PREVALENCE AND RISK FACTORS ASSOCIATED WITH OCCURRENCE OF BLOOD PROTOZOAN DISEASE IN CATTLE OF RAMGRAM MUNICIPALITY, NAWALPARASI DISTRICT, NEPAL

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RECOMMENDATION

This is to recommend that the thesis entitled "**Prevalence and risk factor associated with occurrence of blood protozoan disease in cattle of Ramgram municipality, Nawalparasi district, Nepal"** has been carried out by Ganga Phuyal for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under our supervision. To the best of our knowledge, this thesis work has not been submitted for any other degree in any institutions**.**

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On the recommendation of supervisor **"Dr. Mahendra Maharjan"** this thesis submitted by Ganga Phuyal entitled **"Prevalence and risk factor associated with occurrence of blood protozoan disease in cattle of Ramgram municipality, Nawalparasi 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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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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ABSTRACT

Haemoparsites have generally been shown to cause destruction of red blood cells resulting in anemia, jaundice, anorexia, weight loss and infertility. The study was conducted from June to August 2017 in Nawalparasi district of Ramgram municipality to show the prevalence and risk factors associated with occurrence of blood protozoan disease in cattle. A total of 150 blood samples of cattle were collected by puncturing jugular vein of cattle. The samples were brought to the DLSO Nawalparasi lab in EDTA containing vials for thin blood preparation and Giemsa staining. Microscopic examination of blood samples revealed that the overall prevalence of haemoprotozoan parasites were 17.33%. Three species of parasites were identified with 12 (8%) *Anaplasma* sp., 10 (6.66%) *Babesia* sp., 4(2.66%) *Theileria* sp.Statistically, the disease was found to be prevalent throughout the region with no significance association between infections as the dependent variables like age, sex, body conditions, herd size and localities. The infection in all ages of cattle and highest percentage of infection occurs in age groups 4-8 years (10%) while lower prevalence of infection occurs in the cattle of eight years above. The study showed high prevalence of infection in crossbreed (6.66%) as compared to local breed (4%) and effect of sex of cattle showed high prevalence in female (13.33%) than male (4%). Good body condition (9.33%) of cattle recorded higher prevalence of haemoprotozoan than poor condition (8%). Herd wise infection was found to be high in >10 herd size (12.22%). Thus awareness programs should be organized to educate farmers about tick borne diseases and tick control measurement in order to establish or maintain enzootic stability.

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Abbreviated form	Details of abbreviations
BPP	Blood Protozoan Parasite
CPDD	Communication Publication and Division
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

1. INTRODUCTION

1.1 Background

Nepal is an agricultural country with poor economy. Agriculture and livestock contributes almost 38% and 11% of the national GDP respectively (World Bank, 2004). Livestock plays an important and integral role to form a substantial component of both individual and national wealth in the agricultural system in Nepal. They have a crucial role in food nutritional security, livelihood regional balance and comprehensively contribute to rural poverty alleviation (ILO, 2004). Among the various livestocks, the cattle helps on cultivation, harvesting and acts as method of transportation in rural areas, moreover, their manure is the fertilizer for soil nutrient, increasingly used for fuel and they provide milk and meat products and cash income through trade (Ferris *et al*., 1992). About 75% of Nepalese farmers practice cattle farming system in all region of Nepal including the Mountain, Hill and Terai belts with variation based on climate, topography and socio economic factors (CPDD, 2013). Different breeds of cows have been recently introduced and are now famous for commercial purpose among the farmers with modern technology. Holstein and Jersey are exotic (DLS, 2010) which are used to cross with local breed by artificial insemination.

Nepalese farmers are still practicing the traditional way of cattle farming. Although cattle are performing a vital role, maximum number of cattle are emaciated and frequently affected by several types of diseases due to poor knowledge about cattle farming system. Health status and hygienic condition of animals are degrading day by day (Bhandari *et al*., 2011). Both diseased and healthy animals are being kept in the same shed which increase the chance of transmission of disease and parasite among the cattle (Bhandari *et al*., 2011).

1.2 Cattle diseases

Cattle diseases have been identified as one of the major problem which have disrupted the development of the industry in and have caused substantial economic loss to the poor subsistent farmers in the developing countries (Dhama *et al*., 2014). The economic losses include treatment cost, lowering of productivity, and reduction in fertility, culling of animals and investing usual time, labor and resources for reduced performance (Debnath *et al*., 1990). Some infectious agents like bacteria, viruses, fungi and mycoplasma are known to interfere with cattle health (Dhama *et al*., 2014). Haemorregic Septicemia, Mastitis, bovine tuberculosis, Brucellosis are the major prevailing alignments of bacterial diseases of Nepalese cattle causing heavy economic losses (Thakuri *et al*., 1992). Besides other diseases commonly to affect the cattle, diverse and manifold types of viruses resulting from different grades of infections as foot and mouth disease, Blue tongue disease Viral diarrhea are responsible for marked deleterious effects that tend to lower the overall production both by way of morbidity and mortality (James and Rushton, 2003).

1.3 Tick born disease

Arthropod causes no disease itself but is capable of infecting its hosts by transmitting the different viral, rickettsia, bacterial and protozoan diseases (Kakar *et al*., 2017).They possess attributes like attaching on body, sucking blood slowly and remaining unnoticed for long period of time in their host accounting for their vector potential. Hot and humid climate is favorable for survival and propagation for tick which in turn act as a constant source of infection to susceptible animals (Velusamy *et al*., 2014). Ticks don't only act as vectors for pathogen, the infection itself may also cause direct problem with losses in fertility, weight and milk production and predispose animals to other bacterial and fungal infections (Jongejan and Uilenberg, 2005). Ticks transmit the haemoprotozoan disease and are economically important to cattle industry by causing direct loss and by transmission of microbes (Minjauw and McLeod, 2003). Tick borne diseases especially babesiosis, theileriosis and anaplasmosis are considered as one of the most economic limitations to successful cattle industry in Nepal (Shrestha and Sighn 1999).

1.4 Haemoprotozoan parasitic diseases

Haemoprotozoan parasitic disease has economic importance through significant loss of milk production, infertility, mortality of young stock and exhaustion of draught animals (Sharma *et al*., 2013). The disease is a major constraint on efforts designed to upgrade the cattle production through the introduction of improved breeds of animal.

Theileriosis: *Theileria annulata* and *Theileria parva* are the most pathogenic parasites in cattle, which cause Tropical theileriosis and East coast Fever respectively (Perry and Young, 1993). Tropical Theileriosis is considered to be most prevalent disease of cattle caused by sporozoites form of *Theileria annulata* and is transmitted through *Hyalomma* tick during feeding (Mirzaei, 2007). It is widely distributed in tropical and subtropical zones across Northern Africa, West and East Asia including Indian sub-continent as endemic disease (Chen *et al*., 2000). Theileriosis is one of the most important haemoparasitic diseases in Eastern Nepal (Acharya and Pradhan, 1996; Shrestha and Singh, 2000). Morphologically, *Theileria* sp. are small round, ovoid irregular or bacilliform parasite with an apical complex which is located in erythrocytes as well as lymphocytes cell of their host (Naik *et al*., 2016). They are ranging from clinically in apparent to rapidly fatal (Darghouth *et al*., 1996). The mortality rate for tropical theileriosis can vary from 3% to nearly 90%, depending on susceptibility of the animals while mortality rate from East Coast fever can be up to 100% with case fatality rate of 10-20% in calves (Haque *et al*., 2010). *Theileria parva* causes enlarged lymph nodes, starting with the parotid lymph node, fever, Dyspnea and occasional Diarrhea (Magona *et al*., 2008).

Babesiosis: Babesiosis is caused by haemo-protozoan parasites of the genus *Babesia* and transmitted by ticks of the Ixodidae family (OIE, 2008) and *Boophilus* species as a main vector globally. It is the second most common blood-borne parasites of mammals after the trypanosomes (Telford *et al*., 1993). Babesiosis is also known as piroplasmosis, tick

fever, red water, Texas fever, splenic fever, or tristeza (Ristic, 1988). In 1888, Babes introduced the name of babesiosis in Romania. Later in 1893, Smith and Kilborne (1893) identified causative agent of piroplasma transmitted by arthropod vectors. Ullenberg (2006) and Zweygarth *et al*. (2006) noted in 7 species of babesiosis i.e. *Babesia bovis, Babesia bigemina, Babesia divergens, Babesia major, Babesia ovata, Babesia occultans* and *Babesia jakimovi.* At the same time, Starcovici (1893) identified these parasites as *Babesia bovis* and *Babesia bigemina. Babesia* protozoan parasites were passed transovarially, via the egg, from one tick generation to the next (Gohil *et al*., 2013) then infect all wild and domestic cattle. Babesiosis is generally characterized by extensive erythrocytic lysis leading to anaemia, icterus, haemoglobinuria and mortality (Homer *et al*., 2000). *Babesia microti* is introduced as human babesiosis (Hildebrandt *et al.*, 2013).

Morphologically, *Babesia* sp. is clearly identified in merozoit stage which is about <2.5 to >2.5 micrometer (Laha *et al*., 2015). *Babesia bigemina* is large paired or pyriformed being acute angle or parallel formed to each other, where *Babesia bovis* is small rounded centrally located being obtuse angle to each other (OIE, 2010; Ristic, 1988). They are widely distributed in Africa, Asia, Australia, Central and South America and Southern Europe (Bock *et al.,* 2004). The majority of Nepalese cattle have been infected by *Babesia* sp. in different regions (Adhikary *et al*., 1997).

Anaplasmosis: Anaplasmosis is a vector-borne, infectious haemoprotozoan disease known as yellow bag or yellow fever jaundice like appearance in cattle caused by the rickesttsial parasites *Anaplasma marginale* and *Anaplasma centrale* (Battilani *et al*., 2017). The causative agents of anaplasmosis have been recognized as an important public and animal health issues globally (Battilani *et al*., 2017). Morphological form of *Anaplasma* sp. can be described as small, pleomorphic coccoid to ellipsoidal gram negative organisms present in mature or immature haematopoietic cells, particularly myeloid cells and erythrocytes, in peripheral blood or tissues of mammal (Dumler *et al*., 2001). Anaplasmosis is distributed worldwide throughout tropical and subtropical areas of South, Central and North America, Australia, Asia and Europe (Aubry and Geale, 2011). This pathogen infects red blood cells of cattle and is transmitted biologically by ticks, mechanically by biting flies or blood-contaminated fomites, needles or surgical instruments (Dumler *et al*., 2001) and transplacentally from cow to calf during gestation (Dikmans, 1950). Tick transmission can occur within a stage (transstadial) or from stage to stage (Stich *et al*., 1989). The hot and humid climate is very conducive for the development and survival of potential vectors such as ticks and flies and is a constant source of infection to susceptible animals (Salih *et al*., 2015). Calves less than a year old that are infected with *Anaplasma marginale* usually do not show clinical signs of the disease, but will become carrier host (Radwan *et al*., 2013). Anaplasmosis symptoms are fever that last for 4 to 10 days, anorexia, weight loss, lethargy, cough and increased respiratory rate and pulse rate, abortion, decreased milk production (Samad *et al*., 1984).

1.5 Objectives

1.5.1 General objective

To study the prevalence of blood protozoan diseases and associated risk factors in cattle of Ramgram municipality in Nawalparasi district.

1.5.2 Specific objectives

- To determine the prevalence of blood protozoan parasites of cattle in Ramgram municipality.
- To identify the associated risk factor of blood parasitic disease infection in cattle.

