HAEMO-PROTOZOAN PARASITES OF CATTLE IN GORKHA MUNICIPALITY-7, GORKHA, NEPAL.

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DECLARATION

I here declare that the work presented in this thesis has been done by myself, and has not been submitted anywhere for the ward of any degree. All the source of information has been specifically acknowledged by reference to the authors or institutions.

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RECOMMENDATION

This is to recommend that the thesis entitled **"Haemo-protozoan parasites of cattle in Gorkha municipality-7, Gorkha"** has been carried out by **Gopal khankhawash** for the partial fulfillment of Master's Degree of Science in Zoology with special paper **Parasitolgy.** This is his original work and has been carried out under my supervision. To the best 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 the supervisor Associate Professor Mahendra Maharjan (Ph.D), this thesis submitted by Mr. Gopal Khankhawash entitled "**Haemo-protozoan parasites of cattle in Gorkha municipality-7, Gorkha"** is approved for the examination and submitted to the Tribhuwan University in partial fullment of the requirement for the Degree of Master of Science in Zoology with special paper **Parasitology**.

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CERTIFICATE OF ACCEPTANCE

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
µm	Micrometer
ADS	Agriculture Development Strategy
BPDs	Blood Parasitic Diseases
BPP	Blood Protozoan Parasite
CBS	Central Bureau of Statistics
DLS	Department of Livestock Service
EDTA	Ethylene Diamene Tetra Acetate
ELISA	Enzyme Link Immuno-sorbent Essay
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
ILO	International Labor Organization
IFA	Immunofluorescence of tests
IMR	Institute of Medical research
MoAD	Ministry of Agricultural Development
OIE	Office of International Epizooties
PCR	Polymerase Chain Reaction
TBD's	Tick Borne Disease

ABSTRACT

Haemoprotozoan infections are very common in cattle and cause devastating losses to the livestock industry. The present study was conducted to determine the prevalence of haemo-protozoan parasites in Gorkha municipality- 7. A total of 80 blood samples were collected during the month of May- July, 2017 from the jugular vein of the cattle. All the samples were examined microscopically under the high power magnification (100x) with the help of immersion oil at the central veterinary lab Tripureswar, Kathmandu. The prevalence of haemo-protozoan parasites was found to be 16.25%. The most common haemo-protozoan parasites encountered were *Babesia* sp., *Anaplasma* sp. and *Theileria* sp. with prevalence rate 6.25%, 8.75% and 1.25% The prevalence of various haemo-protozoan parasitic infection in cattle were found statistically insignificant ($\chi 2= 4.5544$, $P>0.1026$). Among the identified blood protozoan parasites, prevalence was higher in above the five years cattle (11.25%) than the less than five years cattle (5%). Maximum male cattle (11.25%) with poor body condition were susceptible for the haemo-protozoan parasitic infection. Likewise, haemo-protozoan parasitic infection was higher in above the three herd size. Statistically, there was insignificance difference between age ($\chi 2= 0.8144$, $P>0.05$), sex ($\chi 2=2.5649e-31$, $P>0.05$) and herd size ($\chi 2= 2.87$, $P>0.05$). Whereas, the prevalence of haemo-protozoan disease in body condition of the cattle were significant difference ($\chi 2=32.348$, $P<0.05$). However, no any activities on health care of cattle with regarding the haemo-protozoan parasites were found. Thus, these indicate a higher prevalence of babesiosis in the cattle of Gorkha municipality-7.

1. INTRODUCTION

1.1 Background

Cattle are the most common type of large domesticated hoofed animals. Within the general term of cattle are cows, bulls, oxen, heifers, steers, bullocks and calves. Various types of cows are used for milk. It includes the Australian Illawarra, Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey and Milking Shorthorn (Bollongino *et al*., 2012). Nepal remains a predominantly agrarian economy. About 66 percent of its population is involved in agriculture, which accounts for 35 percent of the gross domestic product (CBS, 2012). The livestock subsector of agriculture contributes 24 percent of the total agricultural GDP (CBS, 2012), and also plays important roles in human food and nutritional security, livelihood, regional balance, gender mainstreaming, and rural poverty alleviation (ILO, 2004). Livestock farming prevails in all regions of the country, including the Mountain, Hill and Terai belts, with variations based on climate, topography, and socio-economic factors. Nepal has largely a smallholder livestock system under which farmers raise small numbers of livestock in small land holdings.

1.2 Livestock Population and Production in Nepal

Cattle are the main source of traction and manure in Nepal. In terms of animal mass units, it is the largest livestock in Nepal. But the annual growth rate is very minimal (almost zero) compared to other livestock farming. According to MoAD statistics 2017, the livestock population of cattle was about 7,302,808 during 2015/16. Buffalo is the main source of milk and meat in Nepal. Also it is useful as manure and draft power for soil fertility. It is the second largest group of livestock in terms of animal mass units in Nepal. But from the economic point of view, it is more valuable than cattle in Nepal. The production of buffalo milk was around 121,044 metric tons. There were about 5,168,809 buffalo in Nepal during 2015/16. The consumption of sheep's mutton in favor of other meat products declined the annual growth rate for sheep to negative value. According to MoAD statistics 2017, there were about 800,658 sheep in Nepal during 2015/16. Primarily goat is the second most popular source of meat in Nepal. It produces milk too but the goat milk has not gained its popularity and there were about 10,986,114 goats during 2015/16 in Nepal. Pigs are also a major livestock in Nepal. It is done for the purpose of meat production in Nepal. According to MoAD statistics 2017, the populations of pigs were around 1291308 during 2015/16. Chicken is the third most popular source of meat in Nepal. It has gained the popularity due to its economic cost and the positive influence to health comparing with other meat products. During the 2015/16, population of chicken was around the 68630638 (MoAD, 2017). Duck also a source of meat in Nepal but slowly losing its popularity compared to other meat sources. The total population of duck was about 392225 during 2015/16.

1.3 Livestock Diseases

In Nepal, the livestock subsector is declining. The Nepal Agricultural Development Strategy (ADS, 2012) has identified the core reason for the decline as low productivity of animals, mainly due to poor husbandry practices by farmers, the genetic inferiority of local breeds, and the poor condition of animal health. The livestock sector is suffering from a number of disease problems caused by bacteria, viruses, fungi, and parasites. Among these, parasitic diseases i.e. haemo-protozoan and gastrointestinal parasites are most important which affect milk and meat production of animals (Artis, 2006; Gohil *et al*., 2013).Three principal reasons most often cited for the spread of diseases are poor sanitation, improper management, and introduction of new animals into a herd. The prevalence of endoparasite leads to retarded growth rate, lowered output and death of animal due to their undesirable effects on animals (FAO, 1997; Sykes, 1994). Thus, endoparasite becomes one of the main hindrances in the development of livestock production and industry worldwide (Ijaz *et al*., 2009; Khalil-ur- Rehman *et al*., 2009; Bilal *et al*., 2009).

Mastitis is one of the most frequent and costly diseases in dairy cattle (Halasa *et al*., 2007). Bovine parasitic influenza virus type 3 (BIV-3) is a member of respirovirus genus in the family paramixoviridae (Adams *et al.,* 2016). The BPIV-3 is one of the causes of bovine respiratory diseases complex (Snowder *et al*., 1999). This virus may cause tissues damage and immunosuppression resulting in severe bronchopneumonia due to secondary bacterial infection, especially when animals are under stressful conditions (Hannes *et al*., 1997). Gastrointestinal parasites (Nematodes, Trematodes and cestodes) are important parasites of cattle having a negative impact on both animal health and financial returns from production animals. Parasitic diseases offer a great obstacle to livestock, thus causing direct and indirect losses (Harper and Penzhorn, 1999; Kagira and Kanyari, 2001). Gastrointestinal parasitism lowers the productive capacity of animals and may cause death in some cases (Lebbie *et al*., 1994). Some of these are zoonotic and therefore a threat to public health. In cattle, at least 13 different species of coccidia are known to infect cattle. Diarrhea, often bloody, is associated with the presence of *E. zuernii* or *E. bovi*'s, which occur in the lower small intestine, caecum and colon (Taylor and Catchpole, 1994).

1.4 Tick and Ticks Borne Disease of Cattle

Ticks are recognized as important vectors of blood protozoan diseases in livestock. Out of 867 tick species recognized globally, 10 percent of them act as the vectors of pathogens of domesticated animals and human beings (Jongejan and Uilenberg, 2004). Climatic factors, particularly temperature, are considered to be important determinant for tick propagation. Cattle ticks are responsible for severe economic losses in both dairy and beef cattle enterprises in the tropics (Jonsson, 2006). It is obligatory blood-sucking arachnid arthropods infecting mammals, birds, reptiles and amphibians. They are vectors of diseases, causing anemia, dermatitis, paralysis, otocariasis as well as loss of production.

Three families of ticks are established, but two of them Ixodidae (hard ticks) and Argasidae (soft ticks) are well known and of veterinary importance (IMR, 1995). In Nepal, five genera were identified viz. *Boophilus, Hyalomma, Rhipicephalus, Haemaphysalis* and *Ixodes*. Among them *Boophilus microplus* was the most abundant in all agro climatic zones and in most of the farm animals namely bovine, buffalo, goat, pig and rabbit (Shrestha *et al*., 2005).

Haemoparasitic diseases have a global distribution, stretching from the polar circle to the equator. This is due to the fact that their vectors, ticks and blood sucking flies have a global distribution. Tick borne haemoparasistes includes all tick-borne organisms which are visible with light microscope and which occur in the circulating blood as part of their life cycle (Uilenberg, 1995). Among the parasitological problems, the damage caused by tick borne diseases is considered very high (Ghosh *et al.*, 2007). The most important haemoparasites are *Babesia*, *Theileria*, *Anaplasma* and *Trypanosoma.* These haemoparasites are transmitted through ticks (Zahid *et al.*, 2005). Haemoparasites are of great economic loss due to the morbidity and mortality. It is a major threat to food security especially among the livestock dependent communities within the sub Saharan Africa (Kasozi *et al*., 2014). Haemoparsites have generally been shown to cause destruction of red blood cells resulting in anemia, jaundice, anorexia, weight loss and infertility. The occurrence and important of haemoparasite is a reflection of complex interaction involving the causative organisms, vector, the vertebrate hosts and the environment (Akande *et al.,* 2010). Arthropod transmitted haemoparasites diseases are economically important vector-borne diseases of tropical and subtropical parts of the world including Ethiopia. Tick borne haemoparasitic diseases of ruminants are caused by the *Babesia*, *Theileria*, *Anaplasma* and *Trypanosoma* species and all the intracellular parasites species (Sitotaw *et al*., 2014).

