Prevalence of Blood Protozoan Parasite in Cattle of Ghorahi Sub Metropolitan City, Dang

Binita Dangi

T.U. Registration No. 5-2-1156-57-2015

T.U. Examination Roll No. 7930014

Batch: 2079

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

A dissertation submitted

In partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology with special paper Parasitology

---

April, 2025

Prevalence of Blood Protozoan Parasite in Cattle of Ghorahi Sub Metropolitan City, Dang

Binita Dangi

TU Registration No. 5-2-1156-57-2015 M.Sc. Zoology (parasitology)

T.U. Examination Roll No.7930014

Supervisor

Dr. Kishor Pandey

Associate Professor

Co-Supervisor

Janak Raj Subedi

Asssistant Professor

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science in Zoology with special paper Parasitology

---

April, 2025

February 2025

E-mail: dangibinita48@gmail.com

Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu, Nepal
Website: https://www.cdz.tu.edu.np/

Citation: Dangi, B. (2025). *Prevalence of Blood Protozoan Parasite in Cattle of Ghorahi Sub metropolitan City, Dang*. Central Department of Zoology, Tribhuvan University.

Declaration

I hereby declare that the work presented in this dissertation "Prevalence of Blood Protozoan Parasite in Cattle of Ghorahi Sub Metropolitan City, Dang" 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).

Binita Dangi

Exam Roll No.: 7930014

Email: dangibinita48@gmail.com

Date: 24 April 2025

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.
फोन नं. ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology T.U., Kirtipur

Recommendation

This is to recommend that the dissertation entitled "Prevalence of Blood Protozoan Parasite in Cattle of Ghorahi Sub Metropolitan City, Dang" has been carried out by Binita Dangi for the partial fulfilment of Master's Degree of Science in Zoology with special paper Parasitology. This is his/her original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

Dr. Kishor Pandey
Associate Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

Co-Supervisor
Janak Raj Subedi
Assistant Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

Date: 24 April 2025

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

☎ ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology
T.U., Kirtipur

Letter of approval

On the recommendation of supervisor "Dr. Kishor Pandey" and co-supervisor "Mr. Janak Raj Subedi" this dissertation submitted by Binita Dangi entitled "Prevalence of Blood Protozoan Parasite in Cattle of Ghorahi Sub Metropolitan City, Dang" is approved for the examination in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

.....

Kumar Sapkota, PhD

Professor

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Date: 24 April 2025

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.
फोन ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Certificate of acceptance

This dissertation work submitted by Binita Dangi entitled "Prevalence of Blood Protozoan Parasite in Cattle of Ghorahi Sub Metropolitan City, Dang" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

Evaluation committee

Supervisor	Head of Department
Kishor Pandey, PhD	Kumar Sapkota, PhD
Associate Professor	Professor
External Examiner	Internal Examiner
Krishna Das Manandhar, PhD	Mahendra Maharjan, PhD
Professor	Professor

Date of examination: 08/05/2025

Acknowledgements

Firstly, I would like to express my sincere gratitude to my honorable supervisor, Dr. Kishor Pandey, for his continuous guidance, encouragement, and unwavering support throughout this study. Your expertise, insights, and constructive feedback have been instrumental in the development of this research.

I am also deeply thankful to Prof. Dr. Kumar Sapkota, Head of the Department, for providing me with the essential resources and facilities needed to conduct my thesis research.

Additionally, I would like to express my sincere appreciation to all the teachers and staff at CDZ, TU, for their unwavering support, insightful guidance, and motivation throughout my academic journey. Their knowledge and mentorship have greatly contributed to my learning and professional growth.

I would also like to express my sincere thanks to my Co-supervisor Janak Raj Subedi, Prof. Dr. Mahendra Maharjan and Dr Tirtha Raj Ghimire our esteemed faculty teachers, for their valuable guidance, encouragement, and continuous support throughout my research. Their advice and mentorship have been instrumental in refining my study. Further, I would like to thank Dr. Akio Hakamata for donation of microscope to establish microscopic unit in CDZ.