1.6 Significance

Livestock farming in Ramgram municipality of Nawalparasi district is slowly moving towards commercial scale. Many farmers are rearing their livestocks relying only on their traditional knowledge. Animals are being treated symptomatically in this area as farmers are not ready to afford for the treatment of their animals and animal health facility is far away from their access. This may lead many cases remain undiagnosed. As, blood parasitic diseases of cattle cause anemia, fever, anorexia, weight loss and decreased milk production and infertility, in severe cases, parasitic diseases may even cause death of cattle, the study may guide for accurate diagnosing and early treating the animal before the devastating effect of the disease occurs in animal. The study would be useful in understanding the prevalence of blood parasites in cattle of Ramgram municipality, Nepal. No any study has been done to detect prevailing causes of cattle disease in this area. In this context, the aim of this study may reveal the existing status of haemoprotozoan parasitic diseases responsible for illness, weak body condition, low production and infertility. This study would also be helpful for future researchers and investigators, those investigating the diseases of cattle in Ramgram municipality Parasi, Nepal.

2. LITERATURE REVIEW

Haemo-protozoan parasites

Haemoprotozoan parasitic infection causes great economic impact on livestock, affecting 80% of the world's cattle population (Muhanguzi *et al*., 2014). With the introduction of extensive crossbreeding program, the incidence of blood protozoan diseases have increased resulting in high rate of morbidity and mortality in crossbreed and exotic cattle (Sisodia and Mandial, 1986). Haemoprotozoan diseases especially babesiosis, anaplasmosis, theileriosis and trypanosomiasis are considered as the major impairments in the health and productive performance of cattle (Rajput *et al*., 2005). They destroy the red blood cells and cause anemia, jaundice, anorexia, weight loss and infertility in bovine (Akande *et al*., 2010). The direct losses caused by the parasites are attributed to acute illness and death, premature slaughter and rejection of some body parts at meat inspection whereas indirect losses include the reduction of productive potential such as decreased growth rate, weight loss in young growing animal and late maturity of slaughter stock (Hanson and Perry, 1994). Considering such losses, various research works have been conducted on haemo-protozoan parasites of cattle in the world.

2.1 Global Context

2.1.1 Babesiosis

Babesiosis is a tick borne disease caused by *Babesia* sp. that destroys the intra-erythrocytic cells (Telford *et al*., 1993). It is also known as piroplasmosis, tick fever, red water fever, splenic fever or tristeza, and is transmitted only by Ixodid ticks (Ristic, 1988). Starcovici (1893) named these parasites as *Babesia bovis* and *Babesia bigemina*. These parasites commonly cause the diseases in cattle worldwide (Ribeiro and passos, 2002).

Bovine babesiosis is distributed in tropical and sub-tropical area of South America, Africa, Europe, Australia and Asia (Perez *et al*., 1994; Hesterberg, 2007; Stevenson, 2008; Graya *et al*., 2010). High prevalence of *Babesia gibsoni* has been recorded in Southern part of California due to the tick infection (Yamane *et al*., 1993). In New Zealand, Payne *et al*. (1988) screened 61 blood smears and found that five cattle were infected by babesiosis. In United State, *Babesia microti* was mostly found among the human, cattle and other domestic animals (Despommier and Dickson, 1995). Kubelova *et al*. (2012) recorded 16% *Babesia* sp. in Africa. Reda (2012) conducted the study on blood parasite of farm animals in Egypt and reported 19.33% babesiosis in cattle. In Northern center Nigeria, the babesiosis infection rates in cattle were reported to be from 8% (Abdullahi *et al*., 2014; Sam Wobo *et al*., 2016) to 16% (Kamani *et al*., 2010). Moreover, Adua and Idor (2017) reported 7% *Babesia bovis* and 3% *Babesia motasi* in cattle of Nigeria. Similarly, a study conducted by Paul *et al*. (2016) estimated the prevalence of *Babesia* species to be 4.2% while Ugochukwu and Sydney (2014) found that 6.66% cattle were positive for babesiosis. On the other hand, *Babesia bigemina* and *Babesia bovis* had been identified by Matheus (2017) from cattle of Namibia. Seven species of *Babesia*. i.e.

Babesia bovis, *Babesia bigemina*, *Babesia divergens*, *Babesia major*, *Babesia ovata* , *Babesia occultans* and *Babesia jakimovi* have been reported from Tanzania (Uilenberg, 2006; Zweygarth *et al*., 2006). In Egypt, 40% of blood samples were positive for the *Babesia* infection (Hazem *et al*., 2014). *Babesia bigemina* and *Babesia bovis* were highly infectious blood protozoan parasite in Africa (Bock *et al*., 2004; Da Silva *et al*., 2013).

In Iraq, Tareq *et al*. (2016) reported 86.7% infection of *Babesia* sp. in cattle. Durani and Kamal (2008) observed 42% cattle of Pakistan being infected by *Babesia* infection. Khan *et al*. (2004) reported 2.85% *Babesia* infection in cattle from Islamabad. Chowdhary *et al*. (2006) and Abdullah *et al*. (2015) observed 3.33% and 2.27% prevalence of babesiosis from cattle in Bangaladesh respectively. Babesiosis has also been recorded from different geographical region of India. In Southern Rajasthan, Bhatnagar *et al*. (2015) studied infection of *Babesia* parasite of cattle and revealed that 15.65% cattle were infected. Similarly, 14.94% prevalence of *Babesia* sp. has been recorded from southern Kerala, India (Kariyappa *et al*., 2017). The prevalence of *Babesia* sp. was found to be 22.88% in Southern Gujrat, India (Maharana *et al*., 2016).

According to available literature, prevalence rate of babesiosis was higher in female cattle than male cattle of Nigeria (Paul *et al*., 2016), Namibia (Metheus *et al*., 2017), Ethiopia (Fethu *et al*., 2016), Egypt (Sam Wobo *et al*., 2016) Iraq (Tareq *et al*., 2016) whereas prevalence of babesiosis was found to be higher in male cattle than female cattle of Egypt (Reda, 2012) and western Ethiopia (Bihonegn *et al*., 2015).

In relation to age group, a higher prevalence (28%) of *Babesia* sp. has been recorded in cattle of 2-3 year in Egypt (Reda, 2012). In Nigeria, cattle of 4-5 years were highly infected by the *Babesia* sp. with the prevalence rate 28.4% (Sam WObo *et al*., 2016). High prevalence of *Babesia* sp. has been reported in cattle above 8 year of age (27%) followed by 4-7 year (22.4%) and 0-3 year (20.7%) in Southern Ethiopia (Fethu *et al*., 2016). Similarly, the cattle of age group 4-8 year have been identified to be more susceptible to *Babesia* sp. with 18.5% prevalence rate (Metheus, 2017).

In case of breed, Atif *et al*. (2012) showed that cross breed cattle were more infected (10.86%) than indigenous cattle (2.86%) in Pakistan. In contrary, Fethu *et al*., (2016) in Ethiopia, found that the prevalence of babesiosis in local breed (24.5%) were higher than that in Holsteins (16.6%) and hybrid (8.6%). Chaudhary *et al*., (2013) reported 3.22% of *Babesia* infection in crossbreed cattle of India.

2.1.2 Theileriosis

Theileriosis is one of the most infectious diseases caused by intra erythrocytic protozoan parasite of genus *Theileria.* It is transmitted by bite of infective *Hyalonema* tick. Sporozoites of *Theileria* sp. enters into animal body during tick feeding (Oliviera *et al*., 1995) that leads to devastating loss in growth, development, mortality and morbidity of cattle. Theileriosis is widely distributed in Mediterranean Europe, Middle East, India, middle Asia and even in China (d'Oliveira *et al*., 1995). Muhanguzi *et al*. (2014) recorded 5.3% prevalence of *Theilera prava* in Eastern Uganda. Similarly, Keneth *et al*.

(2014) found 6.86% *Theileria prava* while studying epidemiology of haemoparasites in Uganda. Kamani *et al*. (2010) and Abdulahi *et al.* (2014) recorded 12% and 40% *Theileria* infection in dairy cattle of Nigeria respectively. In Egypt, Reda (2012) reported that 23% of cattle were infected by theileriosis.

Tropical theileriosis is one of the most prevalent diseases of cattle caused by *Theileria annulata* (Mirzaei, 2007). Tareq *et al*. (2016) studied epidemiological and haematological changes of cattle and found high infection of *Theileria* sp. (51.4%) from cattle of Iraq. In Pakistan, the study on prevalence of *Theilera* sp. was carried out by Atif *et al*. (2012) and found that 20.51% cattle were infected with *Theileria* sp. while Khan *et al*. (2004) recorded 1.42% prevalence of theileriosis in cattle. Theileriosis has been reported from geographical regions of India such as Punjab, Haryana, Gujrat, Kerala and Madras. Kariyappa *et al.* (2017) and Nair *et al*. (2011) found 26.82% and 16% infection of *Theileria* sp. in cattle of Kerala. In Chhattisgarh, Maiti and Singh (2016) screened tropical theileriosis and found that 23.33% cattle were positive. Washkel and Gaur (2015) identified the prevalence of theileriosis as 51.92% in cattle from India. Kumar *et al*. (2015) observed the prevalence of theileriosis in Punjab and recorded 9.35% infection of the *Theileria* sp. Soundarajan and Rajavelu (2006) recorded 28.2% prevalence of *Theileria annulata* in cattle around Madras. Maharana *et al*. (2016) found 7.08% cattle were infected by *Theileria* sp. in Gujrat. A study carried out by Bhatnagar *et al.* (2015) revealed that 42.26% of cattle were positive for theileriosis in Rajasthan.

In Pakistan, high prevalence of *Theileria* sp. in female (8%) was reported among the cattle of 1-2 years (Atif *et al*., 2012). In India, Naik *et al*. (2016) found that tropical theileriosis was maximum (24.34%) in adult cattle with age of 3 years above followed by 23.80 % in animals of 1-3 years and 14.28 % in 0-1 years. In the same place, the prevalence of tropical theileriosis in cattle with respect to sex was recorded to be 25.45% in female and 17.5 % in male cattle (Naik *et al*., 2016). In Egypt, highly infected (30%) cattle were reported from the age group 2-3 years and less infected (28%) were reported from age group 0-1 years (Reda, 2012).

In Iraq, high percentage of theileriosis has been recorded in crossbreed (52.4%) compared with native breed (50.5%) (Tareq *et al*., 2016). Similarly, Shatri *et al*. (1981) reported highest prevalence in pure exotic breed while lowest prevalence in indigenous cattle in Karnatak state, India. Mahiza (2010) recorded higher infection of *Theileria* sp. in local cattle in Malaysia. But in Tamil Nadu, high prevalence of theileriosis (13%) was found in crossbreed (Velusami *et al*., 2014). In Chhattisgagh India, breed wise prevalence of tropical theileriosis in cattle showed 15.38 % prevalence in Sahiwal, 14.81% in Gir, 23.33 % in Jersey cross and 29.85 % in Holstein Frisian cross (Naik *et al*., 2016).

2.1.3 Anaplasmosis: Anaplasmosis is tick borne haemo rickettsial infection and is known as yellow bag or yellow fever similar to jaundice (Dumler *et al.*, 2001). It is an infectious parasitic disease of cattle affected by numerous species of the blood parasite *Anaplasma* sp. but the most important species are *Anaplasma marginale* and *Anaplasma centrale* (TFRC, 1996).

In Northen Sudan, *Anaplasma* sp. has been recorded with prevalence rate 6.1% in cattle (Ekici and Ferda, 2011). In Uganda, 14.4% infection of *Anaplasma* sp. had been reported (Keneth *et al*., 2014). The prevalence of haemoparasitic infection revealed that 42% cattle were infected with *Anaplasma marginale* in Nigeria (Abdullahi *et al*., 2014). Payne *et al*. (1990) observed tick borne disease and found that 79% cattle were infected with anaplasmosis in Paragyuay. In Nigeria 5.8% cattle were infected with *Anaplasma marginale* (Paul *et al*., 2016). Sam Wobo *et al*. (2016) observed 16.5% infection in Nigeria. After one year, Adua and Idor (2017) recorded 6.16% *Anaplasma marginale* from the same country.