Anaplasmosis is a vector borne blood diseases in cattle caused by the member of genus *Anaplasma*. In cattle, this disease is caused by *A. marginale* and *A. centrale*; later less pathogenic than former (Sajid *et al*., 2014) where as in sheep and goats *A. ovis* is the important causative agent (Radwan *et al*., 2013). Anaplsamosis is not contagious; numerous species of tick vectors (*Boophilus, Dermacenter, Rhipicephalus, Ixodes* and *Hyamomma*) can transmit *Anaplasma* species (Rymaszewska and Grenda, 2008). Not all of these are likely significant vectors in field and it has been shown that strains of *A. marginale* also co-evolve with particular tick strain. After feeding on an infected animal, transmission may occur. Transovarian transmission may occur although even in a single host Boophilus species (Kahn, 2005). Anaplasmosis may also be spread mechanically by infected hypodermic needle, castrating, spaying and dehorning instruments, blood trasnsfusion and embryo transplant. Additionally intra uterine infections also occur in cattle but much less frequency in field cases than in experimental one. *Anaplasma* can transmit by bitting flies to the family Tabanidae (Radostits *et al.*, 2007). It is found in endemic in all six populated continents of the world; mostly in the tropics and subtropics because of the broad range of vectors and difficulties of efficient vector control (Sajid *et al.,* 2014). *Bos taurus* breeds are more likely to develop acute Anaplasmosis than crossed

Zebu, but *Bos indicus* are not commonly affected because of their resistance to heavy tick resistance (Kocan *et al*., 2003). Anaplasmosis is characterized by fever, weight loss, decreased milk production, pale mucous membranes, severe anemia, jaundice, hyper-excitability abortion and mortality without hemoglobinemia and haemoglobinuria during acute phase of the infection (Atif *et al.,* 2013). Tetracycline compounds are effective in treatment if given early in the course of the disease and especially before the parasitaemia has reached its peaks (Lefevre *et al.,* 2010).

Babesiosis is also called piroplasmosis, cattle fever, red water fever or Texas fever. The causative agents of babesiosis are specific for particular species of animals. In cattle: *B. bovis, B. bigemina, B. divergens* and *B. major* (Radostits *et al*., 2007). *B. bovis* and *B. motasi* are known to be pathogenic agents in sheep and goats (Fakhar *et al*., 2012). *Babesia* species is transmitted by hard ticks in which *Babesia* passes transovarially via the egg from the one tick generation to the next (Ijaz *et al*., 2013; Urquhart *et al.,* 1996). Babesiosis occurs throughout the world (Fakhar *et al.,* 2012). However the distribution of the causative protozoa is governed by the geographical and seasonal distribution of the insect vectors. The vector of *Babesia*, *Boophilus microplus* is wide spread in tropics and sub tropics (Chaudhary *et al*., 2010). Bovine babesiosis associated with *B. bigemina* and *B. bovis* are an important disease of tropical and sub-tropical regions of the world. Both species are transmitted by the *Boophilus* ticks, but only tick larvae transmit *B. bovis*, whereas nymphs and adults transmit *B. bigemina* and *B. divergens*. Bovine babesiosis transmitted by *Ixodes ricinus* is widespread. Small ruminant babesiosis is caused by the *B. ovis* (Esmaeilnejad *et al*., 2015). *Bos indicus* breeds of cattle are more resistance to babesiosis than *Bos taurus* (Kamani *et al*., 2014). *Babesia* produces acute disease by two principle mechanism; hemolysis and circulatory disturbance (Carlton and McGavin, 1995).

Theileriosis is a group of tick borne disease caused by *Theileria* species. It is intracellular protozoan parasites infecting leukocytes and erythrocytes of wild and domestic large and small ruminents. In cattle, *T. parva, T. mutans, T. velifera, T. lestoquardi, T. ovis* and *T. separate* (Mandal, 2012). *T. annulata* and *T. parva* are considered to be the pathogenic species of *Theileria* affecting cattle (Kohli *et al*., 2014). *T. lestoquardi* is the most virulent species in sheep and goats (Kahn, 2005). *Theileria* species that infect cattle and ruminants are transmitted by ixodid ticks of the genera *Rhipicephalus*, *Amblyomma, Hyalomma* and *Haemaphysali* (ILO, 2004). *Theileria* sporozoites are transmitted to animals in the saliva of the feeding tick (Mandal, 2012). *T. parva lawrencei* responsible for corridor disease transmitted from buffalo to cattle and *T. parva bovis*, the causing agent of Zimbabwe theileriosis, a more benign from also known as " January disease" (Nambota *et al.,* 1994). Theileriosis occurs when there is much tick activity, mainly during summer but a single tick can cause fatal infection (Hassan, 2010).

Trypanosomosis is an important disease of both humans and animals commonly found in most parts of Africa and South America (Swallow, 2000). The tsetse fly (*Glossina*) is responsible for biological transmission while haematophagus arthropod vectors of the

family Tabanidae, Stomoxynae and Hippoboscidae are responsible for its mechanical transmission (Soulsby, 2012). Transplacental transmission has also been recorded in cattle (Ogwu *et al*., 1992). *Trypanosoma congolense, T. vivax* and *T. brucei* have been reported to cause nagana in cattle while *T. evansi* caused surra in camels (Mbaya *et al*., 2010). In humans, *Trypanosoma brucei* gambiense and *Trypanosoma brucei rhodeseinse* are responsible for human sleeping sickness in West and East Africa respectively, while *T. cruzi*, transmitted by triatomid bugs (*Triatoma magista*) is responsible for transmitting chagas diseases to humans in South America (Solano *et al*., 2000). The *T. brucei* group of trypanosomes (*T. brucei, T. b. gambiense, T. b. rhodesianse* and *T. evansi*) mostly invade tissues (humoral) whereas, *T. congolense* and to a lesser extent *T. vivax* and *T. cruzi* predominantly restrict themselves to the blood circulation (Igbokwe, 1994; Mbaya *et al*., 2011).

1.5 Objectives of the Study

1.5.1 General objective

- To study the haemo-protozoan parasites of cattle in the Gorkha municipality-7, Gorkha.

1.5.2 Specific objectives

- To determine the prevalence of the haemo-protozoan parasites
- To compare the haemo-protozoan parasites in different demographic characters
- To assess the Knowledge, attitude and practices (KAP) of the cattle owner

1.6 Significance of the Study

No any study has been done to detect prevailing cause of cattle disease in this area. This study may guide for accurate diagnosing and early treatment the animal before the devasting effect of the disease occurs in animal. The study would be useful in understanding the prevalence of blood parasites in cattle of Gorkha Municipality-7. In this context, this study may reveal the existing status of haemoprotozoan parasites diseases responsible for illness, weak body condition, low production and infertility. This study would be helpful for future researchers and investigators, those investigating the disease of cattle in Gorkha Municipality-7 Gorkha, Nepal.

2. LITERATURE REVIEW

Haemo-Protozoan Parasites in the Global Context

Haemo-protozoan parasites are organisms that live in the blood of their animal hosts. Babesiosis, anaplasmosis, theileriosis and trypanosomiasis are considered some of the major impediments in the health and productive performance of cattle (Rajput *et al.*, 2005). Ticks are recognized as important vectors of blood protozon diseases in livestock. Out of 867 tick species recognized globally, 10 percent of them act as the vectors of pathogens of domesticated animals and human beings (Jongejan and Uilenberg, 2004). Tick borne disease causes substantial losses to the livestock industry throughout the world (Ananda *et al*., 2006). These have got a serious economic impact due to obvious reason of death, decreased productivity, lowered working efficiency (Uilenberg, 1995). Considering this, numbers of research works have been conducted on haemo-protozoans parasites of cattle. But in Nepal few research works has been conducted regarding the haemo-protozoans parasites of cattle.

Anaplasmosis

Anaplasmosis is an infectious disease of cattle caused by several species of genus *Anaplasma*. *A. marginale* is the most common pathogen of cattle (Smith, 2002). The causative agents can be transmitted biologically by ticks or mechanically by biting flies and blood contaminated fomites, infecting diverse cell types of haematopoietic origin (Dumler *et al*., 2001; Kocan *et al*., 2003). The diseases caused by *Anaplasma* species had been recognized before a century, but are still important public and animal health issues globally (Battilani *et al*., 2017). Large number of works have been conducted to determine the prevalence of bovine anaplasmosis in Africa such as Nigeria (Kamani *et al*., 2010), Sudan (Alkareem *et al.,* 2012; Awad *et al*., 2011), South Africa (Mtshali *et al*., 2004).

In Nigeria, cattle were infected with a wide variety of vector-borne haemo-parasites (Callow, 1978; Swallow, 2000). *Anaplasma marginele* infection was found to be 1.9% in north- central Nigeria (Kamani *et al*., 2010). In south Africa due to distribution of ticks vector *Boophilus decolonatus* and *Rhipicephalus evertsi evertsi* in cattle, they were reported highly infected 87% with *Anaplasma* (Mtshali *et al*., 2004). Similary, Ekici *et al*. (2011) reported 89.9% infection of *Theileria* by the cELISA test from same country. The blood smear examination revealed 5.88% *Theileria* species in Sudan (Alkareem *et al*., 2012). The blood smear examination revealed 5.88% *Theileria* species in Sudan (Alkareem *et al*., 2012). Awad *et al*. (2011) has recorded 6.1% infection of *Anaplasma marginale* from the Northern Sudan.

Anaplasma spp. has been reported from a European country Turkey (Birdane *et al*., 2006; Zhou *et al*., 2016). Blood parasites infections are the most important animal health problems for cattle industries in Turkey. Anaplasmosis has been reported in almost every

region of Turkey (Ozcan, 1961; Goksu, 1970; Mimioglu *et al.,* 1971; Tuzer, 1981). The presence of *A. marginale* (34.41%) was determined by light microscopic examination of Giemsa- strained blood smears from the Turkey (Birdane *et al.,* 2006). Similarly, 29.1% infection of *A. marginale* had been documented from the same country (Zhou *et al*., 2016). While, *Anaplasma marginale* has been reported first time as a tick born disease in Brazil.