Furthermore, I would also like to extend my heartfelt appreciation to Dr. Madhav Rawat, the veterinarian doctor who generously assisted in the fieldwork and sample collection process. Your knowledge and experience in the field were invaluable, and I am incredibly grateful for your time and dedication.

A special thank you to the farmers who allowed me to collect samples from their animals and provided valuable assistance. And special thanks to Sub Metropolitan City Ghorahi Dang for supporting economically in my thesis work. Without their cooperation and trust, this research would not have been possible.

Lastly, to my family and friends, thank you for your endless love, emotional support, encouragement, and for always being there when I needed a boost of motivation. Your companionship and positive energy kept me focused and inspired throughout this journey.

Thank you all for your kindness, support, and contribution to this work.

Abstract

Blood protozoan infections are widespread in cattle and result in significant losses to the livestock industry. This study aimed to assess the prevalence of blood protozoan parasites in Ghorahi Sub Metropolitan City, Dang. A total of 150 blood samples were collected from the jugular vein of cattle between October and November 2024. The samples were examined microscopically under high-power magnification (1000x) using immersion oil at the Central Department of Zoology Laboratory in Kirtipur. Hemoparasites were found in 23.33% of cases. The most common types of these parasites were *Babesia, Anaplasma,* and *Theileria* each with different prevalence rates of 12%, 6% and 5.33% respectively. The occurrence of different blood protozoan infections in cattle was found to be statistically insignificant ($\chi 2$= 5.65, p>0.0596). Among the blood protozoan parasites identified, the age-wise prevalence of blood protozoans' parasites, young cattle had 25.45% prevalence rate and adult cattle were found to have an infection rate of 22.10% with no significant association ($\chi 2$= 0.071325, p-value=0.7894). Sex wise prevalence of blood protozoans' parasites showed that 19.75% of male cattle and 27.53% of female cattle showing statistically insignificant ($\chi 2$=0.86416, p=0.3526). Ward- wise prevalence of blood protozoan parasites showed 18.91% in ward 4, 21.62% in ward 8, while 24.32% in ward 12 and 28.20% in ward 16 with statistically insignificant ($\chi 2$= 1.3097, p-value=0.7268). However, there was no regular healthcare provided to the cattle concerning blood protozoan parasites. This suggests a higher prevalence of blood parasites among the cattle in Ghorahi, Dang.

शोध सारांश

गाईवस्तुहरुमा रक्त प्रोटोजोवा सङ्क्रमण धेरै सामान्य छ र यसले पशुपंक्षी उद्योगमा धेरै ठूलो क्षति पुऱ्याउने गर्दछ । यो अध्ययन घोराही उपमहानगरपालिका क्षेत्रमा रहेका गाईवस्तुहरुमा रक्त प्रोटोजोवाको अवस्था थाहा पाउनको लागि गरिएको हो । प्रस्तुत अध्ययन गर्नको लागि गाईको जुगुलर नसाबाट कुल १५० वटा रक्त नमूना २०२४ को सेप्टेम्बर देखि अक्टोबर महिनामा लिइएको थियो । सबै रक्त नमूनाहरु केन्द्रिय प्राणीशास्त्र विभाग किर्तिपुरको प्रयोगशालामा शुस्मदर्शक यन्त्रको (1000x) सहायताले परिक्षण गरियो । ती नमूनाहरुमा रक्त प्रोटोजोवा परजिवीहरु २३.३३% पाइएको छ। ती संकलित नमूनाहरुमा रक्त प्रोटोजोवा परजिवीहरु *Babesia* spp., *Anaplasma* spp., र *Theileria* spp क्रमश १२%, ६% र ५.३३% परिमाणमा पाइयो । गाईवस्तुहरुमा रक्त प्रोटोजोवा परजिवीको संक्रमण राम्रो संख्यात्मक रुपमा ($\chi2$= 5.65, p>0.0596) प्राप्त गरियो । प्राप्त नमूनाहरु परिक्षण गर्दा, पहिचान गरिएका रक्त प्रोटोजोवा परजिवीहरु उमेर अनुसार युवा गाईहरुमा २५.४५%, वयस्क गाईमा २२.१०% संक्रमण देखियो जसमा कुनै महत्वपूर्ण सम्बन्ध छैन ($\chi2$= 0.071325, p-value=0.7894) । रक्त प्रोटोजोवा परजिवीहरु लिङ्ग अनुसार पुरुष गाईमा १९.७५% र महिला गाईमा २७.५३% संक्रमित देखिए, जो संख्यात्मक रुपमा महत्वपूर्ण फरक होइन ($\chi2$=0.86416, p=0.3526) । त्यसैगरी, वडा अनुसार गाईवस्तुहरुमा रक्त प्रोटोजोवा परजिवीहरुको संक्रमण, वडा नं ४ मा १८.९१%, वडा नं ८ मा २१.६२%, वडा नं १२ मा २४.३२% र वडा नं १६ मा २८.२०% पाइयो, जा संख्यात्मक रुपमा महत्वपूर्ण फरक देखिएन ($\chi2$= 1.3097, p-value=0.7268) । तथापि, नमूना संकलन गर्ने क्रममा, गाईवस्तुहरुको नियमिक स्वास्थ्य परिक्षण नभएको पाइयो । त्यसैले, यो अध्ययनले घोराही दाङमा रहेका गाईहरुमा रक्त प्रोटोजोवा परजिवीहरुको संक्रमण उच्च रहेको देखाउँछ ।

