooytophilum* out of 522 dogs where 258 specked and 264 healthy. No difference prevalence between sick 46.9% and healthy 39.8%. There was no difference with regard to abnormal CBC parameter between seropositive and seronegative clinically healthy dogs (Kohn et al. 2011). Whereas Hidaka district of Japan *Haemaphysalis megapinosa* is dominant vector of tick species of both *A. bovis* and *A. phgocytophilum* for cattle (yoshimoto et al. 2010). In the Northern Kerla South India out of 150 samples 25 were detected positive for *A. marginate* and 5 for *A. bovis* detected by PCR-RFLP and nest PCR techniques (Nair et al. 2013).

In north eastern Free State due to distribution of ticks' vector *Boophilus decolonatus* and *Rhipicephalus evertsi evertsi* in cattle were reported highly infected 87% with *Anaplasma* and 94% *B. bigemina* (Matshali et al. 2004). Similar result has been shown in case of *Anaplasma* in sirajgong Bangladesh (Chowdhury et al. 2006). Prevalence of *Anaplasma marginale* in cattle examined by cELISA and IFA test result shows 62 of 69 samples positive. Which conclude that both cELISA and IFA test can be done for *A. marginale* (Ekici et al. 2011). In northern Sudan overall prevalence of *B. bigemina* 4.0%, *B. bovis* 1.9% and *A. marginale* 6.1% which varies significantly in different age group (Awal et al. 2011).

2.1.5 Trypanosomosis

Trypanosoma induce diseases with variable symptoms depending on the infected host, *Trypanosoma* species and serodeme. Generally, trypanosomosis is characterised by fever, anaemia, cachexia, reduced productivity, and infertility, animals often die from heart failure or opportunistic infections. The infected animals tend to exhibit persistent fluctuating parasitaemia comprising a series of trypanosome waves expressing different variable surface glycoproteins (VSG) (Barbet and McGuire 1982).

Mixed infection of *Trypanosoma vivax* and *Fasciola* spp in an intensively managed dairy farm in Niger where 25% had concurrent infection of *T vivax* and *fasciola* spp in cows (Okaiyeto et al. 2011). By the new diagnostic technique loop – mediated isothermal amplification (LAMP) of the random insertion mobile element (RIME- LAMP) to diagnose camel trypanosomasis revealed that 56.52% positive by using RIME-LAMP whereas only 6.34% positive by parasitological method (Abdebahman et al. 2011). In the Northwest Ethiopia Trypanosoma species were encountered in 6.3% examined donkeys while none of mules positive for trypanosome infection. In donkey three species of Trypanosomes found *T. congolensa* 52.4%, *T. brucei* 28.6% and *T. vivax* 19.05% (Abebe et al. 2010).

In Adamawa state prevalence of *Trypanosoma* in cattle was found 26.67% where males 9.17% and female 17.50% infected (Zubairu et al. 2013). In different state of Malaysia cattle and buffaloes are highly infected with bovin anaplasmosis 77.6% and bovine trypanosomasis 14.7% (Wahab et al. 2012). In kurmuk district Blue Nile state 43% cattle population was infected with two morphologically distinct Trypanosomaes where 33.3% *T. vivax* and 10% *T. congolense* appears (salim et al. 2011). Trypanosomiasis infection in Northern Tanzania found 16.7% pigs were infected with one or more species of *Trypanosoma* where *T. vivax* 3.6%, *T. simiae* 1.8%, *T. congolense* 1%, *T. godfrcy* 2.4% and *T. Brucie* 10.1% infected (Hamill et al. 2013).

2.2 Blood protozoan parasites in national Context

In the Banke districts prevalence of BPP shows that *Babesia*, *Trypanosoma* and *Anaplasma* were detected in buffaloes, whereas only *Anaplasma* in cattle and Babesia in dog were detected (Adhikari et al. 1997). A study conducts in Terai region revealed that 13.25% *Theleria*, 9.64% *Babesia* and 6.02% *Anaplasma* presence in cross breed cattle (Shrestha et al. 1999). Similarly in cross breed cattle of Morang district reveled that *Theleria* 26.18% and *Babesia* 10.18% positive found due to the tick vector *Boophilus microplus*, *Hyallomma anatolicum anatolicum* and *Rhiphicebhalus haemaphysaloides* (Deo et al. 2002).

In the Estern terai region Theleria infection in *Hyalomma* thick vector found 8.62%, 27.35% and 20.63% from Sunsari, Morang and Jhapa respectively. Whereas high prevalence in female *Hyalomma* ticks (Gupta et al. 2013)

Domestic animals of Makawanpur district 6.67% affected with Trypanosomasis where 8.57% cattle, 6.67% buffaloes and 13.3% dogs. While none of the goats and pigs acquired infection. Whereas maximium infection found in rainy seasion with high prevalence in cross breed (Maharjan and Mishra 2006)

3. MATERIALS AND METHODS

3.1 Study area

Nepal is a small landlocked country lying between 80º4' - 80º12' east longitudes and 26º22' - 30º27' North latitude. The country shares boundary with China on North and India on East, West and South. The total land area of the country is 1,47,181 square kilometer. In Nepal there are 28.04 million humans population with 2.25 % annual growth rate. About 65.5% people in the country are engaged in agriculture farming. GeoFigureically country is divided into three regions Mountain region, hilly region and Terai region.

Siraha district is located in the Sagrmatha zone of Eastern development region in between 26º33' - 26º55' North latitude and 86º6' - 86º26' east longitudes. Siraha district shares boundary with Saptari district (Balan River) on east, Dhanusha (Kamala River) on West, Udaypur (Chure Mountain) on North and India (Bihar state) on South. It has 76 to 885 meter of altitudinal range from sea level. It covers 1228 Sq km total area. Out of 106 VDCs in siraha district three VDCs (Marar, Khiraauna and Malhaniya) were selected for the present research. These three VDCs are in range of 6-8 km from Headquarter.

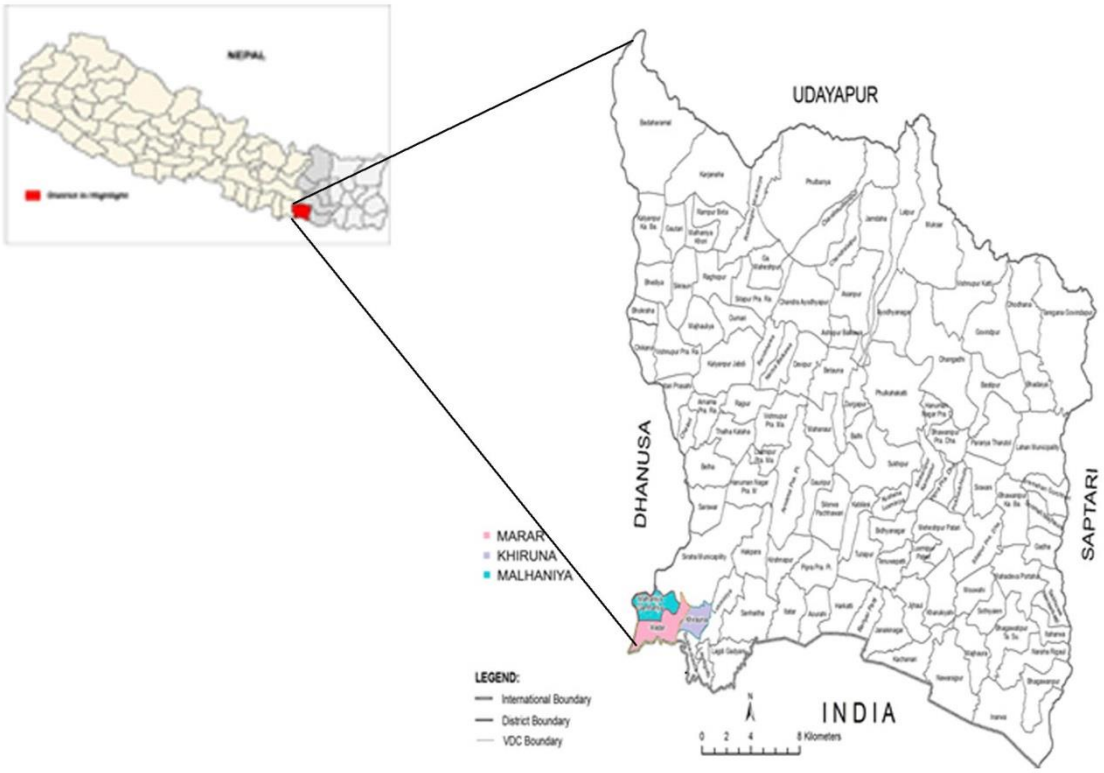

Figure 1 Map of study area

Siraha district is located in low land area where climate varies from maximum 40ºC to minimum 20ºC temperature. Due to the favorable condition ticks and tick borne disease prevalence is expected to be high in the Marar, Khirauna and Malhaniya VDCs.