Number of research have been carried out regarding the prevalence of bovine anaplasmosis in Asian country such as Japan (Yoshimoto *et al*., 2010), Bangladesh (Mohanta *et al*., 2011; Chowdhury *et al*., 2006), China (Yang *et al*., 2013), Pakisthan (Khan *et al.,* 2004) and India (Nair *et al*., 2013; Parmar and Upadhya, 2012; Vahara *et al*., 2012; Kumar and Sangwasn, 2010). *A. bovis* and *A. phgocytophilum* had been reported in the dominant vector of tick species in Japan (Yoshimoto *et al*., 2010). Prevalence of blood protozoans like *Anaplasma marginale, Anaplasma centrale* has been reported in animals of Bangladesh (Ahmed, 1976; Samad and Gautam, 1984). Overall 87% infections by the *Anaplasma* were recovered in Bangladesh (Chowdhury *et al*., 2006). Similarly, Mohanta *et al*. (2011) found 14.63% infection by the *Anaplasma* in the same country. On the other hand, incidence of hemo-parasitic disease was recorded as 18% for the *Anaplasma* in Pakistan (khan *et al*., 2004). Several *Anaplasma* species have been identified in tick vectors of domestic and wild animals in China, including *A. marginale, A. ovis, A. phagocytophilum, A. bovis* and *A. capra* (Yang *et al*., 2013). The occurrence of *Anaplasma* spp. was investigated by PCR in domestic small ruminants. They were found to be highly infected with *Anaplasma* spp. i.e. 46.2% (Yang *et al*., 2013). Haemoprotozoan parasites cause economically important vector-borne diseases of tropical and subtropical parts of the world including India (Salih *et al*., 2015). Prevalence of haemo-protozoan parasites have been reported in animals of different parts of India (Nair *et al*., 2013; Parmar and Upadhya, 2012; Vohora *et al*., 2012; Kumar and Sangwasn, 2010). In the northern kerala India, 16.6% sample was found positive for the *A. marginale* (Nair *et al*., 2013). Likewise, Parmar and Upadhya (2017) had reported 3.77% infection by the *Anaplasma* from the Uttarkhand. While the seasonal incidence of haemo-protozoal disease in crossbred cattle was found to be 37% infection in Gujarat (Vahara *et al*., 2012). On the other hand, 46.9% of *A. marginale* had been recorded by the Kumar and Sangwasn (2010) from the Hariyana, India.

Babesiosis

Babesiosis is one of the tick-borne protozoan diseases of cattle, which figure prominently in the list of serious diseases of livestock industry all over the world (Afzal *et al*., 1999). It is caused by microscopic parasites that infect red blood cells and spread by certain ticks. Bovine babesiosis and other tick borne diseases are responsible for more than 50% losses in the crossbred cattle (Chaudhry *et al*., 2010). *Babesia* is intraerthrocytic haemo-protozoan affecting animal's erythrocytes (Zintl *et al*., 2003). Victor Babes who at the end of the 19th century first discovered the micro-organsm in erythrocytes of cattle in

Romania and associated them with bovine hemoglobinuria or red water fever. The most commonly encountered clinical signs induced by these parasites include high grade fever, anemia, hemoglobinuria, ataxia, and sometimes death (Bock *et al*., 2004).

In Africa, huge number of research has been conducted regarding to the prevalence of *Babesia* spp. such as in Nigeria (Okorafor and Nzeako, 2014; Kaman *et al*., 2010) and central region of Syria (Terkawi *et al.,* 2012). Cattle were infected with a variety of vector-borne haemo parasites in Nigeria (Callow, 1978; Swallow, 2000). Most important genera of Babesia i.e. *B. bigimina and B. bovis* have been recorded (Makala *et al*., 2003; Mtshali *et al*., 2004; Kamani *et al*., 2010). Okorafor and Nzeako (2014) found to be 0.56% infection by the *Babesia bigemina* in Oyo state of Nigeria. While, 16% overall prevalence of *Babesia* had been reported from the North-Central Nigeria (Kamani *et al.* 2010). On the other hand, 24.64% overall prevalence of *Babesia* was identified by the nPCR from the central region of Syria (Terkawi *et al*., 2012).

Haemo-protozoans have been reported from different country of Europe such as Turkey (Zhou *et al*., 2016; Kursat *et al*., 2008) and Slovakia (Kubelova *et al*., 2008). By the PCR results revealed that 11.2% infection of *B. bigemina* from the Turkey (Zhou *et al*., 2016). Similarly, *B. bigemina* and *B. major* have been reported with prevalence rate 0.77% and 0.51% from the East back sea region of Turkey (Kursat *et al*., 2008). While, *Babesia canis* is the most frequently agent of canine babiosis in Slovakia and they had found 14.4 and 2.3% prevalence of *B. canis* from the eastern and southwest Slovakia (Kubelova *et al*., 2008).

Considering the prevalence of haemo-protozoans few works have been accompanied in South America such as Brazil (Canever *et al*., 2013; Brito *et al.,* 2013). Infection of cattle was investigated by the amplification of the gene from the *B. bovis*. They were reported 95.1% infection of *B. bovis* from the Southwestern Brazil (Brito *et al*., 2013). Similarly, *Babesia bigemina* had been found with prevalence rate 63.6% from the same study area (Canever *et al*., 2013).

In Asian countries, large number of works have been carried out regarding prevalence of haemo-protozoans such as Thailand (Terkawi *et al*., 2012), Taiwan (Tsai *et al*., 2017), Vietnam (Sivakumar *et al*., 2003), Bangladesh (Mohanta *et al*., 2011), Srilanka (Kirupanathan *et al.,* 2016), China (Hong *et al*., 2007), India (Parmar and Upadhya, 2017; Bhat *et al*., 2016; Muraleedharan, 2015 *et al.,* 2006). The bovine babesiosis disease has been transmitted by the tick in the Thailand. Terkawi *et al*. (2012) had reported *B. bovis* and *B. bigemina* with the prevalence rate 11.2% and 3.6% by the PCR. In the case of Taiwan, *B. bovis* 1.9% and *B. bigemina* 0.6% were detected from dairy cows (Tsai *et al*., 2011). On the other hand, *B. bovis* and *B. bigemina* had been reported and B. bovis being the most common among them in Vietnam (Sivakumar *et al*., 2013). In the China nine species of *Babesia* have been recognized in livestock such as *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.,* 2007). Kirupanathan *et al*. (2016) identified that 47% of samples were positive for the *Babesia* in Srilanka. *Babesia* parasites are economically important vector-borne diseases of tropical and subtropical parts of the world including India (Salih *et al*., 2015). In Uttarkhand, India infection of Babesiosis has been recorded with prevalence rate 9.62% (Parmar and Upadhya, 2017). Similarly, Bhat *et al*. (2016) had reported 1.48% infection of *Babesia* bigemina by the examination of the *Rhipicephalus microplus* female ticks from the Punjap, India. Likewise, six species of haemo-protozoan i.e. *B. bigemina* and *B. bovis* in cattle and buffaloes, *B. motasi* in sheep and goats, *B. ovis* in sheep and *B. canis* and *B. gibsoni* in dogs had been documented from the Karnata state of India (Muraleedharan, 2005).

Theileriosis

Bovine theileriosis is caused by the protozoan parasite of *Theileria* spp. (*Theileria annulata* and *Theileria parva*) which are round ovoid rod like or irregular shaped organism found in lymphocytes, histiocytes and erythrocytes (Soulsby, 2012; Durrani *et al.,* 2008). Theileriosis species are tick-borne haemo-protozoan parasites of vertebrates that have a major important on livestock production, mainly cattle and small ruminant in tropical and subtropical areas (Mehlhorn and Schein, 1984). *Theileria* species are apicomplexan-haemoprotozoan parasites transmitted by Ixodidae ticks (Preston, 2001; Silva *et al*., 2010).

Infection of the *Theileria* has word wide in distribution and is characterized by the anemia, icterus, homogloninuria and death and as result they have a high economic impact in several parts of the tropical and temperate countries (Wagner *et al*., 2002). Tropical theileriosis is one of the most prevalent diseases of cattle caused by *T. annulata* (Mirzaei, 2007). Large number of research works have been conducted regarding the haemo-protozoans of cattle in an Africa such as Sudan (Salih *et al*., 2005), Nigeria (Okorafor and Nzeako, 2014) and Ethopia (Gebrekidan *et al*., 2016). In Sudan *Hyalomma A. anatolicum* nymphs were infected with 49.6% *Theleria* (Salih *et al*. 2005). In Nigeria cattle were infected with a wide variety of vector-borne haemo-parasites (Callow, 1978, Swallow, 2000). Among them, *Theileria* (*Theileria parva* and *Theileria veilifera*) were the less extent (Makala *et al*., 2003; Mtshali *et al*., 2004; Kamani *et al*., 2010). By the microscopic examination 16% infection of *Theileria mutans* had been identified from the North-Central, Nigeria (Kamani *et al*., 2010). Similarly, Okorafor and Nzeako (2014) found 0.56% infection of *Theileria parva*. On the other hand, by the molecular characterization of *Theileria orientalis*, they had detected 2.2% infection in local breed cattle from Ethiopia (Gebrekidan *et al*., 2016).

Few researches have been carried out to determine the prevalence of bovine theileriosis in Europe like Turkey (Aktas *et al*., 2007; Kursat *et al*., 2008; Zhou *et al.,* 2016). A survey had been conducted in eastern Turkey for the prevalence of *Theileria* by using both microscopy and PCR. They had reported the Theileria spp. with 41% prevalence rate

(Aktas *et al*., 2006). Similarly, Zhou *et al*. (2016) found 18.9% infection by the *T. annulata* from the same country. While in the east black sea region of turkey, 1.2% *T. annulata* has been reported (Kursat *et al*., 2008).

Large numbers of research works have been conducted regarding the *Theileria* spp. in an Asia. Such as Taiwan (Wang *et al*., 1998), Vietnam (Inoue *et al*., 2001), Iraq (Hadi *et al*., 2012), Pakisthan (Khattak *et al*., 2014; Khan *et al*., 2004), China (Ando *et al*., 2010), India (Muraleedharan *et al*., 2005; Singh *et al*., 2012; Kohli *et al*., 2014; Kumar *et al*., 2015; Parmar and Upadhya, 2017). In Taiwan 3.8% incidence of *Theileria* had been reported by using the PCR technique (Wang *et al*., 1998). On the other hand, Inoue *et al*. (2001) had used the piroplasma surface protein gene specific polymerase chain reaction and it's revealed that 27.5% infection by the Theileria in Vietnam. Hadi *et al*. (2012) had observed the abdomen area of the hard tick *Hyalomma a. anatolicum* and they found to be 43% infection by the *Theleria* form the Iraq. Pakistan being a tropical country provides optimal climatic conditions for growth and multiplication of ticks. Tick fauna of Pakistan is rich in number of genera and species (Rasul and Akhtar, 1975). In Pakistan theileriasis is common livestock diseases, which is transmitted by the ticks (Abdussalam, 1959). Incidence of the haemo-parasitic diseases i.e. *Theileria* was recorded as 28% in Pakisthan (Khan *et al*., 2004). Similarly, the prevalence of the *Theileria annulata* had been examined by the Giemsa stained slides in large ruminants from two district of the Pakistan and 5.2% parasitic infection were recorded (Khattak *et al.*, 2014). *Theileria uilenbergi* had been identified as one of the causative agents of theileriosis in china and it was reported by the Abdo *et al.* (2010). In India the annual loss reported due to tropical theileriosis is approximately US$ 800 million (Devendra, 1995). Muraleedharan *et al*. (2005) had reported that 90% of the crossbred cattle were infected by the *Theilerai annulata*. Similarly, examination of Giemsa-stained peripheral blood smears had exhibited that 14.65% of cattle were infected with *Theileria annulata* from Punjab, India (Singh *et al*., 2012).While 9.35% prevalence of *Theileria* had been documented from the same area (Kumar *et al.,* 2015). Likewise, Kohli *et al*. (2014) had reported that 27.2% theilerial infection by using the microscopic examination of blood smears. Similarly, 84.62% prevalence of the *Theleria* was recorded by the Parmar and Upadhya *et al*. (2017) from the same area.