Table of contents

List of tables

List of figures

List of abbreviations

Abbreviated form	Details of abbreviations
BPPs	Blood Protozoan Parasites
BTH	Bovine Theileriosis
TBD	Tick-Borne Diseases
TBHD	Tick-Borne Hemolytic Disease

1. Introduction

1.1 Background

In Nepal, an agricultural country in South Asia, more than 66% of the population is employed in agriculture. The livestock sector contributes to the country's socio-economic development by providing nutrition and a source of income for rural households (Thapa et al., 2025). The cattle industry contributes significantly to the national economy in many developing nations by offering jobs, revenue, and food. The livestock industry in emerging nations is struggling with low productivity as a result of changes in demographics, socioeconomic position, and climatic conditions. It has also become both a cause and a victim of unfavorable climate change effects worldwide (Khanal et al., 2022). According to the report, the population of cattle was about 4,750,329, milking cows were 9,165,79 and the production of cow milk was 1,214,046 in Nepal (MoALD, 2023). The cattle are among the many livestock that aid in farming, harvesting, and transportation in rural areas. Their manure also serves as a fertilizer for soil nutrients and is increasingly being utilized as fuel. They also produce milk and meat, contributing to financial income through trade (Ferris et al., 1992). Cow is also worshipped by the people of Nepal as the goddess Lakshmi (goddess of wealth and prosperity) by the Hindus. The cow population was approximately 998,960, with an annual growth rate of 1.8%, and the production of cow milk was 468,913 metric tons, growing at an annual rate of 3% (Sharpe et al., 2013). Livestock is an important source of nutritional nutrients in developing nations like Nepal and other Asian countries, and it is an essential part of small-scale crop-livestock mixed agricultural systems. As urbanization and income levels rise, it is anticipated that the demand for animal products would rise in developing nations (Khanal et al., 2022).

1.1.1 Livestock diseases

Livestock diseases are alarming recently in Nepal and there has been a challenge to eradicate the disease to keep the cattle safe. Livestock diseases are grouped into four categories: zoonoses, foodborne diseases, endemic diseases, and epidemic diseases. Endemic diseases are present in specific geographical locations and influenced by various factors. Some of the common endemic diseases are Tick-Borne diseases, Brucellosis, Foot and Mouth disease, Leptospirosis, Endemic Gastrointestinal Parasites, Bluetongue diseases, Trypanosomiasis, and epidemic include classical swine fever, African swine fever, and foot-and-mouth disease (Otte et al., 2008).