In the present study blood samples were collected from Marar VDCs 110 sample whereas 50 cattle, 40 buffaloes and 20 goats, from Khirauna100 samples whereas 50 cattle, 35 buffaloes and 15 goats, from Malhaniya 100 samples whereas 50 cattle, 35 buffaloes and 15 goats. The sample of cattle, buffaloes and goats were collected from various age with local and cross breed (Annex)

According to District profile progress report 2012 of District Livestock Service Office (DLSO), Siraha the total number of LS is 1002666. Among them cattle 24.49%, buffaloes 13.29%, goats 59.96%, sheep 0.2% and pig 2.04% with majority of the local breed. The district profile revealed that 55,208 Metric Tons milk and 9,258.2 Metric tons meat production.

3.2 Material use

The materials used during the study have been listed below.

3.2.1 Laboratory tools:

i.	Cotton, tissue paper	ii.	Coupling jar
iii.	Forceps	iv.	Globes
v.	Microscopes	vi.	Needle and sticks
vii.	Slides, cover slips, slide box	vii.	Slide staining tray
ix.	Syringes	x.	Timer
xi.	Sampling vials	xii.	Vaccutainer with EDTA

3.2.2 Chemicals

i.	Methanol/Ethanol	ii.	Distilled water
iii.	Giemsa staining reagents	iv.	Sprits
v.	Formalin solution	vi.	Immersion oil.

3.3 Blood Sampling

A total of 310 Blood samples from 130 cattle, 115 buffaloes and 65 goats' were collected from three VDCs. Blood sample were collected puncturing jugular vein of selected

animals and brought to the DLSO Siraha laboratory in EDTA containing vials for smear preparation and staining.

3.3.1 Preparation of Blood Smears

For thin smears, one drop blood was taken and placed near one end of clean and dry glass slide. Spreader slide inclined about 45º was pushed along horizontal from the one end to another to get thin smear with clear tail. The more acute the angle between the slides, and the more slowly the spreader slide was moved, the thinner the film would be. The resulting film was dried rapidly by waving it in the air, and was fixed with methanol for 2 minutes.

3.3.2 Giemsa's stain solution preparation

In order to make Giemsa stain preparation one volume of Giemsa standard solution was placed in nine volume of phosphate buffered water at PH 7.2. The solution was filtered and kept in a stopped bottle of amber colour and diluted with distilled water. The PH of water controlled by buffering with 3.0 gm. 1-1 Na_2HPO4 and 0.6 gm. 1-1 KH_2PO4.

3.3.3 Giemsa staining

Air dried and methanol fixed blood smear slides were put into coupling jar containing working solution of Giemsa stain for 25-35 minutes. The stained slides were washed gently in current tap water and air- dried.

3.3.4 Microscopic Examination

The stained slides were examined under high power magnification (10x by 100x) with the help of immersion oil. Staring from tail end of the slides to the whole field parasites encountered and focused for photograph. Following morphological characters to identify the parasite as well as using the literature review (Mark Vet Manual)

Babesia has a pear-shaped, located in pairs, round, oval or irregular depending on the stage of development of the parasite in erythrocytes

Theileria has size 1-2 μm and a rod-shaped, oval or coma in erythrocytes.

Anaplasma occurs intracelularly in two morphologically distinct ultrastructure forms dense-cored cells and reticulate cells in blood cells.

Trypanosoma are flagellate protozoans that inhabit the blood plasma, the lymph and various tissues of their hosts.

Photo 1 Cattle and Buffalo of study area

Photo 2 Ticks Infection In cattle

Photo 3 Ticks Infection in Buffalo

Photo 4 Blood in Urine

Photo 5 DLSO, Siraha

Photo 6 Photography of BPP

4. RESULTS

A total of 310 blood samples from domestic animals brought to the DLSO, Siraha seeking treatment with illness were collected and examined for the blood protozon parasites. The domestic animal comprised of 130 cattle, 115 buffaloes and 65 goats from Marar, Khirauna and Malhaniya VDCs of Siraha district.

4.1 Blood protozoan parasite in cattle, buffaloes and goats of Marar, Khirauna and Malhaniya VDCs.

Out of 310 blood samples examined using thin blood smear revealed 56(18.06%) prevalence of blood protozoan parasites in domestic animal of Siraha district. Among them cattle and buffaloes were found to be equally infected by BPP, whereas goats were comparatively less infected i.e. 13.84%, cattle, buffaloes and goats were almost equally infected with *Babesia* species. In case of *Theileria*, cattle and goats were equally infected i.e. 4.61%. Whereas buffaloes were found to be comparatively more (6.08%) infected by *Theleria*. Similarly *Trypanosoma* infection was found maximum in cattle and buffaloes i.e. 5.3% and 4.3% respectively compared to goats (1.5%). In case of *Anaplasma* all three species of domestic animals were found to be infected in the range of 1.57% to 3.07% (Table 1). Prevalence of blood protozoan parasites in three different domestic animals were statistically not significant (p=0.951, $X^{2} = 1.623$ at df =6)

Table 1. BPP in cattle, buffaloes and goats of Marar, Khirauna and Malhaniya VDCs.

Animal Species	Blood Protozoan Parasites				Total
	Babesia	***Theileria***	***Trypanosoma***	***Anaplasma***	
Cattle (130)	8 (6.15%)	6 (4.61%)	7 (5.38%)	4 (3.07%)	25(19.23%)
Buffalo(115)	7 (6.08%)	7 (6.08%)	5 (4.34%)	3 (2.60%)	22(19.13%)
Goat (65)	4 (6.15%)	3 (4.61%)	1 (1.57%)	1 (1.57%)	9(13.84%)
Total(310)	19(6.12%)	16(5.16%)	13(4.19%)	8(2.58%)	56(18.06%)

Age of the host played important role harboring the parasitic disease. Age wise analysis showed that 4-8yrs domestic animals were found to be infected maximum by *Babesia* and *Theleria* compared to other age group. In case of domestic animals above 8yrs age group blood parasitic infection was found almost similar range. Interestingly *Tryapanosoma* was found comparatively high in old aged cattle and buffaloes (Figure 2). Although there is variation in parasitic infection among various age group statistically insignificant association was observed (p=0.454, x^2=5.728 at df =6).

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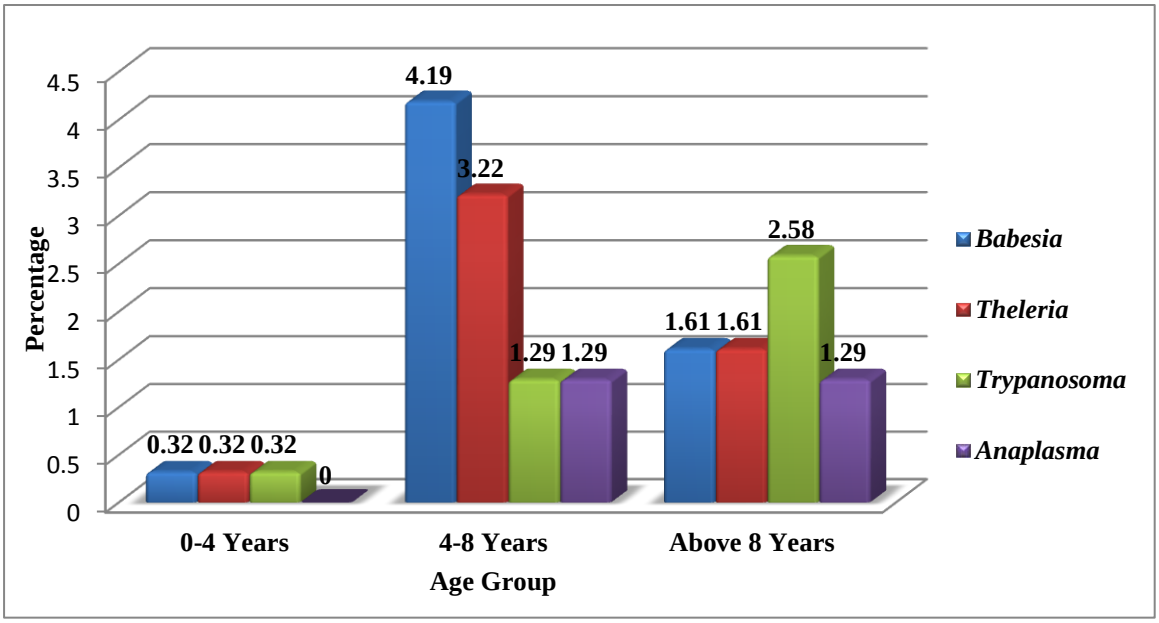

Figure 2 Distribution of BPP in cattle, buffaloes and goats animals of different age groups.