Trypanosomiasis

Trypanosomosis is an important disease of both humans and animals commonly found in most parts of Africa and South America (Swallow, 2000). It is a parasitic disease caused by different species of unicellular parasites and found in the blood and other tissue of vertebrate including livestock, wildlife and people (Uilenberg, 2002). Major trypanosomiasis species affecting cattle are *T.congolense*, *T. vivax* and *T. brucei* (Teter, 2011).The tsetse fly (Glossina) is responsible for biological (cyclical) transmission while haematophagus arthropod vectors of the family, Tabanidae, Stomoxynae and Hippoboscidae are responsible for its mechanical transmission (Soulsby, 2012). Trypanosomiasis reduces the work efficiency of oxen and discourages the introduction of

drought animals in to the crop farming (Omotainete *et al*., 2000). It can be diagnosed based on either detection of the parasite by the light microscope conjugation with observation (Paris *et al*., 1980). Considering this, number of research has been carried out regarding the haemo-protozoans of cattle in Africa, such as in Ethopia (Leta *et al.,* 2016; Alemu and Alemenh, 2017), Euganda (Nabulime *et al*., 2014), Nigeria (Ameem, 2008; Zubairu *et al*., 2013) and Niger (Okaiyeto *et al*., 2011).

In Amhara region of North West Ethopia, trypanosome was considered as an important disease of cattle (Sinshaw *et al*., 2004; Chernet *et al*., 2006). In Ethopia *T. congolense* and *T. vivax* had been reported with the prevalence rate 45.5 and 44.3% (Leta *et al*., 2016). Similarly Alemu and Alenenh (2017) had identified that 6.67% sample were positive for the *Theileria*. In Uganda, 5% prevalence of Theileria was reported by the Nabulime *et al*. (2014). In Nigeria cattle were infected with a wide variety of vector-borne haemoparasites (Callow, 1978; Swallow, 2000). The most economically important trypanosomes spp. was *Trypanosoma vivax, T. congolense* and *T. brucei* in Nigeria. Ameen (2012) had identified the infection of Trypananosoma with the prevalence rate 3.9%. Similarly, 26.67% infection of Theileria had been identified form the same country (Zubairu *et al*., 2013). On the other hand, Okaiyeto *et al*. (2011) reported the 25% infection by the Theileria form the Niger. Few researches have been conducted in the Europe regarding the prevalence of haemo-protozoans. Zhou *et al.* (2016) were recovered the 5.6% infection of Theileria from the Turkey. On the other hand, Bovine trypanosomasis had been recorded with prevalence rate 14.7% from the different state of the Malaysia (Wahab *et al*., 2012).

National Context:

Haemoprotozoan parasites were reported by the Yadav (2015), Gupta *et al*. (2013), Maharjan and Mishra (2006), Deo and Neupane (2002), Shrestha and Singh (2000), Adhikari *et al*. (1997) and Ratala *et al.* (1990) respectively from the different districts.
Blood protozoan diseases constitute the greatest hindrance to the growth of cattle production in the Terai region of Nepal. Theilerial infections have been reported in Hyalomma tick with the prevalence rate 8.62%, 27.35% and 20.63% from the Eastern region of Terai i.e. Sunsari, Morang and Jhapa respectively (Gupata *et al*., 2013). Similarly, Deo and Neupane (2002) had identified that tick vector *Boophilus microplus, Hyallomma anatolicum* and *Rhiphicebhalus haemaphysaloides* were infected by the *Theleria* (26.18%*), Babesia* (10.18%) in cross breed cattle from Morang. Likewise, Shrestha and singh (2000) observed the sample by microscopically and they had been reported 13.2% *Theileria*, 9.64% *Babesia* and 6.02% *Anaplasma*. While in Siraha district, Yadav (2015) recognized that cattle were infected by *Babesia*, *Theileria*, *Trypanosoma* and *Anaplasma* with the prevalence 6.15%, 4.61%, 5.38 and 3.07% respectively.
A causative agent of bovine Babesiosis, *Babeisa bigemina* were detected in cross breed cattle from the Chabahil, Kathmandu (Ratala *et al*., 1990). Likewise blood protozoan parasites i.e. *Theileria* (6.67%) had been reported form cattle in Makawanpur (Maharjan and Mishra, 2006). While Adhikari *et al.* (1997) found that *Babesia, Anaplasma* and

Trypanosoma in thin blood smear of one cattle, three buffaloes and one dog, whereas only *Anaplasma* in cattle and *Babesia* in dog were detected in Banke.

3. MATERIALS AND METHODS

3.1 Study Area and Animal Population

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. About 65.5% people in the country are engaged in agriculture farming. Geo-graphically Nepal has been divided into three regions Mountain region, hilly region and Terai region.

Gorkha district has located in the Gandaki Zone of the Western Development Region of Nepal and surrounded by Dhading, Tanahun, Lamjung, Manang and Chitwan districts and it touches the border of Tibet. It covers an area of 3,610 square kilometers, with elevations ranging from 228 – 8163 meters, and a varied climate. Its geographical location is 28°17′24″N longitude and 84°41′23″E latitude.

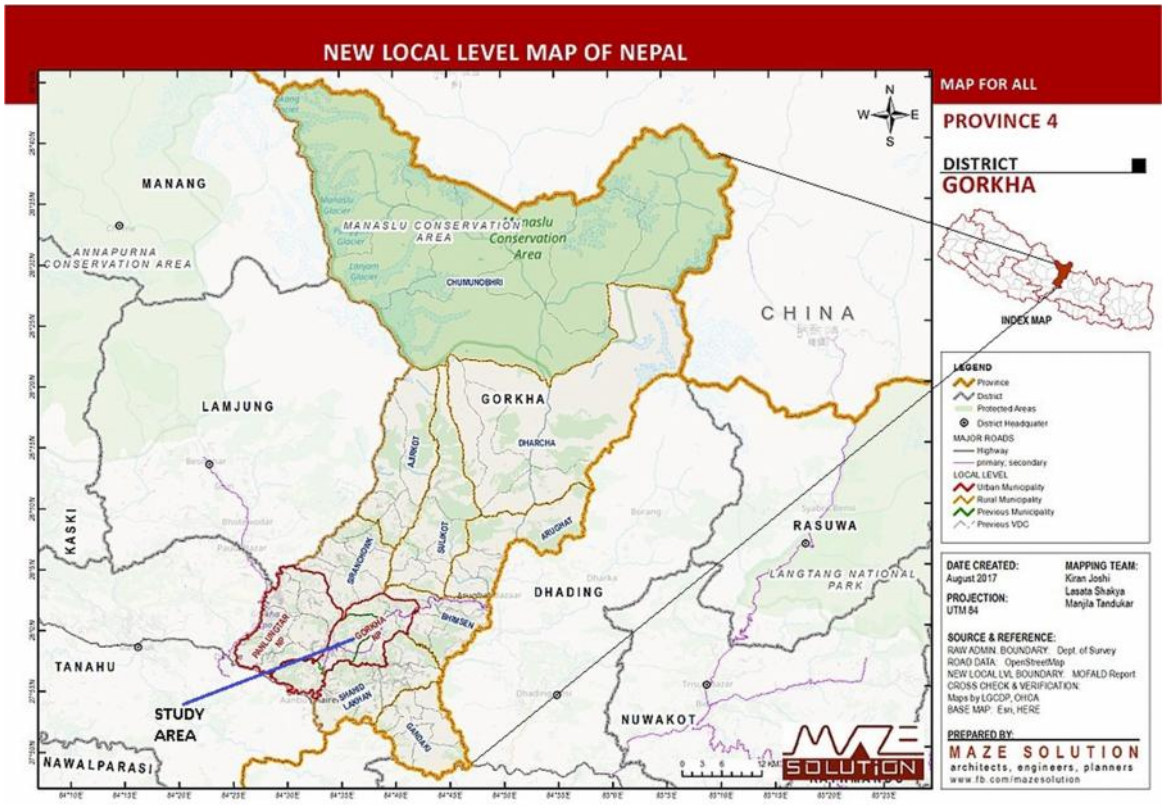


Fig.1: Map of study area

Gorkha district is located in mid hill region where temperature varies from maximum 30ºC to minimum 4ºC. Due to the favorable condition for the ticks, the prevalence of tick borne diseases had been expected in the Gorkha municipality-7. In the present study blood samples were collected from 80 cattle of Gorkha municipality-7, whereas the samples of cattle were collected from various age groups of local cattle (Annex).

According to the MoAD (2017), total number of livestock was 788606 of the Gorkha district. Among them cattle 88508, buffaloes 80560, goats 140508, sheep 29760 and pig 12025, fowl 436320 and duck 925 with majority of the local breed. The district profile report revealed that 18121 Metric Tons milk production and 4951 Metric tons meat production (MoAD, 2017). Different fodder had been used for the cattle such as, Sal (*Shorea robusta*), Tanki (*Bauhinia purpurea*), Harrao (*Terminalia chebula*), Barro (*Terminalia bellerica*), Amala (*Phyllanthus emblica*), Tite pati (*Artenisia vulgaris*), Dapdabe *(Garuga pinnata),* Siris *(Albizia lebbek),* Tanki *(Bauhinia longifolia),* Koiralo *(Bauhinia variegate),* Khasru *(Quercus semecarpifolia),* Kutmero *(Litsea polyantha),* Bakaino *(Melia azedarach),* Rukh katahar *(Artocarpus interga),* Badahar *(Artocarpus lakoocha),* Khanayo *(Fiscus cunia),* Kavro *(Ficus infectoria),* Kimbu *(Morus indica),* Shyal phurso *(Grewia optiva),* Gidari *(Premna integrifolia),* Dubo *(Cybodon dactylon),* Siru *(Imperata cylindrical),* Amriso *(Thysanolaena maxima),* Kera *(Mus paradisiac)* and so on.