1.1.2 Tick and ticks borne disease of cattle

Ticks and diseases carried by ticks are prevalent in tropical and subtropical areas. Because they cause blood loss, damage to hides and udders, and morbidity and mortality in animals, tick spreads are concerning (Ghosh et al., 2007). Tick-borne diseases impact 80% of the global cattle population and it is severing in the South Asian countries like Nepal, India, Pakistan and Bangladesh (Cayla et al., 2019). Cattle health and productivity are thought to be significantly hampered by hemoprotozoan illnesses, including Babesiosis, Anaplasmosis, Theileriosis, and Trypanosomiasis (Bhatnagar & Bhardawaj, 2015). The warm, humid weather provides perfect conditions for the growth and survival of potential vectors such as flies and ticks, which continuously infect susceptible animals (Velusamy et al., 2015a). Tick-borne protozoan infections in cattle and buffaloes cause hemoprotozoan diseases such anaplasmosis, theileriosis, and babesiosis. When blood-feeding flies such as Tabanus and Stomoxys bite an animal, they can transmit trypanosomiasis, which leads to symptoms like loss of appetite, weakness, weight loss, reduced milk and meat production, diminished working ability, abortion, and even death. The dairy business is severely impacted financially by these illnesses, which result in countless losses and significant obstacles for the sector globally (Krishna murthy et al., 2016). The presence and importance of hemoparasites highlight the complex relationships between the organisms responsible for the disease, their vectors, vertebrate hosts, and the environment. In general, hemoparasites are known to destroy red blood cells, resulting in anemia, jaundice, loss of appetite, weight loss, and infertility (Akande et al., 2010). Blood parasite-related diseases are common, especially in tropical and subtropical regions around the world. Blood parasite disease transmission is highly influenced by the health of the cattle (host), disease agents, vectors, and the surrounding environment, which includes the region's climate, geography, sociocultural, and socioeconomic aspects (Ritonga et al., 2020). Vector- borne hemoprotozoan infections, including babesiosis, trypanosomosis, and theileriosis, are known to cause severe clinical sickness in cattle, among other economically significant bovine diseases (Singh et al., 2012).

Anaplasmosis is a vector-borne blood disease caused by species from the genus *Anaplasma* (Rickettsiales: Anaplasmataceae). In cattle, the disease is caused by *Anaplasma* (*A.*) marginale and *A.* centrale (Kocan et al., 2003). Anaplasmosis can cause icterus, fever, weakness, anemia, jaundice without hemoglobinemia or hemoglobinuria, decreased milk supply, weight loss, abortion, hyperexitability from cerebral anoxia, and

sudden death, among other clinical signs and symptoms (Sajid et al., 2014). The *Anaplasma* genus consists of Gram-negative intracellular bacteria, including species such as *Anaplasma marginale*, *Anaplasma centrale*, *Anaplasma ovis*, *Anaplasma phagocytophilum*, *Anaplasma bovis*, and *Anaplasma platys* (Tay et al., 2014). The obligate intracellular rickettsial organism belonging to the genus *Anaplasma* causes an infectious tick-borne disease of ruminants called anaplasmosis. The disease is clinically marked by symptoms such as fever, loss of appetite, reduced milk production, worsening anemia, jaundice, dark-colored urine, pale mucous membranes, difficulty breathing, and constipation. It is sporadic in temperate regions and prevalent in tropical and subtropical countries (Belal et al., 2015).