4.2 Comparison of blood protozoan parasite in three different VDCs of Siraha district.

The prevalence of blood protozoan parasitic infection was slightly varied in three VDCs. Khirauna and Malhaniya VDCs were found to be equally infected i.e. 5.48% with BPP. While Marar VDC found to be comparatively more (7.09%) infected. Cattle and buffaloes were almost equally infected in all three VDCs. Goats were comparatively less infected in all three VDCs (Table 2). Prevalence of blood protozoan parasite in VDCs were statistically not significant (p= 0.436, x^2=5.883 at df=6).

Table 2 BPP in cattle, buffaloes and goats of three different VDCs of Siraha district.

Animal species	VDCs		
	Marar	Khirauna	Malhaniya
Cattle (130)	10 (7.69%)	7 (5.38%)	8 (6.15%)
Buffalo (115)	9 (7.82%)	6 (5.21%)	7 (6.08%)
Goat (65)	3 (4.61%)	4 (6.15%)	2 (3.07%)
Total(310)	22(7.09%)	17(5.48%)	17(5.48%)

Babeisa infection was slightly variable in domestic animal of three different VDCs. Cattle of Marar and Malhaniya VDCs were found to be equally infected i.e. 2.3%, whereas in Khirauna VDC infection showed comparatively less (1.53%).The infection rate is almost similar in buffaloes of three VDCs. But in case of goats babesiosis infection rate showed consisting i.e. (3.07%) high in Khirauna VDC compared to other two VDCs. (Figure 3)

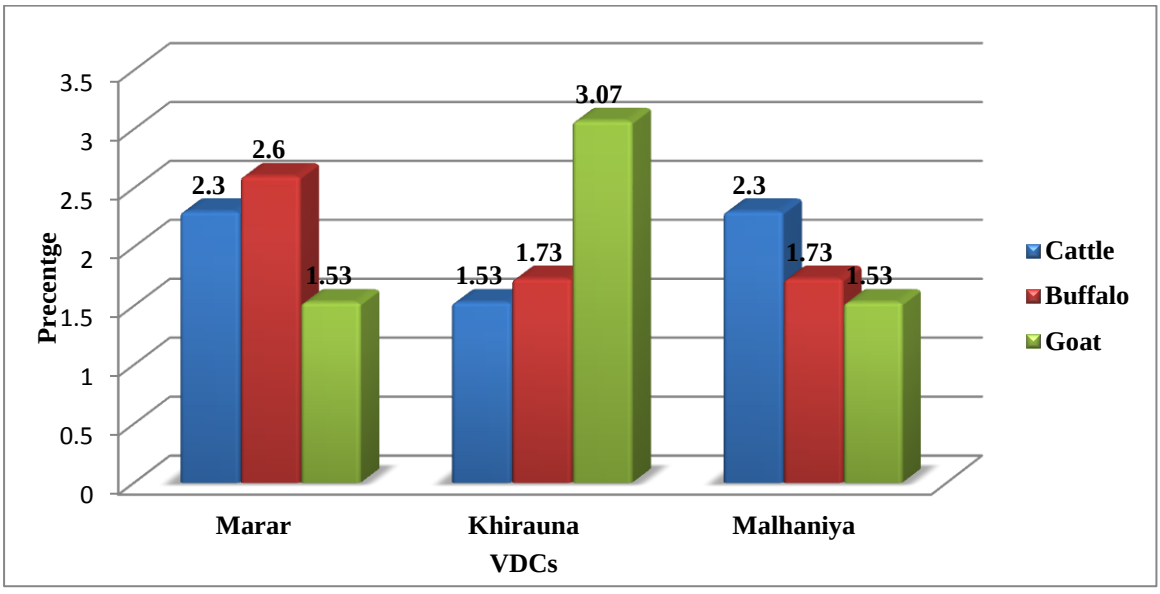

Figure 3 prevalence of *Babesia* in different VDCs.

Theleria infection was found maximum in all domestic animals of Marar VDC. Where as in case of goats *Theleria* infection was not found in Malhaniya VDC, but infection was high in cattle (1.53%) and buffaloes (1.73%) whereas cattle and buffaloes are less infected in Khirauna VDCs. (Figure 4)

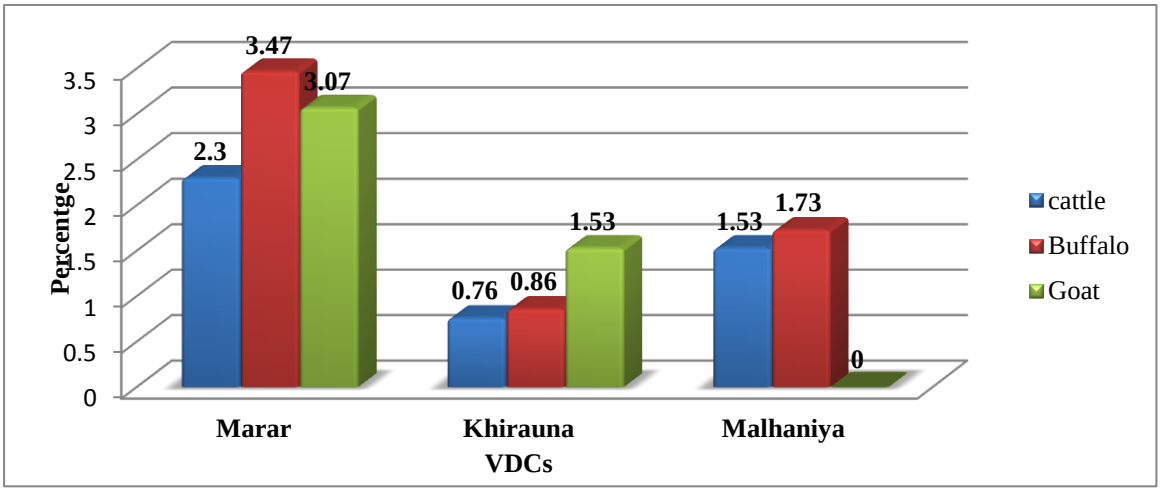

Figure 4 Prevalence of *Theleria* in different VDCs.

All domestic animals were highly infected with trypanosomasis infection in Malhaniya VDC. *Trypanosoma* was not found in goats of Marar and Khirauna VDCs. In case of cattle of Marar and Malhaniya were equally infected i.e. 1.53%. Buffaloes were highly infected with *Trypanosoma* in Khirauna VDC (1.76%) compared to Marar and Malhaniya (Figure 5).

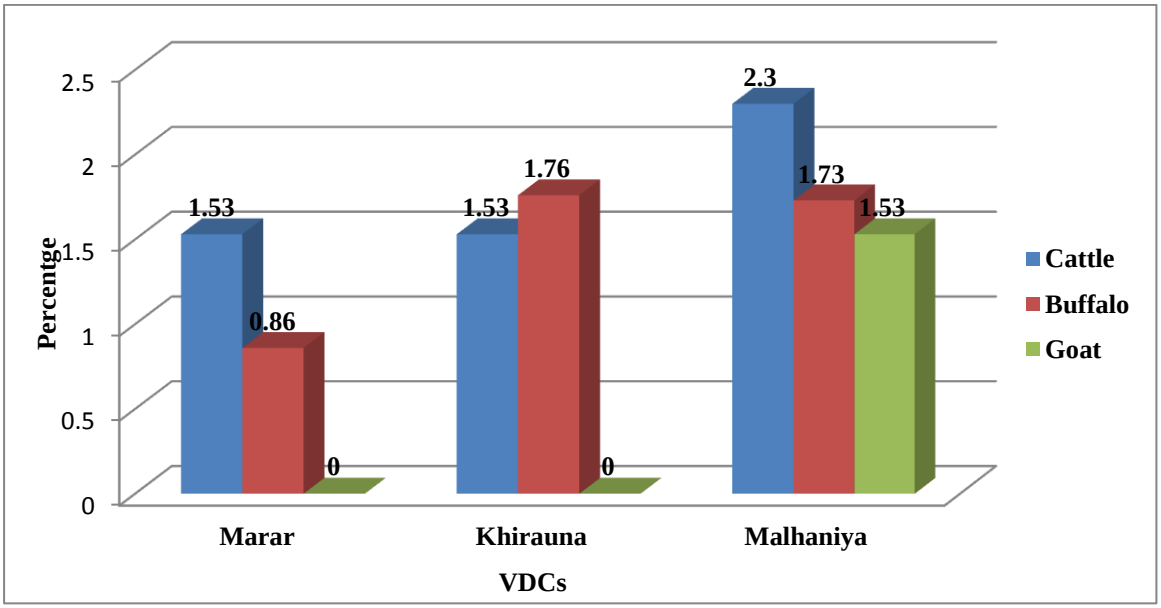

Figure 5 prevalence of *Trypanosoma* in different VDCs.