3.2 Materials

3.2.1 Equipment

I. Cotton, Tissue paper	II. Coupling jar
III. Forceps	IV. Gloves
V. Microscopes	VI. Needle and Sticks
Vii. Slides, Cover slips, Slide box	VIII. Slide staining tray
IX. Syringes	X. Timer
XI. Sampling vials	XII. Vaccutainer with EDTA and stand

3.2.2 Chemicals

I. Methanol/ Ethanol	II. Distilled water
III. Giemsa/ Leishman staining reagents	IV. Sprit
V. Formalin Solution	VI. Immersion oil

3.3 Study Design

3.3.1 Blood Sampling

With the help of authorized technical staff, blood samples were collected during the month of May- July, 2017 from the jugular vein of the 80 different age group of cattle. Samples were collected with sterile syringe and immediately poured in to the Ethylene Diamene Tetra Acetate (EDTA) and samples were collected in field condition.

3.3.2 Preparation of Thin Blood Smears

For thin smears, one drop of blood was taken and placed near one end of clean and dry glass slide, inclined about 30˚ was pushed along horizontal from the one of clean 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 then dried rapidly by waving it in the air and was fixed with methanol for 2 minutes.

3.3.3 Giemsa's Stain Solution

The Giemsa's stain solution was used in stained preparation. For protozoan parasites, stained preparation might be required for the study of internal character for identification of the species. The Giemsa-stained preparation was commonly employed for this study. In order to make Giemsa's stain preparation, one volume of Giemsa standard solution was placed in nine volume of phosphate buffered water at PH 7.2. The solution was then filtered and kept in a stopped bottle of amber colour, and diluted with distilled water. The PH of water used for diluting the stain might be controlled by buffering with 3.0gm 1-1 Na_2HPO4 and 0.6gm 1-1 KH_2PO_4.

3.3.4 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.

3.4 Microscopic Examination

Examination of smears of peripheral blood was the simplest diagnostic method. The preparations were examined under high power magnification (10x by 100x) with the help of immersion oil. Starting from tail end of the slides to the whole field, any suspicious object was centered and focused for a detailed diagnosis.

3.4.1 Identification Procedure for Blood Parasites

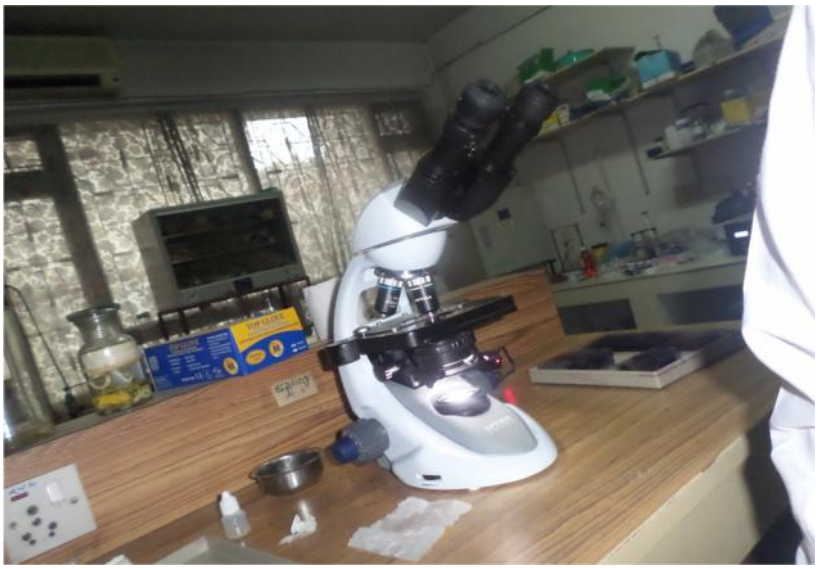
Almost all erythrocyte and field of all slides were always observed under microscopic examination. The characters of the individual parasites were sufficiently given specific attention to stain, shape, size, colour, position (i.e. attachment to erythrocytes), characteristic appendages, inclusion bodies, the membranes etc. during the identification of blood parasites. Diagnosis of the blood parasites in the smears was based on the description of Soulsby (2012) and other. Thin blood smears were used in estimating the stage of development and the severity of the disease.

Babesia: It may be pear shaped or round, usually centrally located in the erythrocytes and often found in pairs that are at an obtuse angle to each other (OIE, 2010; Ristic, 1988; Soulsby, 2012).

Theileria: It forms in red blood cells are mainly rod-shaped, 1.5- 2µm by 0.5-1µm; however, round , oval, comma and ring shaped form may also occur in erythrocytes (Soulsby, 2012).

Anaplasma: Morphologically the genus *Anaplasma* can be described as a gram negative, small, often pleomorphic coccoid to ellipsoidal organisms that reside within cytoplasmic vacuoles, either singly and more often in compact inclusions. In mammalian hosts they are present in mature or immature haematopoietic cells, particularly myeloid cells and erythrocytes, in peripheral blood or tissues as well as organs of the mononuclear phagocyte system (Dumler *et al*., 2001, Soulsby, 2012).

Photograph 1 Cattle of study area



Photograph 2 Central veterinary lab Tirpureswar

Photograph 3 Microscopic examination of blood smears

3.5 Questionnaire Survey

For data collection, a questionnaire was developed and presented before administration to gather information regarding the possible risk factor including age, sex, body condition and Herd size. The selected farmers were individually interviewed using questionnaire which targeted the household heads or their representatives. Both open and closed ended questions were included in the questionnaire administered to the respondents in order to seek information on household socio-economic characteristics such as knowledge of tick infection, manage-mental aspects of cattle farming system, use of anti-parasitic drugs.

3.6 Statistical Analysis

The obtained data were analyzed according to the prevalence of blood protozoan parasites and associated to risk factors (age, sex, herd size and body condition) of cattle. The analyzed data was interpreted by representation with table and graph. Prevalence was assessed by using statistical software R version 3.4.1 where descriptive stastistics was expressed as proportion with 95% confidence interval. For Chi-Square p-value and significance was determined when $P<0.05$.

4. RESULTS

4.1 Prevalence of Haemo-Protozoan Parasites of Cattle of Gorkha Municipality- 7, Gorkha.

A total of 80 cattles belonging to Gorkha municipality- 7, Gorkha were screened for haemo-protozoan parasites. The blood samples were collected from the jugular vein and samples were brought to the central veterinary lab, Tripureswar, Kathmandu and microscopically examined using giemsa stain method.

Table1. Prevalence of haemo-protozoan parasites of cattle of Gorkha municipality ward no. 7, Gorkha.

Variable	Category	Prevalence	χ2	P-value
Age	≤5 yr	4(5%)	0.055109	**0.8144**
	>5 yr	9(11.25%)		
Sex	Male	9(11.25%)	2.5649e-31	**1**
	Female	4(5%)		
Body condition	Good	0	32.348	**1.25e-08**
	Poor	13(16.25%)		
Herd size	≤3	7(8.75%)	2.87	**0.090**
	>3	6(7.5%)		

Haemo-protozoan parasitic infections were analyzed on the basis of demographic characteristics. Age wise infection of haemo-protozoan parasites showed highly prevalent in greater than five year age group with insignificant difference. Similarly, sex wise prevalence also showed insignificant, although the prevalence was found comparatively high in males. Likewise herd size wise infection of haemo-protozoan of haemo-protozoan parasites showed almost equal prevalence among the cattle kept in large size (>3) and small herd size (<3).

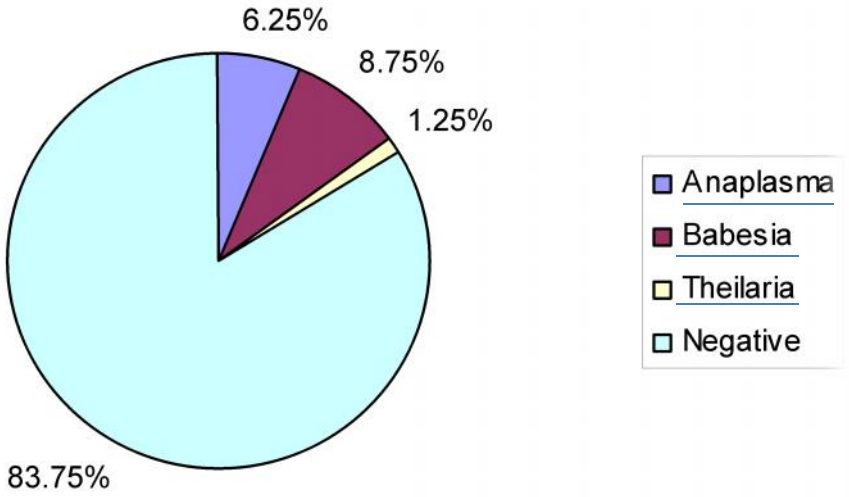


Fig.1: Prevalence of haemo-protozoan parasites of cattle

Among the haemo-protozoan parasites, *Anaplasma* sp., *Babesia* sp. and *Theileria* sp. were found with prevalence 6.25%, 8.75% and 1.25% respectively. Although the prevalence of *Anaplasma* sp. and *Babesia* sp. were higher as compared to *Theileria* sp. The significant different was not found in the prevalence of haemo-protozoan parasites (P-value=0.1026, $\chi 2$=4.5544 at df=2).

4.2 Comparative Prevalence of BPPs in Gorkha Municipality -7, Gorkha.

Among the three different genera of blood protozoans parasites identified in cattle, prevalence of each parasite were demographically characterized. Age, sex, body condition as well as herd size wise comparisons were prioritized.

4.2.1 Age wise comparision of prevalence of blood protozoan parasites

Age wise prevalence of *Anaplasma* sp. revealed comparatively high in less than five years with insignificant association to that of age group ($\chi 2$= 0.3555, P-value= 0.551 at df=1). While the prevalence of *Babesia* sp. in two different age group showed simply different from prevalence of *Anaplasma* sp. The prevalence was found to be comparatively higher among the cattle with age group >5 years, although the statistical difference was not significant ($\chi 2$= 0.8557, P-value= 0.3579 at df=1). Whereas prevalence of *Theileria* sp. was comparatively less prevalent than the other BPP and *Theileria* sp. was higher with age group greater than five years, however statistically difference was not significant ($\chi 2$= 0.3314e-32, P-value= 1 at df=1).