Babesia belongs to the family Babesiidae, within the phylum Apicomplexa. It is classified under the class Sporozoasida, order Eucoccidiorida, and suborder Piroplasmorina (Levine, 1971). The genus *Babesia* contains intraerythrocytic protozoan parasites that cause babesiosis. These parasites can infect a range of domestic and wild animals, and occasionally, they also affect humans. Ticks are the disease's primary vector of transmission and distribution. Babesiosis has a significant economic impact on the cattle sector (Bock et al., 2004). Millions of cattle in tropical, subtropical, and temperate regions of the world are primarily affected by babesia after the rainy and winter seasons; these diseases are most commonly observed during the summer (Farooqi et al., 2017). These three species (*B. bigemina, B. bovis, and B. divergens*) cause babesiosis, and their distribution depends on the presence of ixodid tick species, which are known to spread the parasites across bovine herds. In general, the severity of Babesiosis progression varies and is often related depending on the host's age and immune system strength, the presence of other infections, and/or genetic factors. Pyrexia, anemia, hemoglobinuria, icterus, malaise, lethargy, and anorexia are common signs of acute *Babesia* infections in many hosts, although chronic infections are typically asymptomatic (Schnittger et al., 2012). *Babesia* protozoan parasites infect all cattle, both domestic and wild, after being passed down from one generation of ticks to the next through the egg (Gohil et al., 2013) Trypanosomosis caused by *Trypanosoma evansi* is an important livestock disease are mostly spread by vectors (Barros et al., 2017), which go through intricate developmental cycles. In insects, trypanosomes called stercorarians form in the back of the digestive tract. One example is *Trypanosoma cruzi*, a parasite that causes Chagas disease and is commonly found in Latin America (Barros et al., 2017). According to reports,

Trypanosoma congolense, T. vivax, and T. brucei cause a disease called nagana in cattle, while *T. evansi* causes a disease called surra in camels (Mbaya et al., 2011). Human sleeping sickness is caused by *Trypanosoma brucei gambiense* in West Africa and *Trypanosoma brucei rhodesiense* in East Africa, while chagas illnesses are spread to humans in South America by T. cruzi, which is spread by triatomid bugs (Triatoma magista) (Solano et al., 1999). High fever, appetite loss, paralysis, neurological signs, immunodeficiency, and eventual mortality between two weeks to four months are the symptoms of surra. Surra can lead to various health problems in animals that cause financial losses, such as reduced milk production and weight, lower value when selling or slaughtering livestock, infertility, death, decreased ability to work or provide draught power, weakened immune systems (which can lead to more diseases and make vaccines less effective), and the cost of treatment (Holland et al., 2001)

Theileriosis is a widespread protozoan parasitic disease that affects dairy cattle and buffaloes around the world. Cattle and buffaloes are vulnerable to bovine tropical theileriosis, a tick transmitted blood parasite disease caused by *Theileria annulata*, which significantly impacts livestock productivity and the dairy industry. Cattle and buffaloes are afflicted by a number of *Theileria* species; However, the most economically damaging and significant species are *T. annulata* (which causes Bovine Tropical Theileriosis), *T. parva* (responsible for East Coast fever), and *T. orientalis* (which leads to Oriental theileriosis). These diseases are transmitted by ixodid ticks from the *Hyalomma* and *Rhipicephalus* genera (Krishna Moorthy et al., 2021). *Theileria spp.* are present in their host's lymphocytes and erythrocytes. Infections caused by the parasite can range from quickly lethal to clinically undetectable (Darghouth et al., 1996). One of the common protozoan illnesses spread by Ixodidae ticks is theileriosis. Due to its high rates of illness and mortality, it poses a major threat to livestock productivity globally. It poses a particular risk to exotic cattle and their offspring (Irshad et al., 2010).

1.2 Statement of problem

Hemoparasites including *Babesia*, *Theileria*, *Anaplasma* and *Trypanosoma* can cause diseases that impact the health of cattle, which are crucial for farmers because they give milk, meat, and assistance with farm chores. These parasites, which are carried by insects like flies and ticks, can seriously harm cattle's health by causing fever, weight loss, and even death. Although these illnesses are prevalent in many places, particularly in tropical and subtropical regions, the current evidence regarding their prevalence is lacking.

Developing effective treatment and preventative strategies is challenging if the number of cattle afflicted and the most prevalent parasites are unknown. The goal of this study to assess the occurrence of these blood protozoan parasites in cattle of Ghorahi Sub Metropolitan City Dang, so that farmers can be supported and animal health can be better protected.