In Pakistan, Khan *et al*. (2004) studied prevalence of haemoprotozoan and the infection of *Anaplasma marginale* was recorded to be 75.75% in cattle. Similarly, Rajput *et al. (*2005) recorded 51% animal were infected by *Anaplasma* sp. in Pakistan. But Atif *et al*. (2012) found 9.71% *Anaplasma* sp. during his study in Pakistan. Bhatnagar *et al*. (2015) noticed 42.07% cattle infected by *Anaplasma* sp. In the same way, Murthy *et al*. (2016) observed 2.7 % prevalence of *Anaplasma marginale* in Karnatak state India. Maharana *et al*. (2016) found 3.93% anaplasmosis in Gujarat.

The parasitic prevalence of *Anaplasma* sp. in female cattle was higher than that in the male (Rajput *et al*., 2005). In India, Chaudhary *et al*. (2013) reported high infections in cattle of age group of 3 years above. Similarly, Chakrabarti (2002) also found high prevalence of cattle over 3 years. In Diyala district, male were highly infected (22%) with the age of 4 years above (Tareq *et al*., 2016). Chowdhary *et al.* (2006) recorded high infection (69.99%) in crossbreed cattle in Bangaladesh. Similarly, breed wise prevalence was recorded high (10.86%) in crossbreed in Pakistan (Atif *et al*., 2012). Tareq *et al*. (2016) found that 23% of native breed cattle were highly infected by the *Anaplasma* sp. in Iraq.

2.2 National Context

2.2.1 Babesiosis: Babesiosis has been recorded in different regions of Nepal (Mishra, 2003). In Eastern Terai, Shrestha and Singh (1999) recorded 9.64% prevalence of *Babesia* sp. from dairy cattle. Deo and Neupane (2002) conducted study on bovine piroplasmosis in Morang district and revealed 10.8% babesiosis in cattle. Bohara and Shrestha (2016) recorded 0.64% *Babesia* infection in cattle from Midwestern region. In Siraha, high prevalence of *Babesia* sp. (4.8%) has been recorded at the age of 4-8 years of cattle while low prevalence (0.32%) was recorded at 0-4 years (Yadav, 2015). Shrestha and Singh (1999) examined for blood protozoan disease among crossbreed dairy cattle of Eastern Terai and revealed 9.64% infection of *Babesia* sp. Yadav (2015) found higher infection in local breed (3.22%) than crossbreed cattle (2.9%).

2.2.2 Anaplasmosis: In Banke, Adhikari *et al*. (1997) recorded *Anaplasma* sp. in cattle. Shrestha and Singh (1999) reported prevalence of anaplasmosis in cattle as 6.02% in Eastern Terai. In Midwestern Terai, Bhoara and Shrestha (2016) revealed 5.8% *Anaplasma* infection in cattle. Yadav (2015) studied haemoprotozoan in Siraha district

and recorded 3.07% *Anaplasma* sp. in cattle. High prevalence of anaplasmosis (1.29%) was recorded in cattle of 4-8 years age group and above 8 years while low infection (0.32%) was found in 0-4 years (Yadav, 2015). In Siraha, Breed wise prevalence was recorded to be 10.76% in crossbreed cattle and 8.46% in local breed (Yadav, 2015). Similarly, Shrestha and Singh (1999) recorded 6.02% anaplasmosis infection from crossbreed cattle in Midwestern region.

2.2.3 Theileriosis: In Eastern Terai, the occurrence of *Theileria* sp. was recorded to be 13.25% (Shrestha and Singh, 1999). The *Theileria* infection in Sunsari, Morang and Jhapa were recorded to be 8.62%, 27.35% and 20.63% respectively (Gupta *et al*., 2013). While cattle of Morang district showed overall prevalence of 10.18% theileriosis (Deo and Neupane, 2002). Similarly, Shrestha and Singh (1999) surveyed haemoprotozoan parasite in eastern Terai and revealed that 13.25% cattle were infected by *Theileria* sp. Yadav (2015) reported high prevalence of *Theileria* sp. (3.22%) among the cattle of age group 4-8 years in Siraha. Female cattle were more infected than the male cattle in Eastern Nepal (Gupta *et al*., 2013). In relation to breed, prevalence of *Theileria* sp. infection was higher in crossbreed cattle (2.9%) than local breed cattle (2.25%) in Siraha district (Yadav, 2015).

3. MATERIALS AND METHOD

3.1 Study Area

The study was conducted from June to August 2017 in and around Ramgram municipality of Nawalparasi district. Nawalparasi district is located in Lumbuni zone of Western developmental region in between 27°31'59.99" North latitude and 83°39'59.99" East longitudes. It is surrounded with Rupandehi district on the west part, Tanahu district on the north, Chitwan district and Indian boarder on the east and southern parts. The population of Nawalparasi district estimated to 643,508 (CBS 2011). District head quarter of Nawalparasi is located at Ramgram, which is about 9 km from Mahendra highway and is located 147 km west of Kathmandu. Nawalparasi area covers 2162 square kilometer and has altitude ranging between 91 meter and 1936 m above sea level. Previously, Parasi area consisted of different VDCs as well as Ramgram municipality. In September 2017, Sukrauli, Amraut and Banjaria VDCs were merged into Ramgram municipality to form ward no.10, 14 and 15. The climate varies from maximum 43°C to minimum, 1.5°C. Due to favorable conditions tick and tick borne disease prevalence is expected to be high in Ramgram municipality. Furthermore, this area is well known by livestock production which can be estimated at about 17228 cattle, 106802 buffaloes, 267735 goats, 11356 sheep and 51876 pig and people of this area are highly motivated to adopt cattle farming in commercial scale which accounts for 25737 milking cow in Nawalparasi district (DLS, 2015).

Fig1: Study area, Nawalparasi district.

In the present study, 60 blood samples were collected from Ramgram municipality, 36 samples were taken from Sukrauli (10), 39 from Amraut (14) and 15 from Banjaria (15).

The samples of cattle were collected from various ages, sexes, herd sizes, breeds and body conditions.

The study was done with the help of DLSO technicians and respective technical staff of Animal Service Center and Sub Centers of the study areas. Preliminary works were done prior to the survey works.

3.2 Material Use

Following materials were used for sample collection and for entire laboratory process.

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 viii. 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

3.3 Study Design

3.3.1 Sample size and blood sampling

With the help of authorized technical staff of DLSO, Parasi, 150 blood smears of cattle were collected from three wards with Ramgram municipality during the study period. In the present study, 60 blood samples were collected from Ramgram municipality, 36 samples were taken from Ramgram 10 (Sukrauli), 39 from Ramgram 14 (Amraut), and 15 blood samples collected from Ramgram 15 (Banjaria).

3.3.2 Sample collection and preservation

Before sample collection, questionnaire with a majority of close-ended questions was administered to the farmer or farm manager with questions related to this study. Sterilized syringe with separate needles were used to prick the jugular Vein. Blood samples were collected in closed tube with 4 ml in ethylene diamante tetra acetate (EDTA) containing vacutainer and stored in iceboxes at ≈ 4ºC and collected samples were immediately

brought in district livestock services office (DLSO) Nawalparasi for microscopic examination.

3.3.3 Thin blood smears preparation

A drop of blood approximately 4 mm in diameter was placed on one side of the slide. The drop was then spread by using another slide (called here the "spreader"), placing the spreader at a 45° angle and backing into the drop of blood. The spreader was caught the drop and expanded by capillary action along its edge. The resulting film was dried rapidly by waving it in the air and was fixed with methanol for 2 minutes and attained with Giemsa for 25-30 minutes in coupling jar. The stained slide was washed by flooding very briefly under current tap water and was placed in upright position to drain and air dried.

3.3.4 Giemsa's stain solution preparation

One volume of Giemsa's 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 has been controlled by buffering with 3 gm 1-1 Na2HPO4 and 0.6 gm. 1-1 KH2PO4, 0.2 gram Kcl and Nacl.

3.3.5 Giemsa staining

Air dried by gentle shaking and methanol fixed for 2 minute. The blood smear slides were subjected to coupling jar containing working solution of Giemsa stain for 25-35 minutes. Then stained slides were washed gently in current tap water an air dried.

3.3.6 Microscopic sample examination

The stained smears were examined microscopically by using oil immersion under high power lens (x100) of a light microscope with the help of immersion oil. Starting from tail end of slides to the whole field, any suspicious object was centered and focused for a detailed diagnosis.

3.3.7 Identification of blood parasites

The characters of individual parasites were sufficiently given specific attention to stain, shape, size, colour, position (ie, attachment to erythrocytes), characteristic appendages, inclusion bodies, the membranes during the identification of blood parasites (Salih *et al*., 2007. Diagnosis of blood parasites in the smear was based on the descriptions of (Soulsby 1982; Bowmann, 2009). Both thin and thick blood smear were used in estimating the stage of development and the severity of the disease.

* In Giemsa-stained thin blood films, *Anaplasm*a sp. appear as dense-cored cells and reticulate cells in homogeneously staining blue-purple inclusions 0.3–1.0 μm in diameter (OIE, 2015).

* Microscopically, the species of *Babesia* sp has a peared shaped, located in pairs, round, oval or irregular depending on the stage of the parasite in erythrocytes.

* *Theileria* sp. has size 1-2 micrometer Rod, ovule or comas shaped in blood.

3.3.8 Questionnaire survey

For data collection, a developed pretested questionnaire was prepared to gather information regarding the possible risk factors including age, sex, breed, 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, managemental aspects of cattle farming system, use of antiparasitic drugs.

3.4 Statistical Analysis

The obtained data were analyzed according to prevalence of blood protozoan parasites and associated to risk factors (age, sex, herd size, breed 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 statistics was expressed as proportion with 95% confidence interval (CI). For Chi-Square Test, results were expressed in percentage with P-value and significance was determined when $P<0.05$.

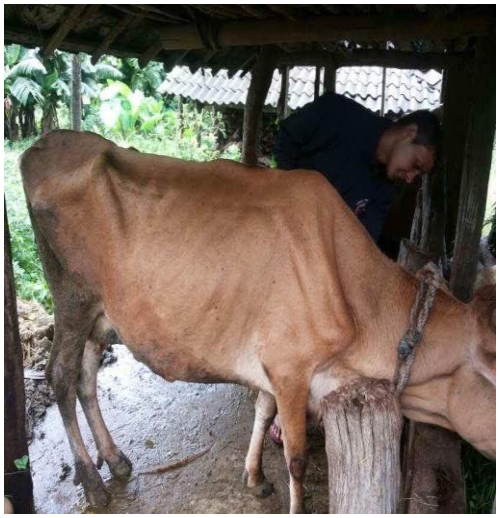

Photo 1 Cattle of study area

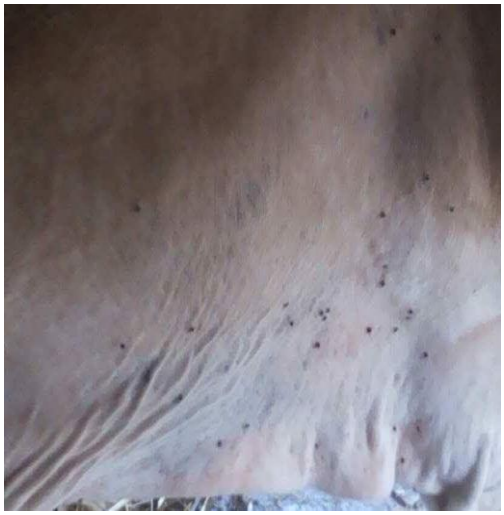

Photo 2 Ticks infection in cattle

Photo 3 DLSO Nawalparasi

Photo 4 Photography of blood parasites.

4. RESULTS

4.1 Prevalence of blood protozoan parasites of cattle in Ramgram municipality in Nawalparasi district.

A total of 150 cattle belonging to different localities of Ramgram municipality were screened for blood protozoan parasites. The blood samples collected from jugular vein were microscopically examined by using Giesma stain method. The result revealed that 26 (17.33%) cattle were found to be positive for blood protozoan parasites.

Table1: Prevalence of blood protozoan parasites in cattle of Ramgram municipality.