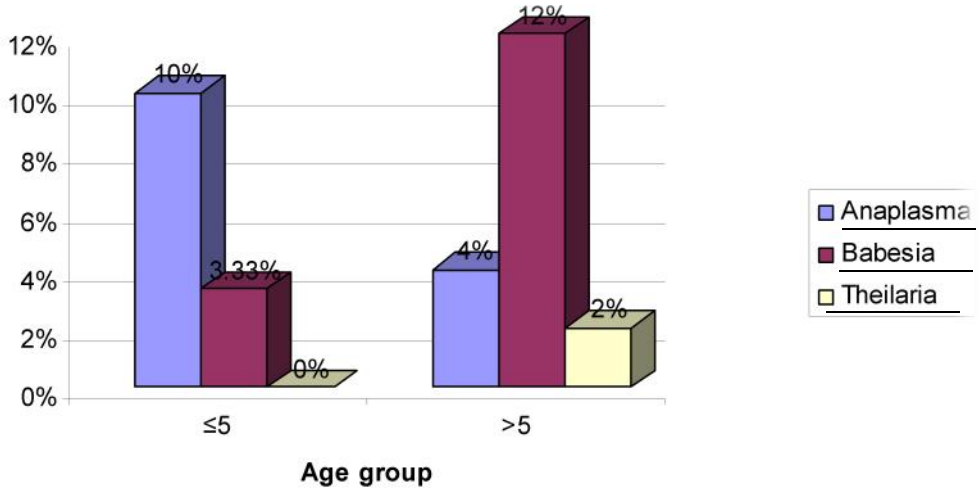


Fig. 2: Age wise comparision of prevalence of *Anaplasma* sp., *Babesia* sp. and *Theileria* sp.

4.2.2 Sex wise comparision of prevalence of blood protozoan parasites

Sex wise prevalence showed that *Anaplasma* sp. had equally prevalent than the other haemo-protozoans. *Anaplasma* sp. were highly prevalent in male with insignificant ($\chi 2$= 0, P-value= 1 at df=1) association the sex group. On the other hand prevalence of the *Babesia* sp. was comparatively high in male as compare to other, although significant difference was not occur with linked to the sex groups. In the age group prevalence of *Babesia* sp. was higher in the male with statistically insignificant ($\chi 2$= 0.25962, P-value= 1 at df=1). While the prevalence of *Theileria* sp. were less abundant than the other haemo-protozoans in sex group with insignificant ($\chi 2$= 6.7264e-31, P-value= 1 at df=1) distribution.

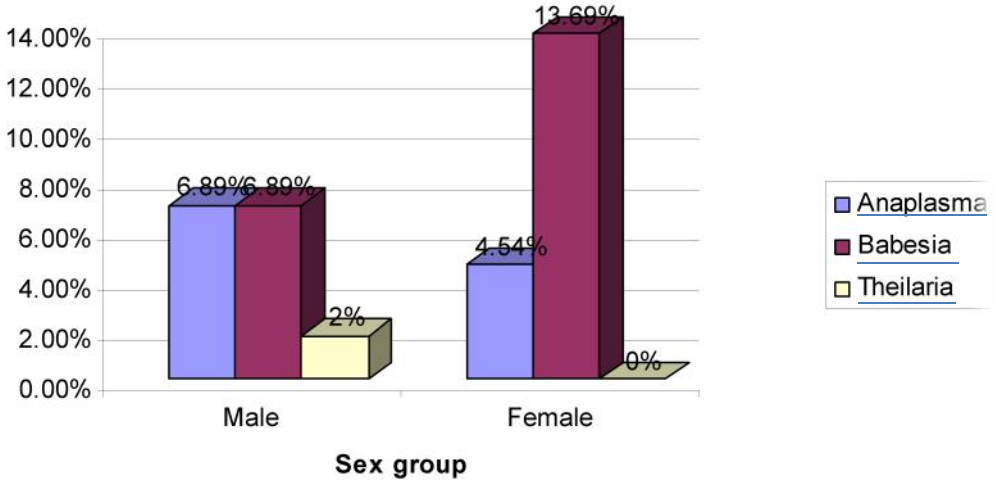


Fig. 3: Sex wise comparision of prevalence of *Anaplasma* sp., *Babesia* sp. and *Theileria* sp.

4.2.3 Body condition wise comparision of prevalence of blood protozoan parasites

Body condition wise prevalence of haemo-protozoans revealed that *Anaplasma* sp., *Babesia* sp. and *Theileria* sp. were highly prevalent in the poor body condition. In the group of good condition, BPP were totally absent. Among the prevalent of BPP, *Babesia* sp. was the higher with accompanying to body condition and statistically also showed that three genera were significant regarding to the body condition of the cattle.

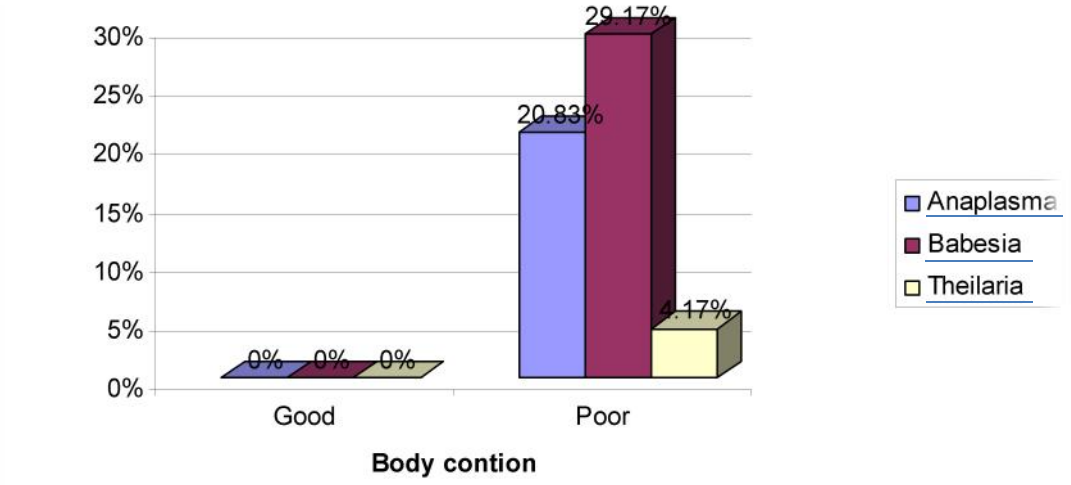


Fig. 4: Body condition wise comparision of prevalence of *Anaplasma* sp., *Babesia* sp. and *Theileria* sp.

4.2.4 Herd size wise comparision of prevalence of blood protozoan parasites

Herd size wise comparison of BPP revealed that *Babesia* sp. was highly prevalent with regard to other BPP. Prevalence of BPP was more prevalent in the having the herd size greater than the three. While In the herd size greater than three, *Babesia* sp. was found high prevalence with insignificant ($\chi 2$= 0.034, P-value= 0.8535 at df=1) association to the herd size. On the other hand prevalence of *Anaplasma* sp. was slightly different as compare to other BPP. The prevalence was found to be higher in greater than three herd size with insignificant ($\chi 2$= 0.3822, P-value= 0.5564 at df=1) association to the herd size. Likewise prevalence of *Theileria* sp. was vast different than the other BPP. Herd size wise prevalence of *Theileria* sp. was high in greater than three herd wise, although statistically insignificant ($\chi 2$= 0.28801, P-value= 0.5915 at df=1) association to the hard size.

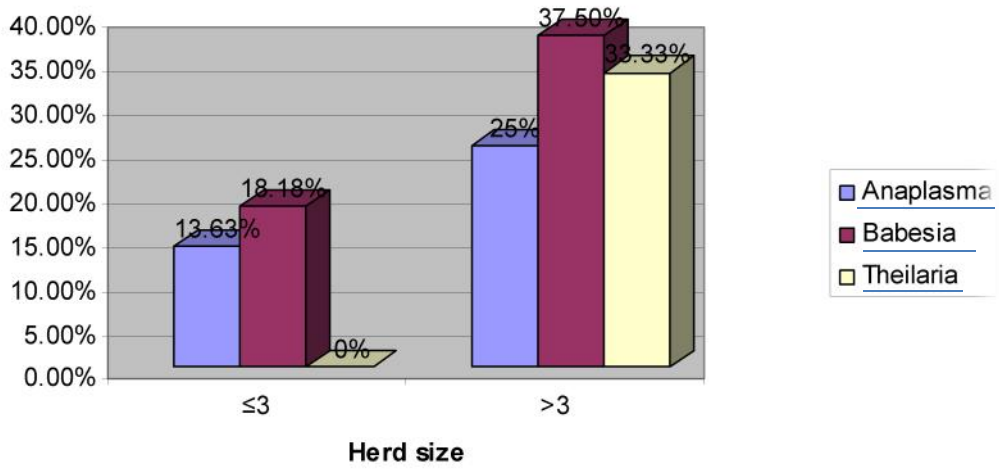
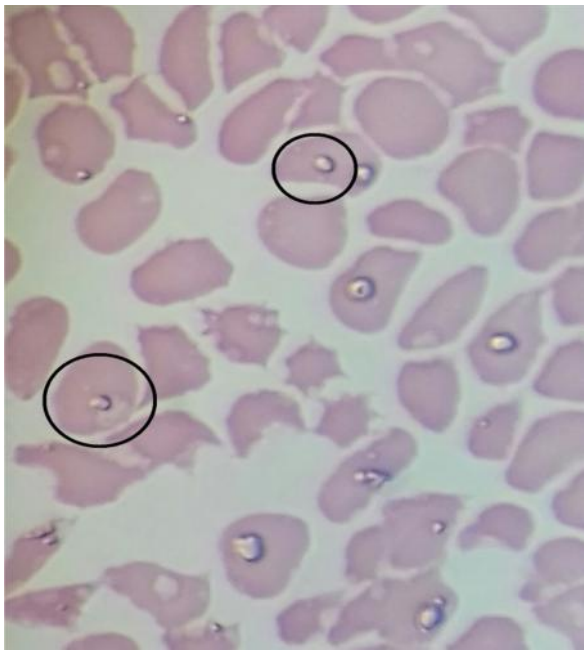


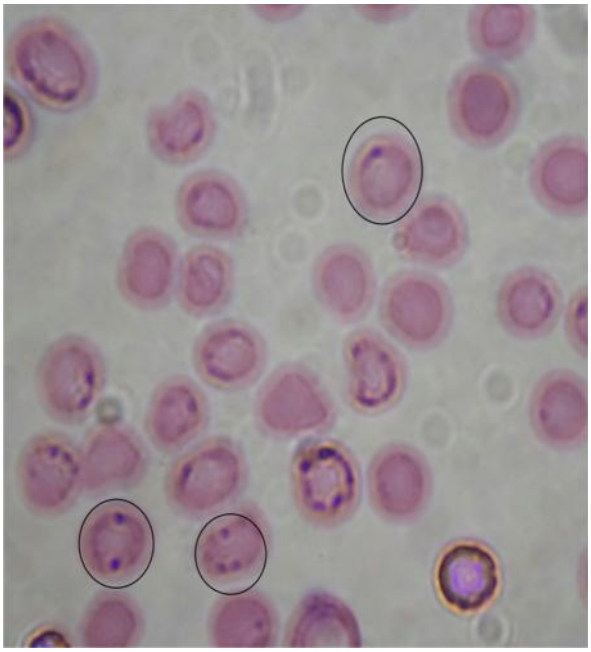
Fig. 5: Herd size wise comparision of prevalence of *Anaplasma* sp., *Babesia* sp. and *Theileria* sp.