1.3 Objectives

1.3.1 General objective

- To determine the prevalence of blood protozoan parasites infecting cattle in Ghorahi, Dang Nepal.

1.3.2 Specific objectives

- To determine the blood protozoan parasite in cattle.
- To find the prevalence of blood protozoan parasites by age-wise, sex-wise and ward-wise.

1.4 Significance of the study

The prevalence of blood parasites in cattle must be investigated in order to comprehend and control the effects of these illnesses on agriculture, the economy, and animal health. Blood parasites including *Anaplasma*, *Theileria* and *Babesia* can cause serious illnesses in cattle, including anemia, weight loss, decreased milk output, and impaired fertility. The welfare of the animals is impacted, but farmers also suffer financial losses as a result of lower output and higher care expenses. By determining the high-risk areas, researchers can implement targeted treatments like as vector control, immunization campaigns, and better treatment approaches. Comprehending the ecological and environmental elements that impact the transmission of parasites also aids in anticipating outbreaks and better controlling them.

2. Literature review

2.1 Blood-protozoan parasites in the global context

Theileriosis, babesiosis, anaplasmosis, and trypanosomosis are blood parasite illnesses that affect livestock (Seangseerattanakulchai & Piratae, 2021). The destruction of red blood cells by hemoparasites has typically been demonstrated to result in infertility, weight loss, anorexia, jaundice, and anemia (Ellis et al., 2003). These hemoparasitic illnesses, which are mostly spread by ticks, can have an adverse impact on animals' health, the cattle sector, and occasionally even people. In addition to being known to cause fever, anorexia, jaundice, an increase in the likelihood of abortions, and sterility, infections can be fatal for farm animals (Abdullah et al., 2019).

Anaplasmosis

Anaplasmosis is a disease that happens when red blood cells get infected by bacteria from the *Anaplasma* group (Maharana et al., 2016). The three species that are most frequently found in cattle are *A. caudatum, A. marginale,* and *A. centrale*. Signs of anaplasmosis include a fever, loss of appetite, tiredness, trouble digesting food, a sluggish stomach, dark brown poop with mucus in it, and low red blood cell levels (anemia) (Weerasooriya et al., 2016). In the Sirajganj district of Bangladesh, about 25.82% of cattle were found to have anaplasmosis. Older cattle (over 3 years old) had the highest infection rate at 34.19%. The disease was also more common in female cattle (28.88%) compared to males (19.20%) a total of 395 were examined (Belal et al., 2015). Out of 200 samples the study revealed 18.5% were Anaplasmosis in Dinajpur district Bangladesh (Kispotta et al., 2017). In Pakistan, lab tests using a microscope showed that 205 animals (41% of those tested) were infected with *Anaplasma* parasites. Among them, 89 had *Anaplasma marginale*, 44 had *Anaplasma centrale*, and 72 had a mix of both types (Rajput et al., 2005). A total of 408 cattle were checked for anaplasmosis to find out how common the disease was overall 6.1% (25/408) were found for bovine anaplasmosis in south western Ethiopia (Abdela et al., 2018). Anaplasmosis was most common in adult cattle, with 308 cases (71%). Juvenile cattle had a lower rate, with 63 cases (14.6%), and calves had the fewest cases at 61 (14.1%). Among adult males, 45 (28.3%) were infected. Out of the 156 infected females (68.1%), adult females made up the largest group with 114 cases 71.7% (Article, 2021) in Nigeria.

The species-specific PCR results. Out of the 15 cattle that were analyzed, 8 (53.3%) tested positive for A. bovis, 12 (80.0%) some animals were positive for *A. phagocytophilum*, and 6 of them (40%) had a dual infection. None of the animals tested positive for *A. centrale* or *A. marginale*. in Japan (Ooshiro et al., 2008).