In the case buffaloes infection of anaplasmosis were found equally in all VDCs. *Anaplasma* was found highly infected in cattle of Marar and Khirauna VDCs but not found in Malhaniya. But in case of goats anaplasmosis infection was found only in Khirauna VDC. (Figure 6)

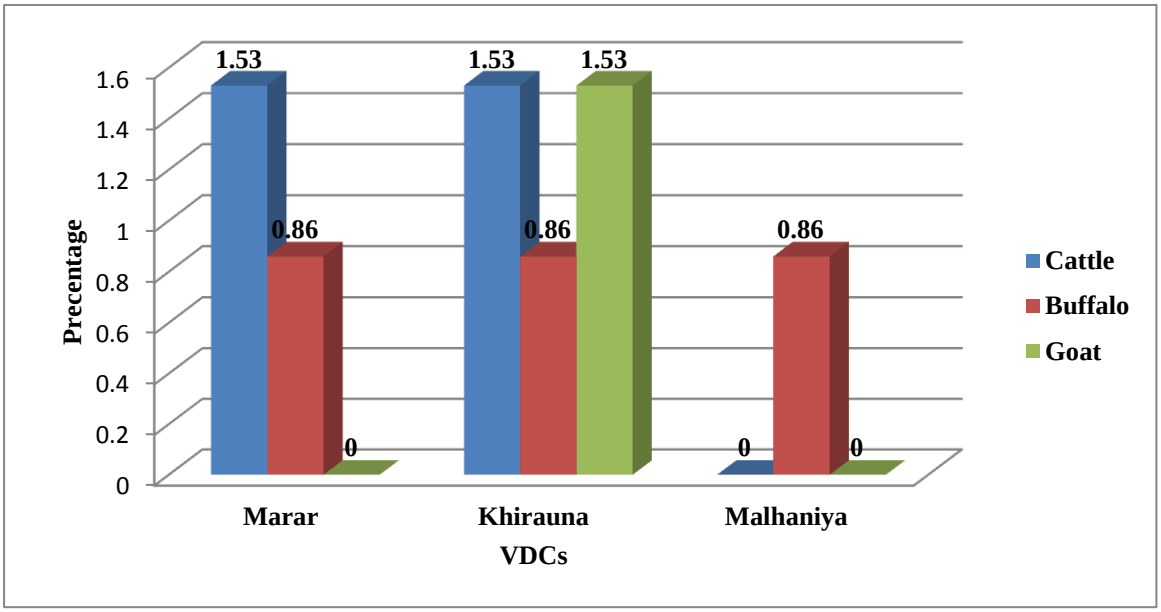

Figure 6 Prevalence of *Anaplasma* in different VDCs.

4.3 Comparison of Blood Protozoan parasites in local and cross breed cattle, buffaloes and goats.

Blood protozoan parasitic infection was variable in local and cross breed domestic animals. Out of 18.06% BPP infection in cattle, buffaloes and goats high infection was found in cross bread (10.32%) than local breed (7.74%). Among them cross breed cattle and buffaloes were found highly infected, but in case of goats local breed were more infected. (Table 3) Prevalence of blood protozoan parasite was not statically significant in local and cross breed. ($p=0.605$, $x^2=1.847$ at $df=3$).

Table 3 BPP in local and cross breed cattle buffaloes and goats.

Animal species	Cross	Local
Cattle	14 (10.76%)	11 (8.46%)
Buffalo	15 (13.04%)	7 (6.08%)
Goat	3 (4.61%)	6 (9.23%)
Total	32(10.32%)	24(7.74%)

Infection rate of BPP were varied in between local and cross breed cattle, buffaloes and goats. In case of babesiosis infection was found comparatively high in local breed (3.22%) than the cross breed (2.9%). But in case of other three protozoan parasites, cross breed domestic animals were found comparatively more infected. *Theleria* infection was found 2.25% and 2.9% in local and cross breed whereas *Trypanosoma* infection rate was found to be 1.29% and 2.9% in local and cross breed cattle, buffaloes and goats. While *Anaplasma* infection was found 0.86% and 1.61% in local and cross breed cattle, buffaloes and goats. (Figure 7)

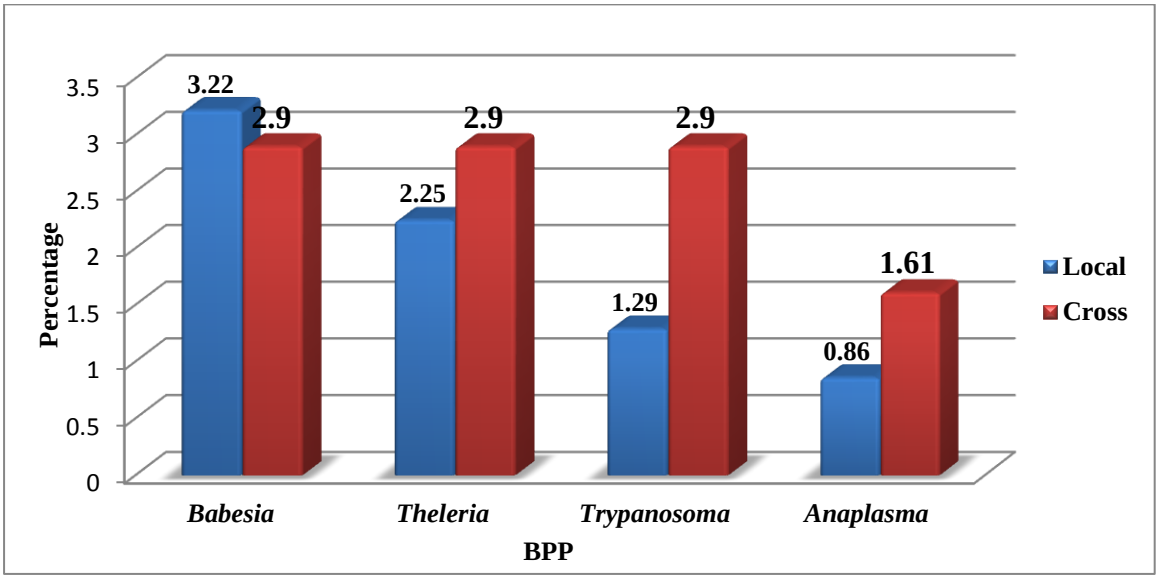

Figure 7 Prevalence of BPP in local and cross breed.

Photo 7 Blood smears showing *Babesia*

Photo 8 Blood smears showing *Anaplasma*

Photo 9 Blood smears showing *Theleria*

Photo 10 Blood Smears showing *Trypanosoma*

5. DISCUSSION

Livestock is subsector of agriculture whereas various domesticated livestock such as cattle, buffalo, goats, pigs' horse and dogs etc. are kept for livestock products. Due to the emergence and reemergence of various diseases in livestock, this sector is facing tremendous negative effect. Among various diseases BPDs are important ones responsible for livestock health deterioration mainly in Terai belt. Due to the favorable breeding of ticks and other vectors.

Tick borne diseases (TBD's) are responsible for substantial losses to the livestock industry throughout the world (Ananda et al. 2009). Hot and humid climate is highly favorable for the development and survival of ticks (Kohli et al. 2014). The most TBD's in India are Theleriosis, Babesiosis and anaplasmosis, which are caused by vector *Hyalomma* spp. and *Boophilus* spp. mostly in rainy season (Minjaw and Mcleol 2000). In Malaysia Theleriosis, leucocytozoonosis, Plasmodiasis, Babesiosis and Trypanosomasis was reported in domestic animals (Awathani et al. 2014). In the case of cattle of South Africa Anaplsmosis and Babesiosis has been reported. Similarly BPD's has also been reported from Bangladesh (Choudhary et al. 2006). The diseases are also prevalent in Nepal.

Present study revealed that overall prevalence of BPD's among livestock of Siraha district was 18.06% which is comparatively less than BPDs prevalence (32.91%) in Makwanpur district (Mishra 2003). BPDs have been reported from various part of Nepal from Banke (Adhikari et al. 1997), Salyan (Shakya et al. 1996), Morang (Deo 2002) and other areas of countries. Due to The similar climatic factor the diseases has been reported from various state of India Punjab (Iqbal et al. 2011), Rajasthan (Bhatnagar 2015), and Ludhiyana (Sahil et al.2014). The disease has been reported from the other Asian countries China (Hong et al. 1997), Thiland (Terkawi et al. 2011), Taiwan (Tasi et al. 2011), Malaysia (Wahab et al. 2013) and other part of the world.