Parameter of cattle		Prevalence of blood parasites(N=150)	**χ2 value**	**P value**
Sex wise	Male	(6) 4%	0.374	**0.5406**
	Female	(20) 13.33%		
Age wise	0-4	(8) 5.33%	2.1029	**0.3494**
	4-8	(15) 10%		
	8-12	(3) 2%		
Herd size	<10	(7) 4.66%	0.048	**0.8251**
	>10	(19) 12.22%		
Breed	Local	(6) 4%	0.6264	**0.8904**
	Jersey	(6) 4%		
	Holstein	(4) 2.66%		
	Crossbreed	(10) 6.66%		
Body condition	Good	(14) 9.33%	5.9741e-31	**1**
	Poor	(12) 8%		
Locality	Ramgram	(12) 8%	1.8062	**0.6132**
	Sukrauli (10)	(5) 3.33%		
	Amraut (14)	(7) 4.66%		
	Banjaria (15)	(1) 0.66%		

Blood protozoan parasitic infections were analyzed on the basis of demographic characteristics. Sex wise infection of blood protozoans showed highly prevalent in female than male but statistically insignificant difference was observed. Age wise prevalence showed maximum prevalence in between age group 4-8yrs.which was also shown to be statistically not significant. Blood protozoan parasitic prevalence with respect to herd size showed comparatively high among >10 herd but the distribution wasn't significant. On the other hand, Jersey and Local were infected equally but high prevalence was recorded in cross breeds. Cattle with good condition were found to be highly infected by blood protozoan parasites. This clearify that the infection of blood parasite among the breed and body condition was not statistically significant. Ramgram municipality covers almost 19 wards. Most of the samples were taken from Ramgram. Maximum no. of cattle in Ramgram municipality was found to be highly infected. Sukrauli (10) and Amraut (14) were found equally infected but less in Banjariya (15). Prevalence of blood parasites in these localities was found statistically insignificant association.

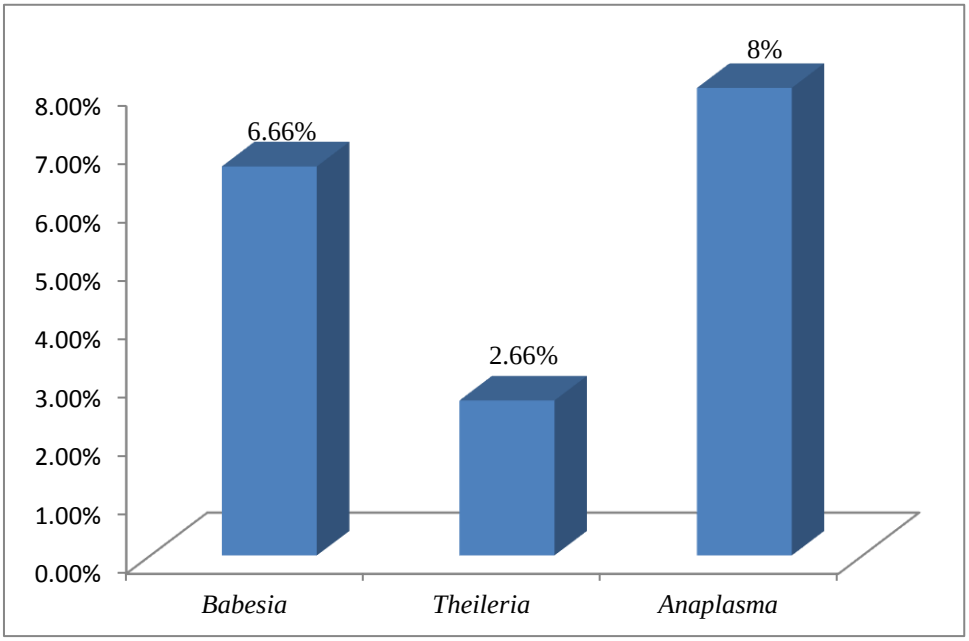

Fig1: Prevalence of blood parasites of cattle of Ramgram municipality in Nawalparasi.

Out of 150 blood samples examined in the Ramgram municipality of Parasi revealed three species of blood parasites infecting cattle. They were highly infected by anaplasmosis. The infection of *Babesia* sp. was comparatively less than *Anaplasma* sp. and prevalence of *Theileria* sp. was found lowest among the cattle.

4.2 Prevalence of babesiosis, theileriosis & anaplasmosis in Ramgram municipality, Nawalparasi District.

Babesiosis: Babesiosis is malaria like infection of cattle and other livestock in the disease of cattle and other livestocks. The parasite feed on RBC and destroys it, which causes passage of red or blackish urine causing Red water fever. The primary *Babesia* sp. that infects cattle includes *B. divergence*, *B. bigmina*, *B. bovis* and *B.major*.

Table2: Prevalence of babesiosis in cattle of Ramgram municipality in Nawalparasi.

Parameter		Prevalence rate	**χ2**	**P value**
Sex	Male	(4) 8.88%	0.12755	**0.72**
	Female	(6) 5.71%		
Age	0-4	(3) 4.615%	1.3518	**0.5087**
	4-8	(5) 7.247%		
	8 above	(2) 12.5%		
Herd size	<10	(7) 6.08%	0.0166	**0.8974**
	>10	(3) 8.57%		
Body condition	Good	(6) 7.31%	0.00048	**0.9825**
	Poor	(4) 5.88%		
Breed	Local	(4) 10%	2.668	**0.4457**
	Jersey	(1) 3.125%		
	Holstein	(3) 10.71		
	Crossbreed	(2) 4%		

Babesiosis infection was found comparatively high in male cattle of 8 years or above. Although the age and sex wise statistical association wasn't significant. Most of the cattle over the 10 herd size were found highly infected as compared to less than 10 herd size with insignificant relationship. The infection of babesiosis and body condition also have shown to be not significant Holstein were found highly infected as compared to local and Jersey.The relationship between breed and blood parasite showed insignificantly associated.

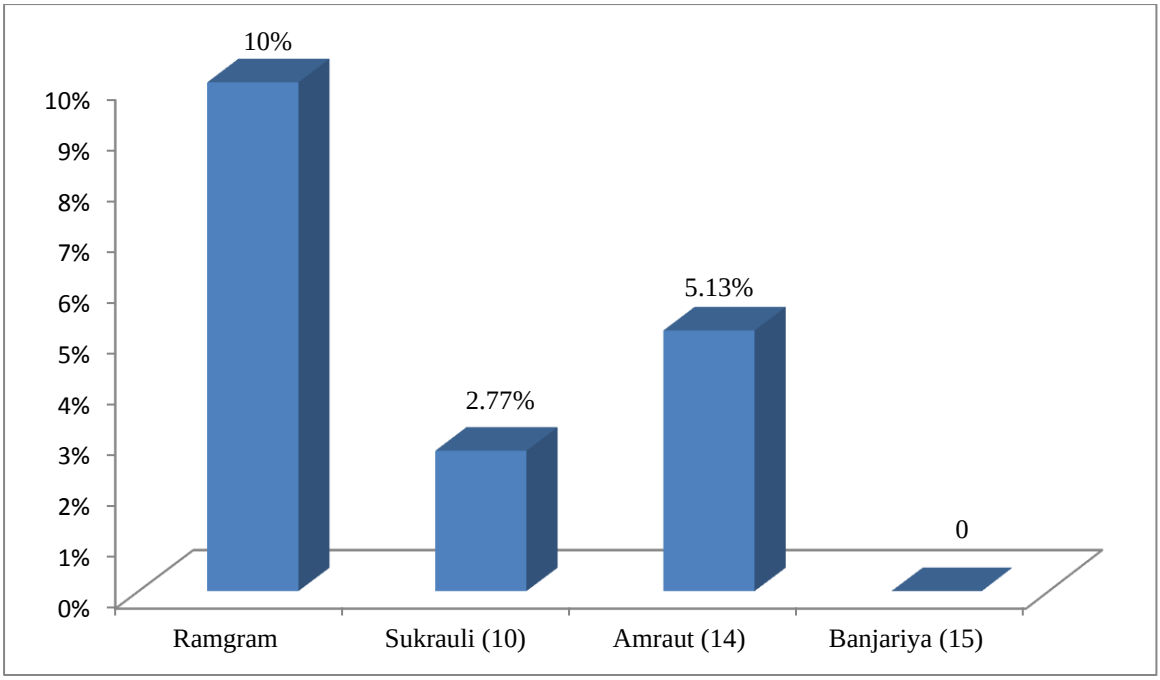

Fig2: Prevalence of Babesiosis of different locality of Ramgram municipality in Nawalparasi

The *Babesia* sp. infection was slightly variable in different localities in Ramgram municipality. Haemoprotozoan parasitic infection in Sukrauli (10) was found comparatively less while none of the babesiosis infection was found in Banjariya (15). The prevalence of babesiosis in Ramgram municipality was statistically not significant. ($P=0.3374$, $\chi 2 = 3.3749$, $df=3$)

Theleriosis*:* As *Babesia* sp., *Theileria* sp. parasites also infect the RBC of large no. of ruminants. It is one of the most economically devastating diseases of livestock all over the world. *Theileria* sp. parasites enter the bovine host during blood feeding by infective tick vectors, which rapidly invade mononuclear leukocytes.

Table3: Prevalence of theileriosis in cattle of Ramgram municipality in Nawalparasi.

Parameter		Prevalence rate	$\chi 2$	P value
sex	Male	0	0.5993	**0.43**
	Female	4%		
Age	0-4	(1) 1.538%	1.50	**0.47**
	4-8	(3) 4.347%		
	8 above	0		
Herd size	<10	(3) 2.608%	4.6254e-31	**1**
	>10	(1) 2.85%		
Body condition	Good	(1) 1.219%	0.488	**0.4845**
	Poor	(3) 4.411%		
Breed	Local	(1) 2.5%	1.1398	**0.7675**
	Jersey	(1) 3.125%		
	Crossbreed	(2) 4%		
	Holstein	0		

Age and sex wise distribution of theileriosis showed that male cattle of age group above 8 years were found to be free from the disease. Although the higher prevalence was found in between age group 4-8 years, statistically age and sex wise distribution of the disease was found insignificant. Theileriosis infection wasn't found to be associated with herd size as well as body condition. Among the breed, the majority of crossbreed had theileriosis followed by Local and jersey. While Holstein cattle were free from theileriosis infection (table3).

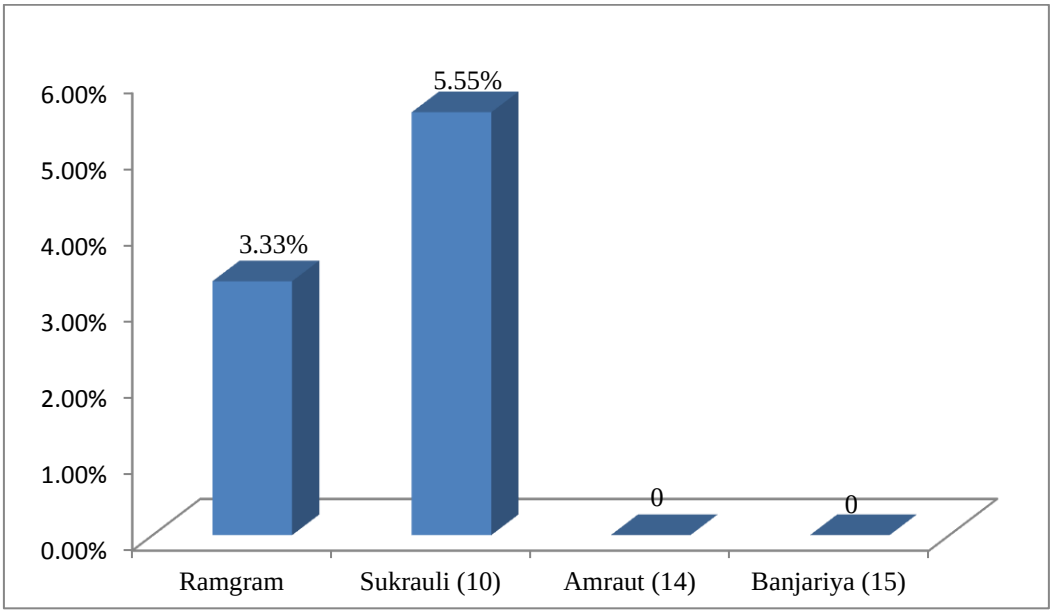

Fig3: Prevalence of theileriosis in different localities of Ramgram municipality in Nawalparasi.