Babesiosis

Babesia is a protozoan that is spread by ixodid ticks that infects erythrocytes or piroplasm. The four species of the *Babesia* genus includes species like *Babesia bovis*, *Babesia bigemina*, *Babesia divergens*, and *Babesia major* that cause the majority of infections in cattle (Wen et al., 2016). Blood samples from 506 cattle were examined to check for the presence of *B. bovis* and *B. bigemina* how the number of positive instances was distributed among the subcounties that were sampled. 25.9% of cows had bovine babesiosis overall (131/506). Seventy of the 131 animals that tested positive for Babesia had a *B. bigemina* infection, meaning the prevalence was 13.8%, and 87 among the animals that tested positive for *B. bovis*. meaning the prevalence was 17.2% in Kenya (Githaka et al., 2022). In Pakistan, Babesiosis is a common and highly significant disease in terms of economic impact. It results in significant financial losses and high rates of mortality among young animals. Out of 479 cattle samples a prevalence rate of 11.89% for *B. bovis* was observed in cattle (Farooqi et al., 2017). Tick fever, or bovine fever (babesiosis), is the most important arthropod-transmitted disease affecting cattle worldwide, primarily due to its economic impact. It remains a constant threat in vast areas of Australia, Africa, South and Central America, and the United States (Bock et al., 2004). Serological testing showed that 79% of cattle tested positive for *B. bovis* antibodies, and 52% tested positive for *B. bigemina* antibodies. Meanwhile, nPCR testing revealed that 71% of cattle were infected with *B. bovis* and 34% with *B. bigemina* protozoa. This is the first report on the prevalence of *B. bovis* and *B. bigemina* in cattle based on both serological and DNA analysis studies conducted in central and southern Portugal (Silva et al., 2009).

Theileriosis

Theileriosis is spread by Hyalomma ixodid ticks. The overall prevalence of theileriosis in Uttarakhand India was found to be 27.2% (82) when blood smears were examined under a microscope. The highest incidence, 45.4%, was observed during the rainy season (Kohli et al., 2014). *Theileria annulata* is the most common piroplasm infection in cattle

throughout Egypt *T. annulata* was present in 16.49% of cattle in the investigated locations overall. The highest prevalence rate was observed in the Alexandria governorate (22.58%), followed by Menofia (14.62%), Beheira (13.91%), Kafr El Sheikh (12.22%), and Qalyubia (16.25%) (Selim et al., 2022). Out of 34 Giemsa-stained blood smears from seemingly healthy cows, 3 samples (8.82%) tested positive for *Theileria* species in Odisha (Sahoo et al., 2017). Numerous studies have been conducted on the hemoprotozoans of cattle in Africa, including countries like Sudan. (Salim et al., 2011). Numerous studies have been carried out on *Theileria* species in Asia, particularly in Taiwan (Wang et al., 1998). In different 5 Asian countries bovine theileriosis have been reported, China has the highest prevalence rate of BTH at 39%, followed by Iran at 33%, India at 31.7%, Pakistan at 21.2%, and Bangladesh at 2.69% (Zaman, 2020). An outbreak of oriental theileriosis occurred in dairy cattle that were imported from Australia to Vietnam (Gebrekidan et al., 2017).

In European countries, According to PCR data, the overall infections of *T. annulata*, *B. bigemina*, *T. orientalis*, and *A. marginale* were, respectively, 29.1%, 18.9%, 11.2%, and 5.6% in turkey (Zhou et al., 2016). In the eastern Black Sea region of Turkey, a 1.2% prevalence of *T. annulata* has been reported (Altay et al., 2008).

Trypanosomiasis

Trypanosoma causes Trypanosomiasis which is alone responsible for one quarter of economic losses due to animal pathologies (Haan, n.d.). *Trypanosoma evansi* has been reported in cattle across Africa, Asia, South America, and Europe from 1909 to 2017. Africa has reported cases of *T. evansi* infection in Egypt, Ethiopia, and Nigeria, with infection rates varying between 6% and 42% (Aregawi et al., 2019). In Asia *T. evansi* infection prevalence ranges from 1 to 61%, with reports from India, Indonesia, Iraq, Malaysia, Philippines, and Thailand (Aregawi et al., 2019). *Trypanosoma* species are the primary cause of canine trypanosomiasis, a complicated disease. Chagas disease is caused by *Trypanosoma* (Schizotrypanum) *cruzi*, the surra (African form) and *Trypanosoma* (Trypanozoon) *evansi* (creating the American form) (Desquesnes et al., 2013). The state of Adamawa reported a 26.67% prevalence of *Trypanosoma* in cattle, with males accounting for 9.17% and females for 17.50% of cases (Ibn Zubairu et al., 2013). The *Trypanosoma vivax* investigation, which involved 130 cows in Semiarid Paraiba, North eastern Brazil, revealed that 60 of the infected cattle had a variety of symptoms, including fever, Anameia, weight loss, hypoglycemia, elevated serum levels of aspartate