4.3 The knowledge, Attitude and Practice (KAP) of Cattle Owner in Gorkha Municipality Ward no. 7, Gorkha.

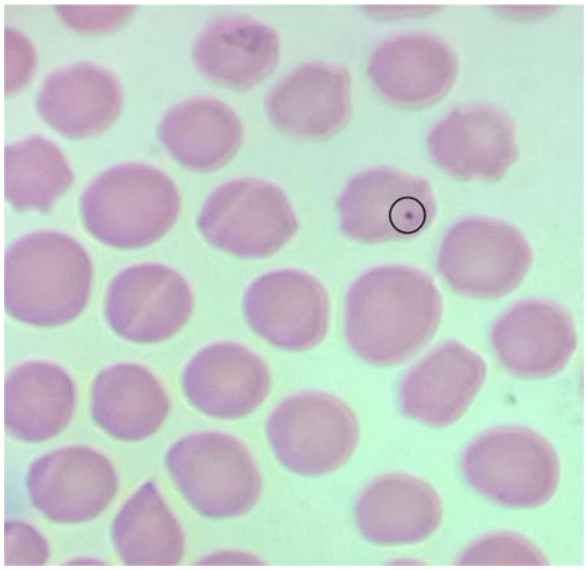
A structured questionnaire survey was carried out among the cattle owners of Gorkha municipality-7. Out of 30 cattle owners survey it was found that all of them had owned only local cattle by the traditional system. Interestingly it was found that none of them had ever taken livestock farming training from any of the veterinary office. Pond water was the major source for the drinking purpose to their cattle. In the studies areas, most of the cattle owners did not use any parasitoids for the ecto-parasite. However cattles were free from the tick and other ecto-parasite. Cattle had left for the feeding in the open ground. Deworming practice was not applied by the cattle owner in the shed. Even almost cattle owners were unknown from the cattle's disease, while these diseases transmit by the tick.



Photograph 4 Blood smear showing *Theileria* sp.



Photograph 5 Blood smear showing *Babesia* sp.



Photograph 6 Blood smear showing *Anaplasma* sp.

5. DISCUSSION

Livestock farming is an important agricultural sub-sector in Nepal. According to the data from Central Bureau of Statistics (CBS, 2012), livestock covers approximately about 24 percent of agricultural Gross Domestic Product (GDP). Generally, animals are born free of diseases or parasites, but they usually acquire these maladies either through contact with diseased animals or due to improper sanitation, feeding, care and management. Fungal, viral, parasitic as well as blood protozoans diseases are the major disease of the cattle. Haemoprotozoan infections are very common in cattle and cause devastating losses to the livestock industry and pose a major threat to the dairy industry throughout the world (Shahnawaz *et al*., 2011). Babesiosis, anaplasmosis, theileriosis and trypanosomiasis are considered some of the major impediments in the health and productive performance of cattle (Rajput *et al.,* 2005). These diseases are transmitted through tick as well as other blood sucking flies in tropical and subtropical parts of the world (Salih *et al*., 2015). Hot and humid climate is highly favorable for the development and survival of ticks vectors (Kohli *et al*., 2014). These haemoprotozoan are economically important vector borne diseases and has always been a formidable barrier to the survival of exotic as well as cross bred cattle particularly (Ananda *et al*., 2009). The worldwide incidence of haemoparasitic infections in cattle has been severally reported by different workers (Laha *et al*., 1989; Thach *et al*., 1996).

In the present study, the haemo-protozoan parasites were found to be 16.25% in cattle of the Gorkha municipality ward no. 7, Gorkha. The similar results have been reported as 18.06% and 16.64% prevalence in Siraha district (Yadav, 2015) and in Tamil Nadu, India (Velusamy *et al*., 2014) respectively. Haemo-protozoans has been documented from the different region of the Nepal such as Terai region i.e. Sunsari, Morang, Jhapa (Gupta *et al*., 2013), Makawanpur (Maharjan and Mishra, 2006), Morang (Deo and Neupane, 2002), Terai region (Shrestha and Singh, 1999), Salyan (Shakya *et al*., 1996), Banke (Adhikari *et al*., 1997), Kathmandu (Ratala *et al*., 1990) etc. The present prevalence rate of haemo-protozoans parasites were less as compared to 23%, 28% and 36.36% reported by the Maharjan and Mishra (2006), Shrestha and Singh (2000) and Deo and Neupane (2002) respectively. It may be due to the climatic variation in between Terai and hilly region. The prevalence rate of present study was higher as compared to 9%, 6.67%,

9.92% reported by the Bhatnagar *et al*. (2005), Velusamy *et al*. (2014) and Okorafor and Nzeako (2014) while less than the reports of Maharana *et al*. (2016), Chowdhary *et al*. (2006), Velusomy *et al*. (2014) and Ananda *et al*. (2009) who revealed 25.7%, 27.88% 37%, 39.39% respectively from various parts of the world. The variation in parasitic prevalence could be due to the climatic factors which directly influence the vector distribution.

With regard to the age-wise prevalence of haemo-protozoans parasites, above the five years cattle were found to be highly infected with the prevalence rate 11.25% with insignificant difference in this study. Which is that similar to the prevalence rate reported 12% by the Yadav (2015). Whereas Velusamy *et al*. (2014) and Anada *et al*. (2009) documented the high prevalence rate in 2-7 and 4-6 years age group. The higher prevalence of haemo-protozoan in young cattle compare to the calf and old age cattle may be due to the grazing habit of young cattle and management practices of the farmers, where high chance of contact with the vectors of these diseases occurs. In this study, sex-wise prevalence of haemo-protozoans parasites was higher in male (11.25%) than the female (5%). It may be due to the reason that male are used as ploughing purpose during the rainy season in the field. Okorofor and Nzeako (2014) reported that female was highly infected. It may be due to the stress of breeds, milking and cyclical hormonal changes in female. Cattles which were infected by haemo-protozoans parasites ultimately have poor body condition body. This result was correlated with the Paul *et al*. (2016) where cattle with moderate body condition were highly infected (6.07%) than the good body condition (4.2%).

All the livestock are equally susceptible for haemo-protozoans parasitic infection. The disease has been reported from cattle, buffalo and goat from Makwanpur district (Maharjan and Mishra, 2006), cattle, goat and dog from the Banke (Adhikari *et al*., 1997), horse form the Kathmandu and mules from the Salyan (Shakya *et al*., 1996) of the country. Various tropical and sub-tropical region of the world suffered by the haemo-protozoans. In Saudia Arabia, camels, cattle, sheep and goat were infected by the BPPs (Khalifa *et al*., 2009). Likewise, BPPs have been reported from the cattle of Sudan (Mohammed *et al*., 2011), Bangledesh (Chowhary *et al*., 2006) etc. Blood protozoan parasites have been reported from deer, cattle, buffaloes and pig of Malayasia (Nurulaini

et al., 2013). Goat, buffalo and dog were infected by the BPP in China (Zhang *et al*., 2011) and cattle from the India (Velusamy *et al*., 2014). Altogether three genera of the haemo-protozoans were reported in cattle from the present study.

Cattle were highly infected by anaplasmosis with the prevalence rate 6.25% in the present study. Which showed comparatively similar to the 6.1%, 5.88% and 6.1% prevalence of BPP, reported by the Maharjan and Mishra (2006), Awad *et al*. (2011) and Birdane *et al*. (2006). The prevalence of haemo-protozoans parasites were found to be higher than 3.77% reported by the Parmar and Upadhya (2017). This could be due to the improper management practices. While infection rate was less than the reports of Kumar and Sangwan (2010), Mtshali *et al*. (2004), Zhou *et al*. (2016), Chowdhury *et al.* (2006), Mohanta *et al*. (2011), Khan *et al*. (2007), Yang *et al*. (2013), Nair *et al*. (2013) and Vohora *et al*. (2012) who revealed 46%, 87%, 89.9%, 29.1%, 87%, 14%, 18%, 46.2% and 16.6% respectively from different part of the world.

Regarding to the age, the prevalence of *Anaplasma* was high in less than five years age group cattle. This result is in agreement of the Yadav (2015), who reported that cattle were highly infected above the five years age group. Besides these, high infection of anaplasmosis in cattle were recorded in above the three years (Chowdhary *et al*., 2006 and Chakrabarti, 2001) and Minnat *et al.* (2016) had documented that above the four years cattle were highly infected with the haemo-protozoans parasites. Adult were highly infected than old while young had no *Anaplasma* (Maharana *et al*., 2016). In Pakisthan, 1-2 years cattle were highly infected (Atif *et al*., 2012). In the present study, male were highly infected by the anaplasmosis with the prevalence rate 6.89%. Similar result was reported by the Minnat *et al.* (2016), where male were highly infected than female. Besides these, the parasite prevailing percentage in female was slightly higher than that of the male (Rajput *et al*., 2005). With regard to the body condition of the cattle, high prevalence rate i.e. 20.23% infection was recorded in the poor body condition. This result was nearly similar to the Kocan *et al*. (2003), where sick cattle were highly infected with prevalence rate 46.9%. Likewise, 25% infection of *Anaplasma* was found in greater than three herd size from the present study.

Bovine babiosis is a tick borne disease of cattle caused by the protozoan parasites of the genus *Babesia*. It is common in Africa, India, Central Asia, Central and Southern America and Australia (Mahoney, 1997; Ristic and Kreier, 1981; Soulsby, 2012; Young

and Morzaria, 1986). With regard to *Babesia*, 8.75% infections of babesiosis were reported from the present study. Which showed comparatively similar to the 9.64%, 10.18%11.2% and 9.2% prevalence of BPP reported by the Shrestha and Singh (2000), Deo and Neupane (2002), Zhou *et al*. (2016) and Parmar and Upadhya (2016). The present prevalence of BPP were found to higher than 5.5%, 6%, 0.56%, 2.5% and 1.48% reported by the Maharjan and Mishra (2006), Yadav (2015), Okrafr and Nzeako (2010), and Bhat *et al*. (2016). While less than the reports of the Kamani *et al*. (2010), Terkawi *et al*. (2012), Brito *et al*. (2012), Cover *et al*. (2013), Kirupantathan *et al*. (2016) and Parmar and Upadhya (2017) who revealed 16%, 24.64%, 95%, 63.6%, 16.63% and 47% respectively. With respect to the age, the prevalence of *Babesia* was found to be higher in the above the five years age group from the present study. Result of present study was nearly similar to the Yadav (2015) who revealed that 4-8 years cattle were highly infected. Besides these, Minnat *et al.* (2016), Adua *et al.* (2017), Reda (2012) and Chowdhury *et al*. (2006) had reported that less than five years age cattle were highly infected as compared to the greater than five years age group cattle. It could be due the less immunity. The prevalence of *Babesia* was reported high in female than the male with regarding to the sex in the present study. This showed that, this result was similar to the Paul *et al.* (2016) who reported the female were highly infected by the haemo-protozoans parasites. It could be due to the presence of *Hyaloma* spp. tick vectors, which involves the trasnsmission of *Babesia*. Besides these, some reports have been revealed that male were highly infected (Bihonengn *et al*., 2015). With regard to the body condition, poor body condition cattle were highly infected with the haemo-protozoans. Similar result had been reported by the Bihonegn *et al.* (2015). Regarding to the herd size, cattle were highly infected in greater than three herd size with prevalence rate 37.50%.