Different species of the livestock are surfing from the BPD's. In the present study cattle and buffaloes were almost equally infected whereas goats were comparatively less infected with BPD's. Similar result had been reported from Makwanpur districts of Nepal (Mishra 2003). In Banke district BPDs was reported from cattle, buffaloes and dog (Adhikari et al. 1997), horse from Kathmand and mules from Salyan (Shakya et al. 1996). Various livestock of other countries were also found to be infected with BPDs. In Mosul

and Erbil district of Iraq sheep, cattle and goats have been reported to be infected with BPD's (Ameen et al. 2012, Hasan 2012). Goat, buffalo and dog were infected with BPDs in China (zhang et al. 2011), cattle were reported from Tawain (Tasi et al. 2011) buffaloes from Malaysia and dear, cattle, buffaloes and pig (Nurulaini et al. 2013, Rahman et al. 2012). Whereas in India BPDs has been reported in sheep and goats of panjab (Iqbal et al. 2011) and cattle of Deharadun (Kohali et al. 2014).

Age wise infection of BPDs in domestic animals showed that above 4yrs animals are highly infected with BPD's than the lower age groups animals. In Makwanpur district also similar result had been reported (Mishra 2003). It could be mainly due to the fact that cattle and buffaloes are kept for long time for milk production.

Distribution of BPDs particularly tick born BPDs posing major threat in animal husbandry most of the developing countries likes, Nepal, India, Bangladesh and other countries of the world. The disease is prevalent in those countries, where the climatic and geoFigureic factors are favorable to breeding of tick vectors. The Published reports showed the most of livestock suffering from Babesiosis, theleriosis and Anaplasmosis in various countries.

Babesiosis caused by *Babesia* species had been reported in various domestic animals. In Nepal it has been reported from cattle, goat, and buffaloes and dog with prevalence rate varies from 5% to 10% (Mishra 2003, Adhikari et al. 1997), 26.18% in cattle (Deo 2002) while in present study almost all domestic animals were found to be equally infected i.e. 6%. While prevalence rate seems to be comparatively higher in Indian and other countries Rajasthan sate of India Babesiosis infection has been reported to be 15.65% (Bhatnagar 2015), in Tikreet city 8.8% reported (Ibrahim et al 2012) and very less prevalence 3.3% reported from Bangladesha (Chowdhury 2006). It was evident that prevalence rate of Babesiosi was slightly variable in indicating its negative impact in livestock production.

Theleriosis is an important haemoparacitic disease of animal. Prevalence of Theleriosis was reported from Siraha district (5.6%) where was higher prevalence reported from Makwanpur district i.e.8.75% (Mishra 2003), and10.18% from Morang (Deo 2002). Theleriosis is problem in livestock industries of other countries. Lahor district of Pakistan reported 39.9% Theleriosis among livestock (Durrani et al. 2008), whereas 27.2% infection has been reported from Dehradun, India (Kohali et al. 2014).It is also reported from the various country of the world from Iran 9.2% (Zaeemi et al. 2011), in Sudan

49.6% (Sahils et al. 2005) and other part of the world.

Domestic animals of Siraha were comparatively less infected (2.58%) with anaplasmosis than the other BPDs which is arthropod borne rickettsial disease. In Banke it was only repoted from cattle (Adhikari et al.1997), in the Makwanpur district high prevalence was reported 9.58% (Mishra 2003).Anaplasmosis has been reported from various states of India from Rajasthan 15.65% prevalence in livestock (Bhatanagar 2015), from Punjab 40.83% (Jaswal et al. 2014). The diseases have also been reported from livestock of Bangladesh. (Talukdar et al.2001).

In BPDs other dreadful diseases of livestock are transmitted by other arthropods such as tes tes flies mostaly in Terai belt where the climatic conditions are humid and hot suitable for this. *Trypanosom* prevalence was high in cattle of Siraha district with overall prevalence in livestock 4.19%. In Makbanpur district it was reported to be 6.66% (Mishra & Mahrjan 2006), In Kathmandu it was reported from dog (Gautum & Ghimire 1988). From the various hot and humid climatic conditions countries it was been reported. In Adamawa State of Nigeria it was 26.67% reported (Zubairy et al. 2013), 14.7% trypanosomasis prevalence was reported from Malaysia (Rahman et al. 2012), 43% cattle were infected in Blue Nile State of Sudan (Salim et al. 2011), and 16.7% was infected in Northern Tanzania (Hamill et al. 2013).

The current study revealed that the cross breed domestic animals were highly infected with BPDs than local breed domestic animals, similar result found in Makwanpur district (Mishra 2006).Whereas it was reported from other part of the world Deharadun district of India cross breed cattle was reported 27.2% prevalence (Kohli et al. 2014). This is due to the climatic conditions hot and humid climate is not suitable for cross breed.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study on blood protozozn parasites in domestic animals of Siraha was carried by using Giemsa stained thin blood smears. Upon Microscopic examination of 310 suspected blood samples, 56(18.06%) samples were found to be positive for blood protozoan. Whereas positive cases the percentage of *Babesia*, *Theleria*, *Trypanosoma* and *Anaplasma* confirmed were 19(6.12%), 16(5.16%), 13(4.19%) and 8(2.58%) respectively.

Blood parasitic prevalence was highest in 4-8 years age group 55.36% and lowest in 0-4 year's age group 5.35% in cattle, buffaloes and goats.

Blood parasitic prevalence was more in cross breed animal i.e. 10.32% than local breed 7.74%. Whereas in the case of goats local breed 9.23% were more infected than cross breed 4.31%. In case of babesiosis local breed was found more infected i.e. 3.22% than the cross breed 2.9%. But in case of other three parasites, cross breed cattle, buffaloes and goats were found comparatively more infected.

Area wise distribution of blood protozoan parasitic infection was slightly varied in three VDCs. Marar VDC comparatively more 7.09% infected while Khiruna and Malhaniya VDCs were equally infected i.e. 5.48%.

6.2 Recommendation

Blood protozoan parasitic disease is highly prevalent in endemic areas of Terai region hence,

i. Blood protozoan parasitic disease is the major problem of cattle, buffaloes and goats of Siraha district, hence BPDs control programme must be include in regular program of DLSO.
ii. DLSO need to be strengthen with well-equipped laboratory in order to diagnose common blood parasitic diseases.
iii. Vector control program needs to organize regularly in order to prevent common blood parasitic diseases in domestic animals.

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ANNEX

List of blood sample collected from the Marar VDCs.

S.No.	Farmer's Name	Ward No.	Animal Species	Age	Breed	Smear code	Symptoms	Remarks
1	Jugeshwar Yadav	2	cow	1yr	JX	A001	Ticks, Weak	
2	Jugeshwar Yadav	2	cow	6yr	JX	A002	High fever	
3	Jugeshwar Yadav	2	cow	7yr	JX	A003	Normal	
4	Bindeshwar Morbaita	2	Buffalo	5yr	Local	A004	Ticks	
5	Bindeshwar Morbaita	2	Buffalo	9yr	Local	A005	Ticks	
6	Ansu Miya	1	Goat	3yr	Local	A006	Weak	
7	Paltu Sah	1	cow	4yr	JX	A007	BPP history	
8	Rajesha Yadav	3	cow	8yr	JX	A008	Fever, Weak	
9	Rajesha Yadav	3	cow	4yr	JX	A009	Tick, Lice	
10	Rajesha Yadav	3	Ox	7yr	Local	A010	Tick, Lice	
11	Sriprasad Yadav	3	Ox	9yr	Local	A011	Ticks	
12	Sriprasad Yadav	3	cow	8yr	Local	A012	Ticks, Weak	Trypanosomais
13	Abdul Rahman	1	Goat	3yr	Local	A013	Fever, Weak	
14	Abdul Rahman	1	Goat	2yr	Local	A014	Normal	
15	Abdul Rahman	1	Goat	2yr	Local	A015	Normal	
16	Abdul Rahman	1	Goat	4yr	Local	A016	Normal	
17	Pawan Morbaita	2	Ox	9yr	Local	A017	Ticks	
18	Pawan Morbaita	2	Ox	9yr	Local	A018	Ticks,fever	
19	Jagdish Morbaita	2	Buffalo	6yr	MX	A019	Lice,	
20	Dlip Morbaita	2	Buffalo	10yr	MX	A020	Blood in Urine	Babesiosis
21	Amarnath Jha	4	cow	6yr	JX	A021	Emaciation	
22	Amarnath Jha	4	cow	2yr	JX	A022	Tick,fever	
23	Nagendra Yadav	4	cow	7yr	Local	A023	High fever	Theleriosis
24	Balram prasad sah	4	cow	2yr	JX	A024	Blood in Urine	
25	Bishnu Yadav	4	Buffalo	10yr	Local	A025	Weight loss	
26	Bishnu Yadav	4	Buffalo	9yr	MX	A026	Ticks,fever	
27	Bishnu Yadav	4	Buffalo	2yr	MX	A027	Ticks,fever	
28	Kuse das Tatma	5	Ox	7yr	Local	A028	Ticks,fever	
29	Kuse das Tatma	5	Ox	7yr	Local	A029	Weight loss	
30	Gobind Jha	5	cow	5yr	JX	A030	Normal	
31	Dilip Paswan	5	Buffalo	9yr	Local	A031	High fever	
32	Tarkeshwar Yadav	5	Buffalo	8yr	MX	A032	weak, fever	
33	Usman Miya	1	Goat	5yr	Local	A033	Ticks, Blood in Urine	Babesiosis
34	Anshu Jha	5	cow	9yr	JX	A034	Blood in Urine	
35	Anshu Jha	5	cow	4yr	JX	A035	Blood in Urine	
36	Anshu Jha	5	Ox	1yr	JX	A036	Weight loss	
37	Opendar Sah	6	Buffalo	7yr	MX	A037	Lymph node inlargment	
38	Opendar Sah	6	Buffalo	2yr	MX	A038	Ticks	
39	Kari Miya	2	Goat	3yr	Cross	A039	Ticks, Lice	
40	Kari Miya	2	Goat	3yr	Cross	A040	Ticks, Lice	
41	Binod Kr. Mukhiya	4	Buffalo	9yr	Local	A041	High fever	Theleriosis
42	Binod Kr. Mukhiya	4	cow	8yr	JX	A042	Lacrimation	
43	Binod Kr. Mukhiya	4	cow	9mo	JX	A043	BPP history	
44	Prmod Kr. Jha	5	cow	7yr	JX	A044	High fever	
45	Prmod Kr. Jha	5	Ox	8yr	Local	A045	Weekness	
46	Prmod Kr. Jha	5	Ox	8yr	Local	A046	Weekness	
47	Rakesha Kr. Paswan	5	Buffalo	9yr	Local	A047	Fever	
48	Rakesha Kr. Paswan	5	Buffalo	1yr	MX	A048	Ticks	
49	Rajaram Yadav	5	cow	6yr	JX	A049	Blood in Urine	Babesiosis
50	Rajaram Yadav	5	Buffalo	7yr	MX	A050	Weight loss	Babesiosis
51	Om Parkas Kharga	6	Ox	5yr	Local	A051	Weak,Ticks	
52	Dev Narayan Yadav	6	cow	7yr	JX	A052	High fever	