The prevalence of *Theileria* infection varied in different localities of Ramgram municipality in Nawalparasi. Sukrauli VDC (10) had higher theileriosis infection was found, (5.55%) compared to Cattle of Ramgram municipality (3.33%). None of the theileriosis infection was recorded from Amraut (14) and Banjaria (15) with insignificant statistical association. ($\chi 2$=2.7397, p=0.4335).

Anaplasmosis*:* Anaplasmosis is an infectious cattle disease cause by a minute parasite (*Anaplasma marginale)* that causes destruction of red blood cells. The parasite can be transmitted from infected animals to healthy animals via insects or surgical instruments. Anaplasmosis characterized by anemia, increase heart rate, emaciation, blood in urine.

Table4: Prevalence of anaplasmosis in cattle of Ramgram municipality in Nawalparasi.

Parameter		Prevalence rate	**χ2**	**P value**
Sex	Male	(2) 4.44%	0.5219	**0.47**
	Female	(10) 9.523%		
Age	0-4	(4) 6.154%	0.7989	**0.6707**
	4-8	(7) 10.144%		
	8 above	(1) 6.25%		
Herd size	<10	(9) 7.826%	1.634e-30	**1**
	>10	(3) 8.571%		
Body condition	Good	(7) 8.536%	2.5115e-31	**1**
	Poor	(5) 7.353%		
Breed	Local	(1) 2.5%	4.3575	**0.2254**
	Jersey	(4)12.5%		
	Holistine	(1)3.571%		
	Crossbreed	(6) 12%		

Anaplasmosis infection was found comparatively high in female cattle of 4-8 years, although the age and sex wise analysis showed insignificant association. Similarly, In case of herd size and body condition, anaplasmosis infection was found almost similar range with insignificance association. Breed wise prevalence showed higher infection in Jersey as compared to other breeds, however this difference was found to be statistically not significant.

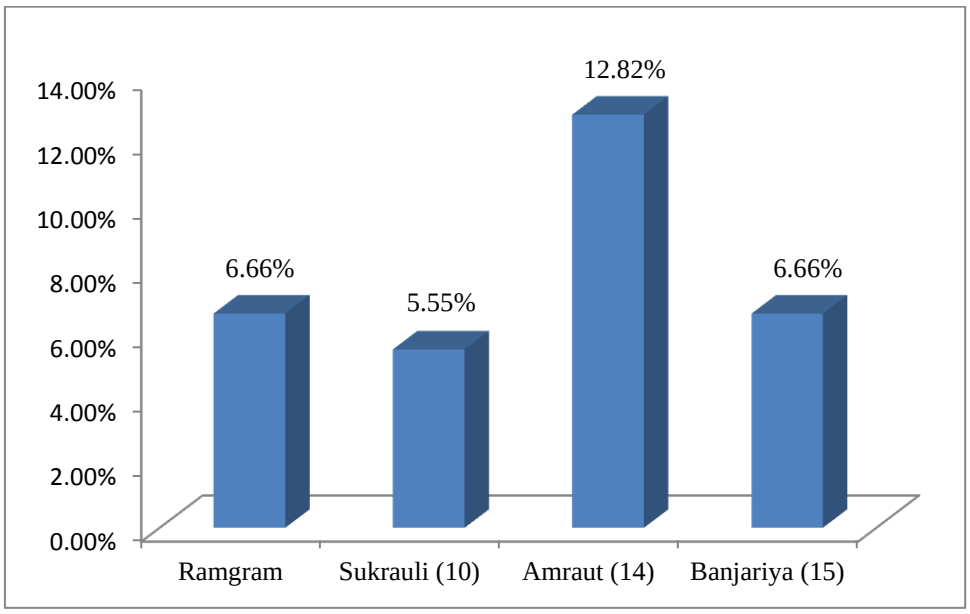

Fig4: Prevalence of anaplasmosis of cattle in different localitties of Ramgram municipality in Nawalparasi.

The above bar graph has depicted increase number of anaplasmosis in Amraut (14) whereas the Ramgram and Banjariya (15) were equally infected. On the other hand, less infection can be seen in Sukrauli (10). Ramgram municipality was less affected with anaplasmosis ($\chi 2$=1.7048 p=0.6359) with insignificant association.

Pre tested questionnaire were administered to 150 cattle farmers. Among them, most of the people (70%) didn't taken livestock farming training and few of them treated their cattle with deworming practice regularly. Out of total deworming cattle, 6 were found to be positive for haemoprotozoan parasites. This was statistically significant ($\chi 2$=30.38, P=3.648e-08). Among the total respondents, most of them (52%) told that they didn't use parasitoid. Awareness level of cattle farmers regarding blood parasites was low, which were considered irregular external parasitoid ($\chi 2$=5.9323, p=0.0148, df =1) showing notable difference between them. Similarly, the greater number of people (121) have knowledge deficit about blood parasite where only 29 out of 150 participants were noticed to be aware of blood parasite transmission. In the same way dramatic difference found in type of housing system where higher number of infected cattle found in open housing in comparison with closed housing. By contrast pond water was used by few farmers (only 40%) for their livestock. The similar Pattern was found for ground feeding (45%) system where greater numbers of cattle were fed via, Trough.

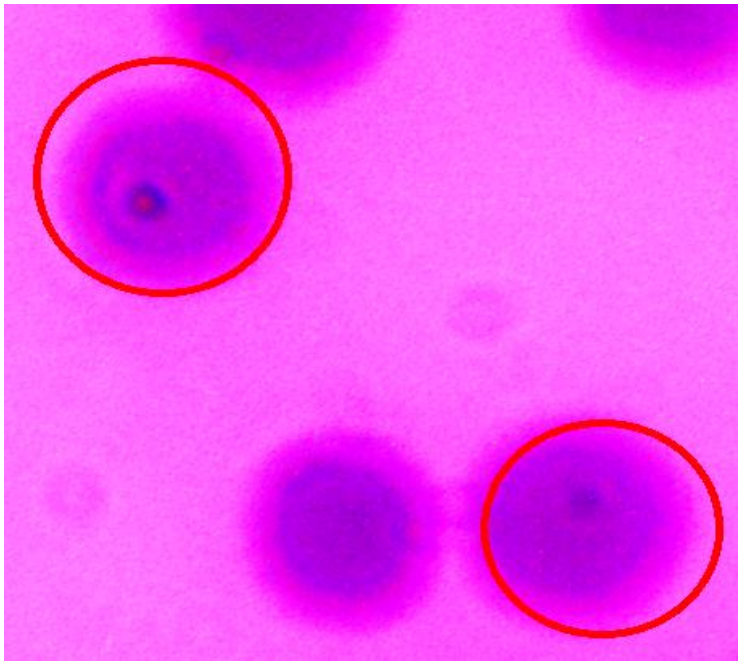

Photo5 Blood smears showing *Anaplasma* sp.

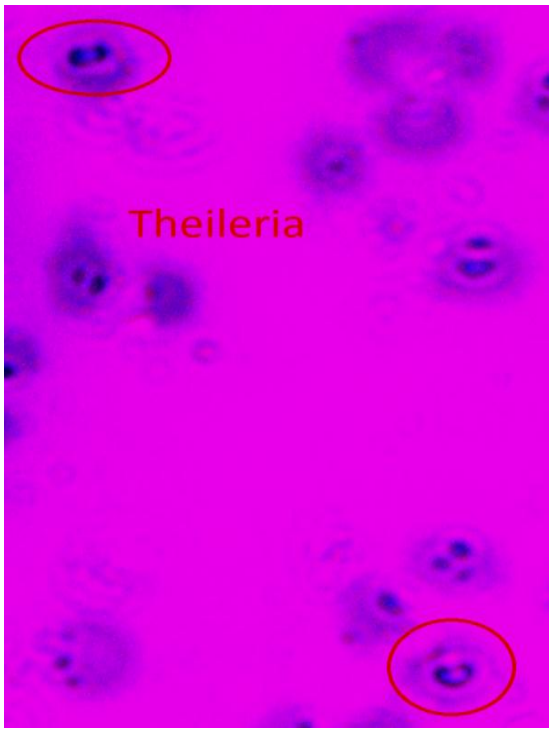

Photo 6 Blood smears showing *Theileria* sp.

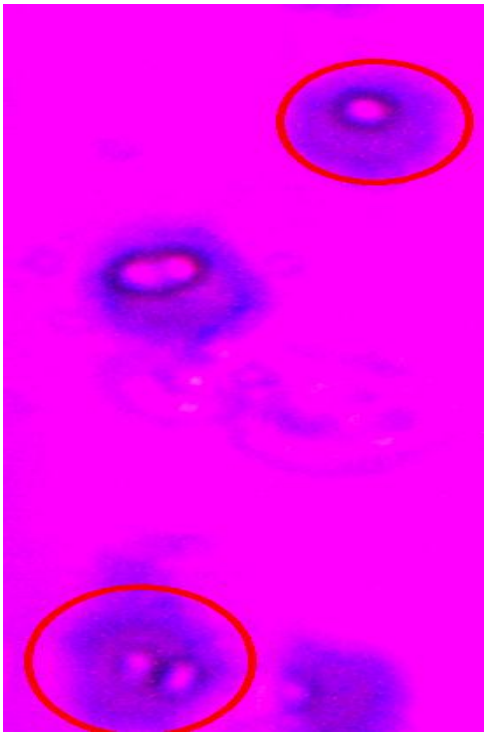

Photo 7 Blood smears showing *Babesia* sp.

5. DISCUSSION

Haemoprotozoan parasitic disease is the most common and infectious tick borne disease. Theileriosis, babesiosis and anaplasmosis are the most destructive diseases of our animal health and the biggest hinderness for successful production.

The present study revealed higher prevalence of blood parasitic protozoan parasites among the cattle of Ramgram municipality. Out of 150 cattle, 17.33% were found to be infected by haemoprotozoan parasites. The overall prevalence is almost similar to the findings of Mishra (2003) and Yadav (2015) who found the prevalence rate in cattle of Makwanpur and Siraha district as 17.5% and 19.23% respectively. Similar result was found by Adua and Idahor (2017) in Nigeria (20%), Velusamy *et al*. (2014) in Tamil nadu (16.66%) and Alim *et al*., (2012) in Bangladesh (16.18%). However the result is comparatively less than several other previous studies done by Sam Wobo *et al*., (2016) and Ugochukwa and Sydney (2014) in Nigeria which was 27.8% and 66% respectively, Tareq *et al*. (2016) in Iraq (86.5%), Chaudhry *et al*. (2013) in Hariyana (27.88%), Murthy *et al*. (2016) in Karnatak, India (43.3%). Similarly, very low prevalence i.e. 9% was reported from Rajasthan (Bhatnagar *et al*., 2015). Low prevalence among the cattle may be due to distribution and density of the reservoir host (Singh *et al*., 2000).The high rates of infection in those places may be due to the high abundance of tick vectors because high temperature and humidity is ideal for survival and breeding of ticks (Velusamy *et al*., 2014).

Sex wise analysis revealed prevalence rate was higher in female cattle (13.33%) compared to male cattle (4%) without significant association in prevalence of haemoprotozoan between two sexes. Similar finding have also been reported previously in Nigeria by Kamani *et al*. (2010), Ademola and Onyiche (2013), Paul *et al*. (2016), in Pakistan by Atif *et al*. (2012) and in Iraq by Tareq *et al*. (2016). This higher prevalence rate in female cattle might be due to the stress of breeding, milking and cyclical hormonal changes.