Theileriosis is caused by the protozoan parasite of *Theileria* spp. (*Theileria annulata* and *Theileria parva*). In the present study, infection of theileriosis was comparatively less than the other haemo-protozoans diseases. 1.25% prevalence of *Theileria* was recorded from the present study. Which is the similar to the 2.2% and 1.2% prevalence of BPP reported by the Gebrekidan *et al*. (2016) and Kursat *et al*. (2008). The present prevalence of BPP was found to be higher than 0.56% which is reported by the Okorafor and Nzeako (2014) from the Nigeria. While less than the Gupta *et al*. (2013), Yadav (2015), Shrestha and Singh (2000), Kamani *et al*. (2010), Aktas *et al*. (2006), Zhou *et al*. (2016), Wang *et al*. (1998), Inoue *et al*. (2001), Hadi *et al.* (2012), Khan *et al*. (2004), Khattak *et al.*

(2014), Muraleedharan *et al*. (2005), Singh *et al*. (2012), Kumar *et al.* (2015), Kohli *et al.* (2014), Parmar and Upadhya (2017) who revealed 6.6%, 8.62%, 27. 35%, 20.63%, 5.6% , 13.2%, 16%, 41%, 18.9%, 3.8%, 27.5%, 43%, 28%, 5.2%, 90%, 14.65%, 9.35%, 27.2% and 84.62% respectively. Greater than five years age group cattle were highly infected with the prevalence rate 2% from the present study regarding the age gruop. Which is nearly similar to the result of Yadav (2015) who revealed the 4-8 years cattle were highly infected with the *Theileria*. Besides these, Minnat *et al*. (2016), Farahan *et al*.(2012) and Reda (2012) reported the less than five years age group cattle were highly infected. Present study revealed that male were highly infected with prevalence rate 2%. This result was similar to the Yadav (2015) who reported the male were highly infected by the *Thileria* and similar result had been reported by the Minnat *et al*. (2016) in which male were highly infected. Besides these, Atif *et al*. (2012) reported that female was highly infected in Pakisthan. In present study, the poor body condition cattle were highly infected with *Theileria*. Similar result has been documented by the Kocan *et al.* (2003) in which sick cattle was highly infected by the *Theileria* with the prevalence rate 46%. With regard to herd size, the theilerial infection were high i.e. 33.33% in greater than three herd size.

Several species of the haemo-protozoans disease were affected to the both-sex, various age groups, different herd size and body condition of cattle. On the basis of the comparative analysis, cattle were highly infected by the *Babesia* sp. with the prevalence rate 8.75%. In the cattle owner, they had lack of management system for the cattle farming. From the present study, identified that farmers were unknown about proper hygiene, husbandry practices and use of anti-parasitic drug. So, there is need of proper education with regard to the cattle farming system among the farmer.

6. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

A study on prevalence of haemo-protozoan parasites had been carried out in domestic cattle of Gorkha by using Giemsa stained thin blood smears. A total of 80 blood samples were collected from the jugular vein of cattle. Samples were examined by microscopically for the haemo-protozoans parasites. Out of the 80 samples, 13(16.25%) samples were found to be positive for blood parasites. *Anaplasma* sp., *Babesia* sp. and *Theileria* sp. were confirmed with prevalence rate 5(6.25%), 7(8.75%) and 1(1.25%) respectively. Whereas *Babesia* sp. was highly prevalence than the other haemo-protozoans parasites. Prevalence of haemo-protozoan parasites was high in greater than five years age group 11.25% and lowest in less than five years age group 5% in cattle of the study area. Male were highly infected by the BPP (11.25%) than the female (5%). The cattle were found to be infected with blood protozoans in poor body condition. The rate of BPP infection in less than and greater than three herd size were found equally infected.
Babisiosis and theileriosis were more prevalent in less than five years cattle although anaplasmosis was high in above the five years cattle. Male were highly infected by the anaplasmosis and theileriosis than the babesiosis. While these three blood parasites were more predominant in poor body condition and above the three herd size cattle.
With regard to the knowledge, attitude and practice (KAP) of cattle owner found that all of them had owned only local cattle by the traditional system, none of them had taken livestock farming training. Pond water was the major source for the drinking purpose most of the cattle owners did not use any parasitoids. Cattle had left for the feeding in the open ground and deworming practice was not applied by the cattle owner during management.

6.2 RECOMMENDATIONS

Based on the outcome of the present study, the following recommendations have been made to reduce the risk of blood protozoan diseases in the cattle.

- Regular screening of cattle for parasitic infection needs to be carried out time to time.
- Knowledge on haemo-protozoan parasites of cattle seems poor among the farmer hence cattle farmers should be made aware of harmful effect of parasitic benefit of dewarming.
- Further research works on molecular level should be carried out.

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8. ANNEX

List of blood sample collected from the Gorkha municipaltity-7.

Identification key For sample	Name of the Farmers	Sex of cattle	Age of cattle (in year)	Symptoms	Parasites	Herd size	Body condition
1	Jagat Bd. Rana	Female	6	Infected with tick	-ve	4	P
2	Jagat bd. Rana	Female	5	Infected with tick	*Anaplasma*		P
3	Jagat bd. Rana	She calf	1		-ve		G
4	Jagat bd. Rana	Male	3		-ve		G
5	Ram bd. Rana	Male	7		-ve	2	G
6	Ram bd. Rana	Male	6		-ve		G
7	Bishnu Panday	Male	5		-ve	3	G
8	Bishnu Panday	Male	5		-ve		G
9	Bishnu Panday	Female	2		-ve		G
10	Sitaram sirmal	Male	4	Infected with tick	-ve	2	P
11	Sitaram sirmal	Male	9	Infected with ticks and weak	*Anaplasma*		P
12	Kumar khatri	Male	7	Infected with tick	-ve	4	P
13	Kumar khatri	Male	8	Infected with tick	*Theileria*		P
14	Kumar khatri	Female	5	Infected with tick	-ve		P
15	Kumar khatri	He calf	1		-ve		G
16	Ghan singh rana	Female	3		-ve	1	G
17	Megh Rana	Male	7	Infected with tick	*Babesia*	2	P
18	Megh Rana	Male	5	Infected with tick	*Anaplasma*		P
19	Tilak bd. rana	Male	6		-ve	2	G
20	Tilak bd. rana	Male	7		-ve		G
21	Bhai kaji thapa	Male	5		-ve	4	G
22	Bhai kaji thapa	Male	8	Infected with tick	*Anaplasma*		P
23	Bhai kaji thapa	Female	6		-ve		G
24	Bhai kaji thapa	He calf	1		-ve		G
25	Naran thapa	Female	6	Infected with	*Babesia*	2	P

				tick			
26	Naran thapa	She calf	1		-ve		G
27	Arjun thapa	Male	9	Infected with tick	-ve	2	P
28	Arjun thapa	Male	9	Infected with tick	*Babesia*		P
29	Ram bd. Pandey	Female	2	Infected with tick	-ve	1	P
30	Kaji ram Bhatta	Male	8	Infected with tick	-ve	4	P
31	Kaji ram Bhatta	Male	6	Infected with tick	-ve		P
32	Kaji ram Bhatta	Female	7	Infected with tick	*Babesia*		P
33	Kaji ram Bhatta	She calf	1		-ve		G
34	Hira Sirmal	Male	7		-ve	2	G
35	Hira Sirmal	Male	7		-ve		G
36	Sanu nepali	Male	9		-ve	3	G
37	Sanu nepali	Male	8		-ve		G
38	Sanu nepali	Female	3		-ve		G
39	Ram Krishna nepali	Male	10		-ve	2	G
40	Ram Krishna nepali	Male	8		-ve		G
41	Babu ram uperkoti	Male	9	Infected with tick	*Babesia*	4	P
42	Babu ram uperkoti	Male	9		-ve		G
43	Babu ram uperkoti	Female	6		-ve		G
44	Babu ram uperkoti	She calf	1		-ve		G
45	Ram Krishna thapa	Male	6		-ve	5	G
46	Ram Krishna thapa	Male	6		-ve		G
47	Ram Krishna thapa	Female	7	Infected with tick	-ve		P
48	Ram krisna thapa	Male	2		-ve		G
49	Ram krishna thapa	He calf	1		-ve		G
50	Raja ram thapa	Male	7		-ve	2	G
51	Raja ram thapa	Male	5	Infected with tick	*Anaplasma*		P

52	Pramod pandey	Male	6		-ve	2	G
53	Pramod Pandey	Male	6		-ve		G
54	Rishi pandey	Male	8		-ve	2	G
55	Rishi pandey	Male	9		-ve		G
56	Badri pandey	Male	6		-ve	2	G
57	Badri Pandey	Male	5		-ve		G
58	Harimaya rana	Male	9		-ve	6	G
59	Harimaya rana	Male	9		-ve		G
60	Harimaya rana	Female	5	Infected with tick	-ve		P
61	Harimaya rana	Male	7	Infected with tick and weak	*Babesia*		P
62	Harimaya rana	Female	5	Infected with tick	*Babesia*		P
63	Harimaya rana	She calf	1		-ve		G
64	Krishna rana	Male	7		-ve	2	G
65	Krishana rane	Male	7		-ve		G
66	BIkash rana	Male	9		-ve	4	G
67	BIkash rana	Male	9		-ve		G
68	BIkash rana	Female	7		-ve		G
69	BIkash rana	She calf	1		-ve		G
70	Min rana	Male	8		-ve	3	G
71	Min rana	Male	7		-ve		G
72	Min rana	female	10	Infected with tick and weak	-ve		P
73	Nil bd. Pulami	Male	7		-ve	2	G
74	Nil bd. Pulami	male	7		-ve		G
75	Phun bd. Pulami	Male	5		-ve	2	G
76	Phun bd. Pulami	Male	5		-ve		G
77	Rod bd. Pulami	Male	4		-ve	2	G
78	Rod bd. Pulami	Male	5		-ve		G
79	Jit bd. Thapa	Male	6		-ve	2	G
80	Jit bd. Thapa	Male	6		-ve		G