53	Dev Narayan Yadav	6	cow	2yr	JX	A053	Lymph node inlargment	
54	Dev Narayan Yadav	6	Ox	5yr	JX	A054	Ticks	
55	Jay Prakash Yadav	1	Buffalo	9yr	MX	A055	High fever	
56	Jay Prakash Yadav	1	Buffalo	2yr	MX	A056	Normal	
57	Hasn Miya	2	Goat	4yr	Local	A057	Ticks, Highfever	Theleriosis
58	Hasn Miya	2	Goat	3yr	Local	A058	Ticks	
59	Arun Kr Mahto	7	Ox	9yr	Local	A059	Ticks,Darkurine	Babesiosis
60	Arun Kr Mahto	7	Ox	9yr	Local	A060	Fever	
61	Jogindra Paswan	7	Buffalo	7yr	Local	A061	Ticks	
62	Md. Saukat Ali	3	Goat	1yr	Cross	A062	Ticks	
63	Md. Saukat Ali	3	Goat	3yr	Cross	A063	Ticks	
64	Md.Kalam	3	Buffalo	8yr	MX	A064	Lymph node inlargment	
65	Jugeshwar Yadav	3	Buffalo	8yr	MX	A065	Ticks, Weighloss	
66	Bishnudev Yadav	3	cow	7yr	Local	A066	Weakness	
67	Sebak Yadav	4	Buffalo	8yr	MX	A067	Weight loss	
68	Lakshaman Das	4	Goat	1yr	Local	A068	Normal	
69	Lakshaman Das	4	Goat	4yr	Local	A069	High fever	Theleriosis
70	Badri Mahto	7	Ox	10Mo	Local	A070	Ticks	
71	Deb Saran Goit	7	Buffalo	7yr	MX	A071	Lacrimation	
72	Deb Saran Goit	7	Buffalo	1yr	Local	A072	Lacrimation	
73	Dhanik Lal Mahto	7	cow	9yr	Local	A073	High fever	
74	Binod Kr Das	7	Buffalo	7yr	MX	A074	Lymph node inlargment	Theleriosis
75	Sukan Miya	2	Goat	5yr	Cross	A075	Weight loss	
76	Sukan Miya	2	Goat	4yr	Cross	A076	Ticks	
77	Lakshaman Yadav	4	Ox	8yr	Local	A077	Fever	
78	Lakshaman Yadav	4	Ox	8yr	Local	A078	Normal	
79	Hari Lal Yadav	8	Buffalo	9yr	MX	A079	Blood in Urine	
80	Sukdew Raut	8	Buffalo	9yr	MX	A080	High fever	
81	Sukdew Raut	8	Buffalo	8yr	MX	A081	Ticks,Lice	
82	Ramsebak Raut	8	cow	6yr	JX	A082	Fever	
83	Ramsebak Raut	8	cow	8yr	JX	A083	Normal	
84	Bhutai Masiata	8	Buffalo	6yr	MX	A084	Fever	
85	Bhutai Masiata	8	Buffalo	1yr	MX	A085	Ticks	
86	Manju Das	8	Goat	2yr	Local	A086	Emaciation	
87	Jagat Lal Yadav	8	Buffalo	9yr	Local	A087	Weight loss	Babesiosis
88	Jagat Lal Yadav	8	Buffalo	11Mo	MX	A088	Ticks	
89	Tetar Miya	1	Goat	3yr	Cross	A089	Weak	
90	Tetar Miya	1	Goat	3yr	Cross	A090	Weak	
91	Muga Lal Yadav	1	Buffalo	9yr	MX	A091	High fever	
92	Muga Lal Yadav	1	Buffalo	5yr	MX	A092	Normal	
93	Sarup Sada	9	cow	8yr	Local	A093	Ticks,fever	
94	Sarup Sada	9	Ox	1yr	JX	A094	Tick	
95	Jagat Parsad Vinbar	9	Buffalo	7yr	Local	A095	Fever Weekness	Anaplasmosis
96	Jagat Parsad Vinbar	9	Buffalo	8mo	Local	A096	Weekness	
97	Pawan Kr Yadav	9	cow	9yr	HLX	A097	Tick	
98	Pawan Kr Yadav	9	cow	9yr	HLX	A098	Ticks,fever	
99	Pawan Kr Yadav	9	Ox	2yr	JX	A099	Ticks	
100	Bishun Kr Mukhiy	1	Buffalo	9yr	Local	A100	High fever	
101	Raj Kr Mukhiya	1	Buffalo	8yr	MX	A101	Blood in Urine	
102	Suta Mukhiya	1	Buffalo	8yr	MX	A102	Fever	
103	Md. Naiem	1	Goat	1yr	Local	A103	Ticks	
104	Ram Parkash Yadav	1	cow	6yr	Local	A104	Blood in Urine	Babesiosis
105	Sikindar Morbaita	2	Buffalo	7yr	Local	A105	High fever	Theleriosis
106	Srinarayan Yadav	2	cow	7yr	JX	A106	Ticks, Weight loss	
107	Bisnudev Yadav	1	Ox	8yr	Local	A107	Fever	
108	Bisnudev Yadav	1	Ox	8yr	Local	A108	Normal	
109	Bisnudev Yadav	1	Buffalo	7yr	MX	A109	Lacrimation	
110	Rijhan Mukhiya	1	cow	9yr	JX	A110	High fever	Theleriosis

List of blood sample collected from the Malahaniya VDCs.