Regarding age wise prevalence maximum infection rate was found in between age group 4-8yrs (10%) followed by 0-4 (5.33%) and 8 year (2%). Similar finding has been recorded in Siraha by Yadav (2015), in Makwanpur of Nepal by Mishra (2003), in Nigeria by Ugochukwu and Sidney (2014), in India by Ananda *et al*. (2009). The cattle belonging to age group 4-8 years are physically active and do not have restriction of grazing in specific grazing ground but do not have passive colostrum immunity as in young cattle which might be the reason behind the higher prevalence rate observed in cattle of 4-8 years as compared to other age group.

Prevalence rate of haemoprotozoan parasite with respect to herd size was found to be higher among >10 herd size. This might be due to close association of infected cattle with healthy cattle as availability of host determines the transmission and distribution of the ticks and parasitic diseases.

The current study revealed that crossbreed cattle were highly infected with haemoprotozoan parasites (6.66%). The result coincides with study of Yadav (2015) and Mishra (2003) done in Siraha and Makwanpur district respectively. Similar result has also been reported from various parts of the world such as Tareq *et al.* (2016) Iraq, Kohli *et al*. (2014), Velusamy *et al*., (2014) and Bhatnagar *et a*l. (2015) from India. The reason behind the high prevalence of haemoprotozoan parasites in crossbreed cattle might be due to difference in innate and acquired immunity, animal husbandry and managemental practices (Velusamy *et al*., 2014; Bhatnagar *et al*., 2015).

On the basis of body condition, cattle were categorized into two group i.e. good and poor body condition. The prevalence was found to be higher among cattle having good body condition (9.33%) as compared to cattle having poor body condition (5.88%). However, the result was found to be insignificant. The reason behind this result might be due to subclinical infection of haemoprotozoan parasites in cattle with good body condition and also negligence of farmers in the care of cattle with good body condition. Locality wise prevalence of haemoprotozoan parasite was found to be high in Ramgram municipality (8%) in comparison to other localities. Most of the samples were collected from the Ramgram municipality furthermore the locality was covered with varieties of herbs and bushes which provides favorable environment for the vectors of haemoprotozoan parasites.

Babesiosis is a haemoparasitic tick-borne disease (TBD) caused by *Babesia bigemina* and *Babesia bovis* (Riek, 1964; Riek, 1966). The result in this study indicated that overall prevalence of 6.6% *Babesia* sp. was similar with finding of Keneth *et al*. (2014) in Uganda. The result of bovine babesiosis coincides with Yadav (2015) who reported 6.15% prevalence of *Babesia* species in Siraha district. Moreover, this finding was in approximate to the finding of Adua and Idor (2017) who recorded 7% prevalence of *Babesia bovis* from Nigeria. However, the finding was higher than that of the study done by Ugochukwa and Sydney (2014) with the prevalence rate 2.2% in Nigeria, 2.85% from Pakistan (Khan *et al*., 2004), 3.33% and 2.27% from Bangaladesh (Chowdhary *et al*., 2006; Abdullah *et al*., 2015) whereas lower than finding of Bihonegn *et al*. (2015) from Ethiopia 11.5%, Durani and kamal (2008) from Pakistan (42%) and Kariyappa *et al*. (2017) from India (14.94%). The discrepancy in the prevalence of bovine babesiosis might be due to different factors like management condition of the focus area, use of acaricides during tick infestation, farming system and proper use of antiparasitic drugs, fluctuations of parasites during chronic course of the disease and in carrier animals.

In the present study, higher prevalence rate of babesiosis was in male (8.88%) cattle as compared to female cattle (5.71%). Even though this difference was not statistically significant, this finding was in agreement with finding of Reda (2012) in Egypt. Similarly, Bihonegn *et al*. (2015) found higher prevalence of babesiosis in male (1.73%) as compared to female (1.32%) in Ethiopia. However, the result was in disagreement with the report of Methus (2017) from Namibia, Atif *et al*. (2012) from Pakistan, Tareq *et al*. (2016) from Iraq. The higher prevalence of tick borne diseases in male animals may be due to the fact that male animals are subjected for ploughing purposes, pulling

carts and stressful work that suppress the immune system of the animals (Bihonegn *et al*., 2015).

In the present study the highest prevalence of babesiosis was noted among cattle of age group above eight years of age (12.5%) followed by 4-8 (7.24%) and 0-4 (4.61%). This result is similar to the finding of Ayaz *et al*. (2013) from Pakistan who reported high prevalence in old animals (13.4%) followed by adult (11.7%) while the lowest being in young animals (5.5%). However, the results disagree with Yadav (2015) who identified that 4-8 age groups of cattle were more susceptible to *Babesia* species when compared to other groups. Cattle above eight years of age do not have passive colostrum immunity and absence of restricted grazing makes them susceptible to the haemoparasitic protozoan infection.

Prevalence of babesiosis was found to be higher among the cattles having >10 herd size which might be due to close association of infected cattle with healthy ones. The prevalence of the disease based on the body condition of the animals was higher for cattle with good body condition (7.31%) with insignificant differences. The reason behind the finding might be due to grazing of cattle together in the grasslands and overnight in the stalls which facilitate transfer of ticks within the herd.

No correlation ($p > 0.05$) was found between locality and presence of blood parasites. Cattle of Ramgram municipality were found to be more infected (10%) than Amraut (5.13%) and Sukrauli (2.77%) while none of the *Babesia* infection was found in Banjariya VDC. This might be due to collection of large no. of samples from Ramgram municipality.

The result of this study showed that 2.66% of the cattle during the study period were positive for theileriosis which is similar to that of Yadav (2015). The result is lower than that of Gupta *et al*. (2013), Mishra (2003) and Deo and Neupane (2002) who found 8.75%, 8.75% and 10.18% prevalence rate of theileriosis from the cattle of Sunsari, Makwanpur and Morang respectively. Similarly, the result is also lower than that of Bhatnagar *et al*. (2015), Maharana *et al*. (2016), Kumar *et al.,* (2015) and Kohli *et al*. (2014) who reported prevalence rate of 42.26%, 7.08%, 9.35% and 45.5% respectively from cattle of India. The rate of infection obtained in this study can be considered to be low with lower occurrence of vector *Hyalomma* sp. in this geographical part of the country. This may be due to regular use of chemical control program on animals and cattle shed which reduced the tick population under field condition.

Sex wise analysis showed 4% female cattle were infected by theileriosis while theileriosis infection was not found in male cattle. This is accordance with Gupta *et al*. (2013) who reported higher rate of infection in female than male in Eastern Terai. Similar reports has been found by Atif *et al*. (2012) from Pakistan, Tareq *et al*. (2016) in Iraq, Naik *et al*. (2016) in India. Higher prevalence in female cattle than male cattle might be due to the stress of breeding, milking and cyclic hormonal changes in female cattle.

The theileriosis infection in cattle of age group 4-8 years was higher (4.34%) than 0-4 years (1.53%) while cattle of age group above 8 years were found to be free from the disease. In the current study, higher susceptibility of adult cattle to theileriosis is found consistent with the findings of Yadav (2015) who reported higher prevalence in cattle with the age of 4-8 years in Siraha. The result is also supported by Naik *et al*. (2016) in India, Abdul *et al*. (2014) in Malaysia but the finding disagree with Atif *et al*. (2012) and Tareq *et al.* (2016). It is a well-known fact that handling of calves for nursing is easier to farmers than handling adult animals. Therefore, it is possible that farmers in this study were able to de-tick calves rather than the adult cattle.

Prevalence of theileriosis in cross breed cattle was higher than Jersey, Local and Holstein. This result is in accordance with Yadav (2015) in Siraha, Tareq *et al*. (2016) in Iraq, Atif *et al*. (2012) in Pakistan, Naik *et al*. (2016) and Velusamy *et al*. (2014) in India. This might be due to unfavorable temperature and environment for crossbreed cattle.

Anaplasmosis is the most prevalent haemo rickettsial disease of cattle in Nepal (Adhikari *et al.* 1997). Anaplasmaosis prevalence was high among the haemoprotozoan parasites in cattle of Parasi area with overall prevalence of 8%. This result was similar to the finding of Mishra (2003) who reported 9.5% prevalence in Makwanpur. Similar finding has been reported (6.1%) from Nigeria (Ekici and Ferda, 2011; Adua and Idor, 2017), 9.71% from Pakistan (Atif *et al*., 2012). However, the prevalence of anaplasmosis in this study is higher than the prevalence rate reported by Yadav (2015) from Siraha and Bohara and Shrestha (2016) from Mid-western Terai which was 3.07%, 5.8% respectively. Moreover, this result was much lower than earlier reports 79% from Paraguay (Payne *et al*., 1990), 70% from Bangladesh (Chowdhary *et al*., 2006). The differences in geographical location, presence and spread of competent vector could actually have played a significant role in these differences (Ntonifor *et al*., 2013).

Anaplasma sp. can cause infections in bovine population of all age categories where severity and mortality rate increases with augmentation of animal age (Richey, 1984). Age wise prevalence of anaplasmosis was higher in cattle of age group 4-8 years (10.144%) while low prevalence rate (6.15%) at the age of 0-4 years. The findings of present study agree with Yadav (2015) who found similar trend of infection rate of 1.9% among the age group 4-8 years. Similar findings were reported by Chowdhary *et al*. (2006) from Bangaledesh, Maharana *et al*. (2016) from India. Both young and adult animals usually develop only a mild form of the disease although various stress factors which can exacerbate this in individual cases (Stoltsz, 1994).

Regarding sex-wise distribution, a higher anaplasmosis infection rate in females (9.53%) as compared to male (4.44%) cattle had been recorded in the present study which agrees with Kispotta *et al*. (2016) from Bangladesh, Atif *et al*. (2012) from Pakistan. However, the results of this study disagree with Tareq *et al*. (2016) who reported 22.9% male were infected with anaplasmosis as compared to (14.4%) female cattle. The higher prevalence of tick borne diseases in female animals may be due to the

fact that female animals are kept longer for breeding and milk production purposes (Kamani *et al*., 2010). On other hand higher prevalence in female animals might be due to hormonal disturbances due to its use in milk production and breeding system which lowers the immune system of the animal (Atif *et al*., 2012).

Prevalence rate of anaplasmosis was found to be higher in cattle of age group 4-8 years (10.44%) while lowest prevalence was found to be at the age group 0-4 years (6.12%).The finding of present study agrees with Yadav (2015) who found higher infection rate among the age group 4-8 years (1.29%) and lower prevalence rate (0.32%) among the age of 0-4 years. This is due to inverse age resistance and stronger passively acquired immunity might be contributing factors behind the lower prevalence of *Anaplasma* sp. in young age group.

Prevalence of anaplasmosis was slightly higher in cattle with good body condition (8.53%) than those with poor body condition (7.35%) with no significance difference. The result deviates with finding of Abdela *et al*. (2017) where poor body condition of cattle had more infection than those with good body condition. This shows that good and poor body conditions of cattle are susceptible to infection with anaplasmosis in those areas where the disease vectors are endemic. Breed susceptibility of anaplasmosis recorded in this study supports the report of Yadav (2015) and Shrestha and Singh (1999). Prevalence of anaplasmosis was found to be higher in Jersey followed by crossbreed, local and holistiene breed cattle with insignificance difference.

In the present study, the highest prevalence of anaplasmosis was recorded in Amraut VDC (12.82%) among the study areas considered for this study. The possible explanation for this might be associated with that Amraut VDC (14) was near to forests and bushes which is believed to be the most suitable habitat for the vector of the *Anaplasma* sp.

Regarding to other practices livestock farming training, regular deworming practices, use of external parasitoid and knowledge of tick transmitted disease were significantly associated with blood parasite. There was lack of awareness among the people regarding the haemoprotozoan parasites and the mode of transmission of these diseases. Cattle in open housing system were found to be more infected with haemoprotozoan parasites than those cattle of closed housing system.

6. CONCLUSIONS AND RECOMMENDATIONS

The present study provides basic information on the prevalence and risk factors associated with occurrence of blood protozoan disease in cattle in and around Ramgram municipality. The study revealed the overall prevalence of blood protozoan parasite was 17.33% by using Giemsa staining technique. The identified parasites were *Anaplasma* sp. *Babesia* sp. and *Theileria* sp. Among the three species maximum prevalence was with anaplasmosis (8%). Prevalence of age related haemoprotozoan parasites showed that 4-8 yrs ages of cattle were more infected. Although female were found to be more infected than male which was not statistically significant. Similarly, herd size as well as body condition of cattle were also found to be insignificantly associated with haemoprotozoan parasites. Breed wise prevalence with respect to crossbreed cattle were more infected in >10 herd size.

Babesiosis infections in male cattle with the age of 8 years above were found to be highly prevalent although theileriosis and anaplasmosis were high in female cattle of 4-8 years age group. In case of body condition, Anaplasmosis and babesiosis were more prevalent in good body condition whereas cattle of poor body condition were found to be more infected by theileriosis. Similarly crossbreed holeistine and Jersey cattle were more infected by theileriosis, babesiosis and anaplasmosis respectively. All three species were more prominent in >10 herd size. Distribution of blood parasitic infection found to be varied in different localities. Cattle of Ramgram municipality were highly infected by babesiosis similarly cattle of Amraut (14) and Banjaria (15) were infected by anaplasmosis and theileriosis.

With response to knowledge, attitude and practice most of the farmers didn't taken livestock farming training and few of them had deworming practice regularly. Awareness level of cattle farmers regarding blood parasites was low. Most of the people have knowledge deficit about blood parasite. In the same way dramatic difference found in type of housing system where higher number of infected cattle found in open housing in comparison with closed housing.

Recommendations

• Seroprevalence study of blood protozoan should be carried out for the accurate identification of the subclinical or carrier status of blood protozoan parasites in cattle.

• Surveillance and monitoring programme for blood protozoan infection in cattle should be carried out regularly in the field.

• All individuals working with cattle should be provided the knowledge about proper hygiene, husbandry practice and use of antiparasitic drugs.

• Effective quarantine law for exotic cattle should be implemented.

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ANNEX-1

List of blood samples collected from the Ramgram municipality

S.N	Name of the owner	Address	Animal Species	Age	Sex	Herd Size	Body condition	Knowledge of haemoprotozoan	Result of microscopic Examination	Remarks
1	Dal Singhar Yadav	Ramgram 4	Cattle	2 yrs	F	4	Poor	No	Negative	Ramgram 4
2	Rajendra Harijan	Ramgram 6	Cattle	7 yrs	F	2	Poor	No	Negative	Ramgram 6
3	Lolai Yadav	Ramgram 2	Cattle	3yrs	F	3	Good	No	*Anaplasma* spp.	Ramgram 2
4	Shambhu Yadav	Ramgram 10	Cattle	6 yrs	F	2	Poor	Yes	*Theileria* spp.	Sukrauli 7
5	Nagendra Upadhyay	Ramgram 2	Cattle	4 yrs	F	5	Good	No	Negative	Ramgram 2
6	Rabindra Chaudhary	Ramgram 6	Cattle	5.5 yrs	M	2	Poor	No	Negative	Ramgram 6
7	Santaram Yadav	Ramgra 14	Cattle	3 yrs	M	2	Poor	Yes	*Babesia* spp.	Amraut 4
8	Ram Prasad Pandey	Ramgram 6	Cattle	7 yrs	F	150	Good	No	Negative	Ramgram 6
9	Ram Prasad Pandey	Ramgram 14	Cattle	9 yrs	F	150	Poor	No	Negative	Amraut 8
10	Ram Prasad Pandey	Ramgram 4	Cattle	4 yrs	F	150	Poor	No	Negative	Ramgram 4
11	Ram Prasad Pandey	Ramgram 10	Cattle	10 yrs	F	150	Poor	No	Negative	Sukrauli 7
12	Narayan Paudel	Ramgram 9	Cattle	9 yrs	F	7	Good	No	Negative	Ramgram 9
13	Nim Prasad Neupane	Ramgram 14	Cattle	4 yrs	F	5	Good	No	Negative	Amraut 3
14	Rambriksh Kewat	Ramgram 8	Cattle	8 yrs	F	2	Poor	No	Negative	Ramgram 8
15	Rishi Chaudhary	Ramgram 1	Cattle	7 yrs	F	7	Poor	No	Negative	Ramgram 1
16	Firaj Ansari	Ramgram 10	Cattle	5.5 yrs	M	3	Poor	No	Negative	Sukrauli 7
17	Sudama Kewat	Ramgram 10	Cattle	2 yrs	M	2	Good	No	Negative	Sukrauli 9
18	Jayaram Sahani	Ramgram 4	Cattle	9 yrs	F	1	Good	No	Negative	Ramgram 4
19	Shambhu Harijan	Ramgram 7	Cattle	6.5 yrs	M	3	Poor	No	*Anaplasma* spp.	Ramgram 7
20	Kedar Harijan	Ramgram 8	Cattle	3.5 yrs	F	4	Good	No	Negative	Ramgram 8
21	Dharma Raj Yadav	Ramgram 10	Cattle	6 yrs	F	6	Poor	No	Negative	Sukrauli 9
22	Chulhai Yadav	Ramgram 7	Cattle	3.5 yrs	M	3	Good	No	Negative	Ramgram 7
23	Surendra Kunwar	Ramgram 14	Cattle	7 yrs	F	2	Poor	No	Negative	Amraut 9
24	Daktar Chaudhary	Ramgram 14	Cattle	7.5 yrs	F	1	Good	No	Negative	Amraut 8
25	Brij Bihari Chaudhary	Ramgram 10	Cattle	3 yrs	M	1	Poor	No	Negative	Sukrauli 9
26	Komal Yadav	Ramgram 14	Cattle	11 yrs	F	2	Good	No	Negative	Amraut 5
27	Arjun Yadav	Ramgram 14	Cattle	3.5 yrs	M	3	Good	No	Negative	Amraut 2
28	Madhav Chaudhary	Ramgram 14	Cattle	10 yrs	M	2	Poor	No	Negative	Amraut 5
29	Mahendra Yadav	Ramgram 10	Cattle	2.5 yrs	M	3	Poor	No	Negative	Sukrauli 9
30	Kashiram Neupane	Ramgram 14	Cattle	8 yrs	F	6	Poor	No	*Anaplasma* spp.	Amraut 7
31	Sukmaya B K	Ramgram 9	Cattle	2 yrs	M	3	Good	No	Negative	Ramgram 9
32	Dipak Thapa	Ramgram 14	Cattle	10 yrs	M	4	Good	No	Negative	Amraut 5
33	Omprakash Neupane	Ramgram 10	Cattle	11 yrs	F	6	Poor	No	Negative	Sukrauli 8
34	Ramnath Chaudhary	Ramgram 12	Cattle	2.5yrs	M	3	Poor	No	Negative	Ramgram 12
35	Fekanidevi Chaudhary	Ramgram 13	Cattle	7.5 yrs	F	2	Poor	No	Negative	Ramgram 13
36	Ramesh Yadav	Ramgram 6	Cattle	4 yrs	M	4	Good	No	*Babesia* spp.	Ramgram 6

37	Shrikrishna Neupane	Ramgram 14	Cattle	7 yrs	F	10	Good	No	Negative	Amraut 4
38	Shrikrishna Neupane	Ramgram 14	Cattle	3 yrs	F	10	Poor	No	Negative	Amraut 4
39	Saraswati Acharya	Ramgram 14	Cattle	3 yrs	F	5	Poor	No	Negative	Amraut 3
40	Ram Bahadur Kshetri	Ramgram 14	Cattle	6 yrs	F	12	Poor	No	Negative	Amraut 3
41	Laxmanjung Hamal	Ramgram 14	Cattle	2 yrs	F	21	Poor	No	Negative	Amraut 3
42	Kamal Dhakal	Ramgram 14	Cattle	6 yrs	F	9	Good	No	Negative	Amraut 4
43	Laxmanjung Hamal	Ramgram 14	Cattle	8 yrs	F	21	Poor	Yes	*Babesia* spp.	Amrau 4
44	Brij Lal Koiri	Ramgram 15	Cattle	5.5 yrs	F	6	Good	No	Negative	Banjariya 2
45	Jagadish Pathak	Ramgram 10	Cattle	4.5 yrs	F	4	Good	No	Negative	Sukrauli 8
46	Haripal Yadav	Ramgram 14	Cattle	5.5 yrs	M	2	Poor	No	Negative	Amraut 4
47	Ramabatar Kewat	Ramgram 15	Cattle	7 yrs	M	4	Poor	No	Negative	Banjariya 3
48	Ram Mangal Ahir	ramgram 9	Cattle	4 yrs	M	2	Good	No	Negative	Ramgram 9
49	Maniram Kewat	Ramgram 6	Cattle	6 yrs	F	3	Poor	No	*Anaplasma* spp.	Ramgram 6
50	RamNaran Chaudhary	Ramgram 12	Cattle	8 yrs	M	3	Good	No	Negative	Ramgram 12
51	Ramprit Konhar	Ramgram 14	Cattle	2 yrs	F	4	Poor	No	Negative	Amraut 3
52	Pujari Yadav	Ramgram 5	Cattle	1.5 yrs	F	6	Poor	No	Negative	Ramgram 5
53	Jaitun Nesa	Ramgram 6	Cattle	7 yrs	M	4	Good	No	*Babesia* spp.	Ramgram 6
54	Jayaram Sahani	Ramgram 10	Cattle	5.5 yrs	M	2	Good	No	Negative	Sukrauli 8
55	Mantira Launiya	Ramgram 14	Cattle	6 yrs	M	3	Good	No	Negative	Amraut 3
56	Rohit Sahani	Ramgram 6	Cattle	3 yrs	F	5	Good	No	Negative	Ramgram 6
57	Bishal Kumar Gupta	Ramgram 12	Cattle	7 yrs	F	4	Good	Yes	*Anaplasma* spp.	Ramgram 12
58	Bibek Kumar Gupta	Ramgram 10	Cattle	5.5 yrs	F	6	Good	No	Negative	Sukrauli 7
59	Tirthraj Koiri	Ramgra 14	Cattle	3.5 yrs	F	3	Good	No	Negative	Amraut 8
60	Punarbasi Gupta	Ramgram 8	Cattle	7 yrs	M	4	Poor	No	Negative	Ramgram 8
61	Ramananda Yadav	Ramgram 14	Cattle	2.5 yrs	M	4	Poor	No	Negative	Amraut 6
62	Jagadish Sahani	Ramgram 6	Cattle	6.5 yrs	F	4	Poor	No	*Babesia* spp.	Ramgram 6
63	Amar Patel	Ramgram 15	Cattle	5.5 yrs	M	2	Good	No	Negative	Banjariya 2
64	Makkhan Yadav	Ramgram 12	Cattle	3 yrs	F	8	Poor	No	Negative	Ramgram 12
65	Bishnu Bhattarai	Ramgram 10	Cattle	4 yrs	F	12	Poor	No	Negative	Sukrauli 9
66	Niraj Prajapati	Ramgram 4	Cattle	6.5 yrs	M	5	Poor	No	Negative	Ramgram 4
67	Sunil K yadav	Ramgram 14	Cattle	7 yrs	F	9	Good	No	*Babesia* spp.	Amraut 4
68	Sanjay Yadav	Ramgram 15	Cattle	4 yrs	F	8	Good	No	Negative	Banjariya 3
69	Rambahadur Yadav	Ramgram 10	Cattle	3.5 yrs	F	4	Good	No	Negative	Sukrauli 9
70	Aakash Dharikar	Ramgram 15	Cattle	6 yrs	F	1	Poor	No	Negative	Banjariya 3
71	Bhairav Koirala	Ramgram 10	Cattle	4.5 yrs	F	3	Poor	No	Negative	Sukrauli 9
72	Rajmangal Yadav	Ramgram 2	Cattle	2.5 yrs	F	6	Poor	No	Negative	Ramgram 2
73	Samyog Kharel	Ramgram 14	Cattle	7.5 yrs	F	7	Poor	No	Negative	Amraut 6
74	Mallu Ansari	Ramgram 6	Cattle	3 yrs	M	4	Poor	No	Negative	Ramgram 6
75	Ramcharan Konhar	Ramgram 14	Cattle	6 yrs	M	4	Poor	No	Negative	Amraut 7
76	Prayog Yadav	Ramgra 10	Cattle	1 .5yrs	F	6	Good	No	Negative	Sukrauli 8
77	Budharan Chaudhary	Ramgram 14	Cattle	7 yrs	M	5	Good	No	Negative	Amraut 3
78	Jagamanta Sahani	Ramgram 15	Cattle	7 yrs	M	3	Poor	No	Negative	Banjariya 3