S.No.	Farmer's Name	Ward No.	Animal Species	Age	Breed	Smear code	Symptoms	Remarks
1	Kamlesh Yadav	1	Ox	3yr	Jx	B001	Lacrimation	
2	Kamlesh Yadav	1	Cow	9yr	Jx	B002	Ticks	
3	Chamak Lal Yadav	1	Ox	9yr	Local	B003	Ticks, Weight loss	Trypanosomasis
4	Chamak Lal Yadav	1	Ox	9yr	Local	B004	Ticks	
5	Ashok Kr Yadav	2	Cow	6yr	jx	B005	Ticks	
6	Ashok Kr Yadav	2	Cow	2yr	Jx	B006	Ticks,Highfever	Theleriosis
7	Gulchan Sha	2	Buffalo	10yr	Mx	B007	Ticks, fever	Babesiosis
8	Gulchan Sha	2	Buffalo	6yr	Mx	B008	Ticks, fever	Babesiosis
9	Gulchan Sha	2	Cow	7yr	Local	B009	Ticks	
10	Lalit Kr Yadav	3	Buffalo	9yr	Mx	B010	Fever, Weak	
11	Lalit Kr Yadav	3	Buffalo	1yr	Mx	B011	Normal	
12	Bijay Raut	3	Cow	5yr	Jx	B012	Fever, Weak	
13	Jibach Khatbe	2	Goat	2yr	Local	B013	Ticks	
14	Jibach Khatbe	2	Goat	3yr	Cross	B014	Ticks, Blood in Urine	Babesiosis
15	Jibach Khatbe	2	Goat	3yr	Cross	B015	Blood in Urine	
16	Jibach Khatbe	2	Goat	1yr	Local	B016	Normal	
17	Lakchaman Yadav	3	Cow	6yr	Jx	B017	High Fever	
18	Pradip Kr Yadav	1	Buffalo	9yr	Mx	B018	Fever, Weak	
19	Pradip Kr Yadav	1	Buffalo	5yr	Mx	B019	Ticks	
20	Pradip Kr Yadav	1	Buffalo	1.5yr	Local	B020	Normal	
21	Dlip Kr Mahto	4	Ox	6yr	Local	B021	Weight loss Darkurine	Babesiosis
22	Dlip Kr Mahto	4	Ox	6yr	Local	B022	Ticks	
23	Madan Kr Yadav	4	Cow	8yr	Jx	B023	Ticks, fever	
24	Madan Kr Yadav	4	Cow	6yr	Jx	B024	Ticks	
25	Madan Kr Yadav	4	Buffalo	9yr	Local	B025	Normal	
26	Ram Sankar Mahto	4	Cow	5yr	Local	B026	Fever, Weak	
27	Sanjip Kr Mahto	4	Buffalo	6yr	Mx	B027	Lymphnode inlargment	
28	Anup Kr Mandal	1	Goat	2yr	Cross	B028	Ticks	
29	Anup Kr Mandal	1	Goat	3yr	Cross	B029	Ticks weak	
30	Bisheswar Kapar	6	Ox	1yr	Jx	B030	Normal	
31	Bisheswar Kapar	6	Cow	9yr	HLX	B031	Ticks, Blood in Urine	
32	Bisheswar Kapar	6	Cow	6yr	HLX	B032	Ticks	
33	Bisheswar Kapar	6	Cow	2yr	HLX	B033	Ticks	
34	Ramsebak Bhandari	6	Buffalo	8yr	Mx	B034	High Fever	Theleriosis
35	Bachu Sha	2	Ox	8yr	Local	B035	Ticks, fever	
36	Bachu Sha	2	Ox	8yr	Local	B036	Ticks	
37	Bachu Sha	2	Buffalo	9yr	Mx	B037	Fever	
38	Binod Sharma	4	Buffalo	6yr	Mx	B038	Fever	
39	Bhusan Dev Thakur	4	Cow	5yr	Jx	B039	Weight loss Darkurine	Babesiosis
40	Upendar Mahto	4	Cow	7yr	Jx	B040	Normal	
41	Upendar Mahto	4	Buffalo	8yr	Local	B041	Ticks	
42	Jhari Barhi	4	Goat	5yr	Local	B042	Ticks, Emaciation	
43	Dipendra Kr Yadav	5	Buffalo	9yr	Mx	B043	Lacrimation	
44	Dipendra Kr Yadav	5	Buffalo	5yr	Mx	B044	Ticks	
45	Dipendra Kr Yadav	5	Buffalo	1yr	Mx	B045	Ticks	
46	Mukesh Kr Yadav	5	Ox	2yr	Jx	B046	Weight loss, Fever	
47	Saroj Kr Yadav	5	Buffalo	8yr	Local	B047	Fever Ticks	
48	Saroj Kr Yadav	5	Cow	6yr	Jx	B048	Ticks	
49	Sikindar Mandal	5	Buffalo	7yr	Local	B049	Lymphnode inlargment	Trypanosomasis
50	Dinesh Sada	5	Goat	1yr	Local	B050	Weight loss	
51	Kishun Lal Yadav	3	Cow	7yr	Jx	B051	Ticks, fever	
52	Kishun Lal Yadav	3	Ox	2yr	Jx	B052	Ticks	
53	Jibach Yadav	3	Cow	7yr	Local	B053	Weight loss	Trypanosomasis
54	Mahabir Yadav	2	Cow	8yr	Jx	B054	Ticks	
55	Mahabir Yadav	3	Ox	2yr	Jx	B055	Ticks	

56	Kisori Yadav	3	Buffalo	9yr	Local	B056	Weight, loss	
57	Dhaneshwar Chamara	3	Goat	5yr	Local	B057	Fever	
58	Raj Dev Yadav	3	Buffalo	10yr	Mx	B058	High Fever	
59	Raj Dev Yadav	3	Buffalo	4yr	Mx	B059	Normal	
60	Kariya Kapar	6	Buffalo	9yr	Local	B060	Ticks, Lacrimation	
61	Khusi Lal Mahto	6	Cow	7yr	Jx	B061	Ticks	
62	Khusi Lal Mahto	6	Ox	9yr	Local	B062	Ticks weightloss	
63	Khusi Lal Mahto	6	Ox	9yr	Local	B063	Tick weightloss	
64	Dhaneshwar Chamara	7	Ox	10yr	Local	B064	Fever	
65	Birendra Mahto	7	Buffalo	8yr	Mx	B065	High Fever	
66	Ram Mahara	7	Buffalo	9yr	Local	B066	Weight loss, Fever	Trypanosomasis
67	Ramdev Chamara	7	Cow	7yr	Local	B067	Ticks Fever	
68	Sambhu Mahto	6	Goat	2yr	Local	B068	Fever	
69	Sambhu Mahto	6	Goat	2yr	Local	B069	Normal	
70	Arun Mukhiya	8	Buffalo	9yr	Mx	B070	Ticks Weak	
71	Sukh Deo Mukhiya	8	Cow	7yr	Jx	B071	Ticks, fever	
72	Sukh Deo Mukhiya	8	Cow	3yr	Jx	B072	Ticks	
73	Ashok Kr Sha	2	Buffalo	9yr	Local	B073	Ticks Fever	Anaplasmosis
74	Hira Lal Mandal	2	Buffalo	10yr	Local	B074	High Fever	
75	Sarwan Yadav	3	Ox	9yr	Local	B075	Ticks	
76	Sarwan Yadav	3	Ox	9yr	Local	B076	Ticks	
77	Siv Sankar Yadav	3	Buffalo	7yr	Local	B077	Ticks, Lession on skin	
78	Siv Sankar Yadav	3	Buffalo	2yr	Mx	B078	Ticks	
79	Siv Sankar Yadav	3	Cow	8yr	Jx	B079	Normal	
80	Siv Sankar Yadav	3	Cow	1yr	Jx	B080	Normal	
81	Sonfi Khatbe	2	Goat	4yr	Local	B081	Ticks weak	
82	Adik Lal Mandal	8	Buffalo	9yr	Mx	B082	Fever	
83	Adik Lal Mandal	8	Buffalo	2yr	Mx	B083	Normal	
84	Umesha Yadav	8	Cow	8yr	Jx	B084	Lymphnode inlagment	Trypanosomasis
85	Kari Yadav	8	Buffalo	9yr	Local	B085	Fever	
86	Kari Yadav	8	Buffalo	5yr	Mx	B086	Normal	
87	Pulkit Chmara	7	Ox	6yr	Local	B087	High Fever	
88	Pulkit Chmara	7	Ox	6yr	Local	B088	Normal	
89	Gangaram sah	8	Buffalo	8yr	Mx	B089	Fever	
90	Domi Yadav	8	Cow	7yr	Local	B090	Ticks	
91	Domi Yadav	8	Cow	5yr	Local	B091	Ticks Weak	Babesiosis
92	Nathuni Yadav	8	Buffalo	9yr	Mx	B092	Fever	
93	Raghuniya Khatbe	1	Goat	3yr	Cross	B093	Ticks, Weight loss	
94	Raghuniya Khatbe	1	Goat	3yr	Cross	B094	Lymphnode inlargment	Trypanosomasis
95	Raghuniya Khatbe	1	Goat	2yr	Local	B095	Normal	
96	Sobhit Mahto	5	Cow	8yr	Jx	B096	Ticks, fever	
97	Sobhit Mahto	5	Cow	3yr	Jx	B097	Ticks	
98	Sobhit Mahto	5	Ox	7yr	Local	B098	High Fever	Theleriosis
99	Rajdev Mahto	5	Ox	7yr	Local	B099	Ticks weak	
100	Rajdev Mahto	5	Cow	8yr	JX	B100	Ticks	

List of blood sample collected from the Khirauna VDCs.

S.No.	Farmer's Name	Ward No.	Animal Species	Age	Breed	Smear code	Symptoms	Remarks
1	Ramdeo Goit	2	Ox	2yr	Local	C001	Ticks,Fever	
2	Maheshwar Goit	2	Cow	5yr	JX	C002	Ticks,Fever	
3	Maheshwar Goit	2	Cow	1.5yr	JX	C003	Ticks	
4	Pawan Kr Goit	2	Buffalo	9yr	Mx	C004	Ticks,Weak	
5	Surendra Thakur	2	Buffalo	9yr	Mx	C005	High Fever	
6	Birendra Takur	2	Buffalo	8yr	Mx	C006	Weight Loss	
7	Ramashis Mandal	3	Buffalo	9yr	Local	C007	Ticks, Darkurine	Babesiosis
8	Ramashis Mandal	3	Buffalo	10yr	Local	C008	Ticks	
9	Ramashis Mandal	3	Cow	8yr	JX	C009	Ticks	
10	Shiv Kr Mandal	3	Ox	3yr	Local	C010	Fever	
11	Shiv Kr Mandal	3	Buffalo	7yr	Mx	C011	Normal	
12	Devnarayan Mandal	3	Cow	9yr	Local	C012	Weight Loss	Trypanosomasis
13	Devnarayan Mandal	3	Buffalo	7yr	Mx	C013	High Fever	
14	Devnarayan Mandal	3	Buffalo	6mo	Mx	C014	Normal	
15	Vihari Paswan	3	Buffalo	9yr	Local	C015	Ticks, weak	
16	Manoj Kr Sah	7	Ox	7.5yr	Local	C016	Darkurine	Babesiosis
17	Manoj Kr Sah	7	Ox	7.5yr	Local	C017	Normal	
18	Bhula Mandal	7	Buffalo	8yr	Mx	C018	Weak	
19	Bhula Mandal	7	Buffalo	9yr	Mx	C019	Emaciation	Trypanosomasis
20	Bhula Mandal	7	Buffalo	3yr	Mx	C020	Normal	
21	Dinesha Yadav	8	Cow	7yr	HLX	C021	Weak	
22	Dinesha Yadav	8	Cow	8yr	HLX	C022	Weak	
23	Harisha Yadav	8	Cow	2yr	JX	C023	Fever	
24	Harisha Yadav	8	Ox	1yr	JX	C024	Normal	
25	Rabindra Mahara	1	Cow	7yr	Local	C025	Ticks Weightloss	
26	Debindra Mahar	1	Ox	6yr	Local	C026	High Fever	
27	Debindra Mahar	1	Ox	6yr	Local	C027	Normal	
28	Baleshwar Goit	1	Buffalo	9yr	Mx	C028	Ticks, Fever	
29	Baleshwar Goit	1	Buffalo	5yr	Mx	C029	Ticks, weak	
30	Jogindra Chamara	1	Goat	2yr	Local	C030	Ticks	
31	Jogindra Chamara	1	Goat	5yr	Local	C031	Ticks, High fever	Theleriosis
32	Jogindra Chamara	1	Goat	1yr	Local	C032	Normal	
33	Siyaram Mandal	7	Buffalo	9yr	Mx	C033	Ticks,Fever	
34	Siyaram Mandal	7	Buffalo	3yr	Mx	C034	Ticks	
35	Saroj Kr Das	7	Cow	6yr	JX	C035	Fever	
36	Saroj Kr Das	7	Cow	1yr	JX	C036	Ticks	
37	Raj Kr Yadav	8	Buffalo	8yr	Local	C037	Ticks, Weak	
38	Raj Kr Yadav	8	Buffalo	2yr	Mx	C038	Ticks,Weak	
39	Raj Kr Yadav	8	Ox	8yr	Local	C039	Emaciation	
40	Raj Kr Yadav	8	Ox	8yr	Local	C040	Emaciation	
41	Manoj Kr Yadav	8	Buffalo	10yr	Mx	C041	Ticks, High fever	
42	Manoj Kr Yadav	8	Buffalo	5yr	Local	C042	Ticks	
43	Bharat Yadav	8	Cow	9yr	Local	C043	Ticks,Weak	
44	Bharat Yadav	8	Ox	2yr	JX	C044	Ticks, weak	
45	Mohit Yadav	3	Buffalo	9yr	Mx	C045	Lymphnode enlargment	Theleriosis
46	Uday Goit	3	Goat	4yr	Local	C046	Darkurine	Babesiosis
47	Saroj Goit	2	Cow	8yr	JX	C047	Ticks,Weak	Anaplasmosis
48	Saroj Goit	2	Cow	2yr	JX	C048	Ticks	
49	Rajesha Goit	2	Cow	7yr	Local	C049	High Fever	
50	Rajesha Goit	2	Ox	1yr	Local	C050	Normal	
51	Birajesha Goit	2	Ox	8yr	Local	C051	Ticks,Fever	
52	Birajesha Goit	2	Ox	8yr	Local	C052	Ticks	
53	Birajesha Goit	2	Cow	7yr	JX	C053	Ticks,Fever	Theleriosis
54	Birajesha Goit	2	Cow	1.5yr	JX	C054	Ticks,Weak	
55	Pawan Kr Yadav	4	Cow	6yr	Local	C055	High Fever	

56	Pawan Kr Yadav	4	Cow	2yr	JX	C056	Ticks	
57	Mahesh Sada	4	Goat	3yr	Local	C057	Ticks, Weak	
58	Rajendra Sada	4	Goat	5yr	Local	C058	Ticks, Weak	
59	Rajendra Sada	4	Goat	2yr	Local	C059	Ticks, Weak	
60	Lalan Kr Yadav	4	Buffalo	7yr	Local	C060	Blood in Urine	Babesiosis
61	Lalan Kr Yadav	4	Cow	5yr	JX	C061	Blood in Urine	
62	Sivsankar Mandal	1	Buffalo	8yr	Local	C062	Ticks	
63	Sivsankar Mandal	1	Buffalo	3yr	Local	C063	Ticks	
64	Guru Dayal Mandal	1	Ox	7yr	Local	C064	Ticks,Fever	
65	Guru Dayal Mandal	1	Ox	7yr	Local	C065	Ticks	
66	Guru Dayal Mandal	1	Buffalo	6yr	Mx	C066	Ticks	
67	Sita Ram Sah	5	Cow	8yr	JX	C067	Ticks,Fever	Anaplasmosis
68	Sita Ram Sah	5	Cow	9mo	JX	C068	Ticks	
69	Aman Miya	5	Goat	3yr	Local	C069	Ticks Weightloss	
70	Aman Miya	5	Goat	6mo	Local	C070	Ticks	
71	Umesha Mandal	6	Cow	5yr	JX	C071	Fever	
72	Nathuni Mandal	6	Buffalo	9yr	Mx	C072	Ticks, Fever	Anaplasmosis
73	Bhola Thakur	6	Ox	4yr	Local	C073	Fever	
74	Bhola Thakur	6	Buffalo	8yr	Local	C074	Lacrimation	
75	Sandesh Mandal	6	Cow	6yr	JX	C075	Ticks,Weak	
76	Sandesh Mandal	6	Ox	1.5yr	JX	C076	Ticks	
77	Paltu Paswan	3	Goat	2yr	Cross	C077	Ticks Weightloss	
78	Paltu Paswan	3	Goat	2yr	Cross	C078	Ticks Weightloss	
79	Paltu Paswan	3	Goat	3yr	Local	C079	Ticks	
80	Ganesh Kr Mandal	3	Ox	7yr	Local	C080	Lymphnode enlargment	Trypanosomasis
81	Ganesh Kr Mandal	3	Ox	7yr	Local	C081	Normal	
82	Ashok Kr Mandal	9	Buffalo	9yr	Mx	C082	Ticks,Fever	
83	Ashok Kr Mandal	9	Cow	7yr	JX	C083	Ticks	
84	Ashok Kr Mandal	9	Cow	2yr	JX	C084	Normal	
85	Shiv Kr Sha	5	Buffalo	10yr	Mx	C085	Weight Loss	Trypanosomasis
86	Shiv Kr Sha	5	Buffalo	4yr	Mx	C086	Normal	
87	Md. Najir Miya	5	Goat	5yr	Local	C087	Ticks Weightloss	Babesiosis
88	Md.Rafik Rien	5	Buffalo	9yr	Mx	C088	Ticks	
89	Mukesh Kr Sah	5	Cow	7yr	JX	C089	Ticks,Fever	
90	Mukesh Kr Sah	5	Ox	8yr	JX	C090	Ticks	
91	Biltu Sah	5	Buffalo	9yr	Local	C091	Fever	
92	Jhapsi Paswan	9	Goat	3yr	Local	C092	Ticks	
93	Jhapsi Paswan	9	Goat	4yr	Cross	C093	Ticks,Fever	Anaplasmosis
94	Dipak Mandal	9	Cow	7yr	JX	C094	Ticks, weak	
95	Dipak Mandal	9	Cow	7yr	JX	C095	Ticks	
96	Asarfi Mandal	9	Cow	9yr	Local	C096	Weight Loss	
97	Asarfi Mandal	9	Buffalo	8yr	Local	C097	Normal	
98	Sita Ram Mandal	1	Buffalo	8yr	Mx	C098	Ticks	
99	Sita Ram Mandal	1	Buffalo	3yr	Mx	C099	Lacrimation	
100	Hajari Mandal	1	Cow	6yr	JX	C100	Blood in Urine	Babesiosis