79	Ramchandra Gupta	Ramgram 14	Cattle	2.5 yrs	F	4	Poor	No	Negative	Amraut 1
80	Shivnarayan Yadav	Ramgram 10	Cattle	6 yrs	F	8	Poor	No	*Anaplasma* spp.	Sukrauli 9
81	Ramdev Dhawal	Ramgram 6	Cattle	3 yrs	M	3	Poor	No	Negative	Ramgram 6
82	Ramsewak Agrahari	Ramgram 10	Cattle	5.5 yrs	F	11	Poor	No	Negative	Sukrauli 8
83	Santosh Thapa	Ramgram 8	Cattle	3 yrs	F	7	Poor	No	Negative	Ramgram 8
84	Hareram Yadav	Ramgram 5	Cattle	7 yrs	F	9	Good	No	Negative	Ramgram 5
85	Om Prakash Chaudhary	Ramgram 10	Cattle	8 yrs	M	4	Good	Yes	Negative	Sukrauli 7
86	Dipak Yadav	Ramgram 10	Cattle	3.5 yrs	F	6	Poor	No	Negative	Sukrauli 7
87	Sarada Dhakal	Ramgram 14	Cattle	7.5 yrs	F	7	Poor	Yes	Negative	Amraut 5
88	Padam Narayan Chaudhary	Ramgram 4	Cattle	4 yrs	M	4	Good	Yes	Negative	Ramgram 4
89	Mahendra Yadav	Ramgram 15	Cattle	12 yrs	F	5	Poor	No	Negative	Banjariya 3
90	Dipak Aryal	Ramgram 10	Cattle	8 yrs	F	7	Poor	No	*Theileria* spp.	Saukrauli 9
91	Bebi Baniya	Ramgram 12	Cattle	6 yrs	F	7	Good	Yes	Negative	Ramgram 12
92	Dip Chandra Yadav	Ramgram 12	Cattle	3 yrs	M	3	Good	No	Negative	Ramgram 12
93	Raj Kumari Yadav	Ramgram 10	Cattle	6 yrs	F	2	Poor	No	Negative	Sukrauli 7
94	Mohan Kewat	Ramgram 14	Cattle	3.5 yrs	M	4	Poor	Yes	Negative	Amraut 9
95	Dhan Bahadur Yadav	Ramgram 15	Cattle	7 yrs	F	3	Poor	Yes	Negative	Banjariya 3
96	Ramkewal Agrahari	Ramgram 6	Cattle	6 yrs	M	2	Poor	No	Negative	Ramgram 6
97	Birbal Yadav	Ramgram 9	Cattle	4 yrs	F	6	Good	Yes	Negative	Ramgram 9
98	Madan Koirala	Ramgram 10	Cattle	8 yrs	F	4	Good	Yes	Negative	Sukrauli 8
99	Rahamad Ali Hussain	Ramgram 8	Cattle	3 yrs	M	2	Good	No	Negative	Ramgram 8
100	Pradip Yadav	Ramgram 9	Cattle	6 yrs	F	1	Poor	No	Negative	Ramgram 9
101	Sanjay Yadav	Ramgram 12	Cattle	7.5 yrs	F	3	Poor	No	Negative	Ramgram 12
102	Chhedi Gupta	Ramgram 10	Cattle	2.5 yrs	M	4	Poor	No	Negative	Sukrauli 9
103	Ramesh Singh	Ramgram 9	Cattle	7 yrs	F	8	Poor	Yes	Negative	Ramgram 9
104	Tikaram Bhandari	Ramgram 2	Cattle	2 yrs	F	12	Good	Yes	Negative	Ramgram 2
105	Ujari Ansari	Ramgram 5	Cattle	5.5 yrs	M	2	Poor	No	Negative	Ramgram 5
106	Maniram Konhar	Ramgram 6	Cattle	4 yrs	M	3	Poor	No	Negative	Ramgram 6
107	Brijlal Koiri	Ramgram 15	Cattle	6 yrs	M	5	Good	Yes	Negative	Banjariya 2
108	Tek Bahadur Karki	Ramgram 10	Cattle	3 yrs	F	2	Good	Yes	Negative	Sukrauli 9
109	Pradip Lal Yadav	Ramgram 14	Cattle	3.5 yrs	M	7	Poor	No	Negative	Amraut 3
110	Kamalabati Kewat	Ramgram 14	Cattle	4.5 yrs	F	3	Good	No	*Anaplasma* spp.	Amraut 4
111	Ali Hussain Dhuniya	Ramgram 14	Cattle	9 yrs	F	1	Good	Yes	Negative	Amraut 4
112	Budh Narayan Chaudhary	Ramgram 10	Cattle	7 yrs	F	1	Good	No	Negative	Sukrauli 7
113	Kalpana Harijan	Ramgram 15	Cattle	9 yrs	F	2	Poor	Yes	Negative	Banjariya 3
114	Shyam Raj Yadav	Ramgram 10	Cattle	3 yrs	M	3	Good	No	Negative	Sukrauli 9
115	Anwar Ali	Ramgram 4	Cattle	7 yrs	F	2	Good	No	Negative	Ramgram 4
116	Sima Beldar	Ramgram 13	Cattle	6 yrs	M	4	Good	No	Negative	Ramgram 13
117	Kamala Adhikari	Ramgram 4	Cattle	5.5 yrs	F	2	Good	Yes	Negative	Ramgram 13
118	Ramlal Malla	Ramgram 10	Cattle	3.5 yrs	F	16	Good	No	Negative	Sukrauli 9

119	Prem Kumar Malla	Ramgram 15	Cattle	6 yrs	F	16	Good	No	Negative	Banjariya 2
120	Raj kumar malla	Ramgram 14	Cattle	8 yrs	F	16	Good	No	Negative	Amraut 6
121	Kisna malla	Ramgram 8	Cattle	3.5 yrs	F	16	Good	No	*Theileria* spp.	Ramgram 8
122	Bhagauti Yadav	Ramgram 10	Cattle	8.5 yrs	F	12	Good	No	*Babesia* spp.	Sukrauli 9
123	Bhagauti Yadav	Ramgram 15	Cattle	6 yrs	F	12	Good	Yes	Negative	Banjariya 3
124	Padma Aryal	Ramgram2	Cattle	3 yrs	F	6	Good	Yes	Negative	Ramgram 2
125	Balaram Adhikari	Ramgram 10	Cattle	6.5yrs	F	4	Good	No	Negative	Sukrauli 8
126	Jivannath Chapagain	Ramgram 14	Cattle	3 yrs	F	6	Good	No	Negative	Amraut 5
127	Chandan Pandey	Ramgram 4	Cattle	5.5 yrs	F	7	Good	Yes	Negative	Ramgram 4
128	Hareram shrestha	Ramgram 1	Cattle	3.5 yrs	F	86	Good	No	Negative	Ramgram 1
129	Hari Narayan Shrestha	Ramgram 10	Cattle	6 yrs	F	86	Good	No	Negative	Sukrauli 8
130	Narayan shrestha	Ramgram 15	Cattle	4 yrs	F	86	Good	No	*Anaplasma* spp.	Banjariya 4
131	Ramlal Shrestha	Ramgram 10	Cattle	7 yrs	F	86	Good	No	*Anaplasma* spp.	Sukrauli 7
132	Paras Shrestha	Ramgram 1	Cattle	2.5 yrs	F	86	Good	Yes	Negative	Ramgram 1
133	Gopal Neupane	Ramgram 11	Cattle	10 yrs	F	5	Good	No	*Babesia* spp.	Ramgram 11
134	Devraj Bhandari	Ramgram 15	Cattle	4 yrs	F	11	Good	No	Negative	Banjaria 2
135	Bal Krishna Adhikari	Ramgram 15	Cattle	1.5 yrs	F	17	Good	Yes	Negative	Banjariya 3
136	Soraj Sapkota	Ramgram 14	Cattle	10 yrs	F	21	Good	Yes	Negative	Amraut 2
137	Soraj Sapkota	Ramgram 10	Cattle	3.5 yrs	F	21	Good	No	*Anaplasma* spp.	Sukrauli 9
138	Krishna Bahadur Khatri	Ramgram 1	Cattle	13 yrs	F	14	Poor	No	*Babesia* spp.	Ramgram 1
139	Dipendra Chaudhary	Ramgram 14	Cattle	3.5 yrs	F	13	Good	Yes	Negative	Amraut 2
140	Dipendra Chaudhary	Ramgram 10	Cattle	2.5 yrs	F	13	Good	Yes	Negative	Sukrauli 7
141	Dipendra Chaudhary	Ramgra 14	Cattle	7 yrs	F	13	Good	No	Negative	Amraut 7
142	Punam Chaudhary	Ramgram 12	Cattle	2.5 yrs	M	2	Good	No	*Babesia* spp.	Ramgram 12
143	Rukmagat Neupane	Ramgram 10	Cattle	3.5 yrs	F	11	Good	No	Negative	Sukrauli 9
144	Rukmagat Neupane	Ramgram 10	Cattle	4 yrs	F	11	Good	No	Negative	Sukrauli 9
145	Krishna Prasad Dhakal	Ramgram 9	Cattle	9 yrs	F	7	Good	No	*Anaplasma* spp.	Ramgram 9
146	Devi Prasad Adhikari	Ramgram 14	Cattle	3 yrs	F	12	Good	No	Negative	Amraut 1
147	Navaraj Acharya	Ramgram 4	Cattle	6 yrs	F	7	Poor	No	*Theileria* spp.	Ramgram 4
148	Mukti Tamang	Ramgram 14	Cattle	4 yrs	F	13	Good	Yes	Negative	Amraut 3
149	Jit Bahadur Yadav	Ramgram 14	Cattle	3.5 yrs	F	7	Poor	No	*Anaplasma* spp.	Amraut 1
150	Amar Singh Kurmi	Ramgram 4	Cattle	3 yrs	M	3	Good	Yes	Negative	Ramgram 4

ANNEX 2.

Questionnaire survey to determine the relationship between risk factors and occurrence of parasite

Name of the respondent: Age…Sex

Address……

1. Have you taken livestock farming training?

a) Yes b) No

2. Do you practice regular deworming?

a) Yes b) No

3. Do you use external parasiticide regularly to your livestock?

a) Yes b) No

4. Do you know that tick transmits disease to animal?

a) Yes b) No

5. Details about the animal sampled

i) Age:

a) Young-stock (0-4 years) b) Adults (5-8 years) c) Old (>8years)

ii) Breed:

a) Jersey b) Holeistein c) Crossbred d) Local

iii) Sex:

a) Male b) Female

iv) Type of feeding system

a) Ground feeding b) Trough feeding

v) Housing system

a) Open b) Closed

vi) Floor pattern

a) Non-cemented b) Partially cemented

vii) Watering system

a) Tap water b) Pond

viii) Herd size

a) Larger (>10 animal) b) Smaller (<10 animal)) and

ix) Body condition

a) Good b) Poor

x) Presence of tick in animal's body

a) Yes b) No