aminotranferase, and even neurological disorders (Batista et al., 2007). Through buffy coat microscopic examination, 1132 cattle from three tsetse- infected districts Dale Sadi (599), Dale Wobara (161), and Hawa Galan (372) were examined to determine trypanosome infections and anemia (haematocrite ≤ 24%). The prevalence of anemia was 39.1% overall, with higher rates in the districts of Dale Wobara and Dale Sadi. Animals with poor or medium body condition, as well as adult and elderly females, showed a statistically higher frequency of anemia. The prevalence of trypanosome infections overall in Western Oromia was 8.57% (97/1132), primarily attributable to *T. congolense* alone (82 cases) in 3 districts or to T. vivax in the Dale Sadi area only (3 cases) (Tasew & Duguma, 2012).

2.2 National context

In the context of Nepal, the research on blood parasite has been increasing on recent days. Tick-borne blood parasitic infections are widespread in cattle and lead to significant losses in the livestock industry. The overall prevalence of tick-borne hemoparasitic (TBHP) infections was found to be 9.12%, with 26 positive samples. Among the positives, the highest number were infected with *Anaplasma* species, followed by *Babesia*, and then *Theileria*, with prevalence rates of 46%, 27%, and 12%, respectively. The highest prevalence was observed in Bhaktapur district (14.47%), followed by Kathmandu (7.31%) and Lalitpur (6.97%). Among the positive samples, 73.07% had a recent history of tick infestations, and 81% showed at least one clinical sign of tick-borne hemolytic disease (TBHD) (Lama, 2023). Out of the 106 samples collected from the Kathmandu Valley, 45 samples (42.5%) tested positive for piroplasm (*Babesia* spp. and *Theileria* spp.) through microscopic examination, while 56 samples (52.8%) were positive via nPCR (Dhakal et al., 2023). Out of 403 cattle of 6 districts, *Theileria* infection was 19.85% (80 cattle were positive) positive by single dilution. The highest seroprevalence (26.06%) was observed in Morang district followed by Siraha (21.43%), Sunsari (19.67%), Jhapa (12.50%), Saptari (8.00%) and Udayapur (7.94%) (Shrestha et al., 2017). In the Makawanpur district, the *Trypanosoma* was detected in 16 out of 240 domestic animals (6.67%). Of these, 9 out of 105 were cattle (8.57%), 5 out of 75 buffalos (6.67%), and 2 out of 15 dogs (13.3%) had the disease. No goats or pigs were infected. The disease was most prevalent in cross-breed animals than in native breeds, and it was discovered in 9 out of 82 cases (10.98%) during the rainy season.

3. Materials and methods

3.1 Study area

This study was conducted in various wards of Ghorahi Sub Metropolitan City in Dang, Lumbini Province, situated in the inner Terai of Midwestern Nepal. (Figure 1) It is the second largest valley in Asia, bordered by the Sivalik Hills and the Mahabharat Range. It is located within the geographical coordinates of 28° 00'0" N to 82°15' 60" E at an altitude of 109 meters to 2914 meters above sea level. The total area of Dang is 2955 square kilometers. Many people in this region are involved in agriculture, animal husbandry and poultry farming etc on both small and large scales. The number of cattle in Dang District is 135,242 and the number of milking cows is 22,450 producing 18,089 metric tons of milk (MoALD, 2023).

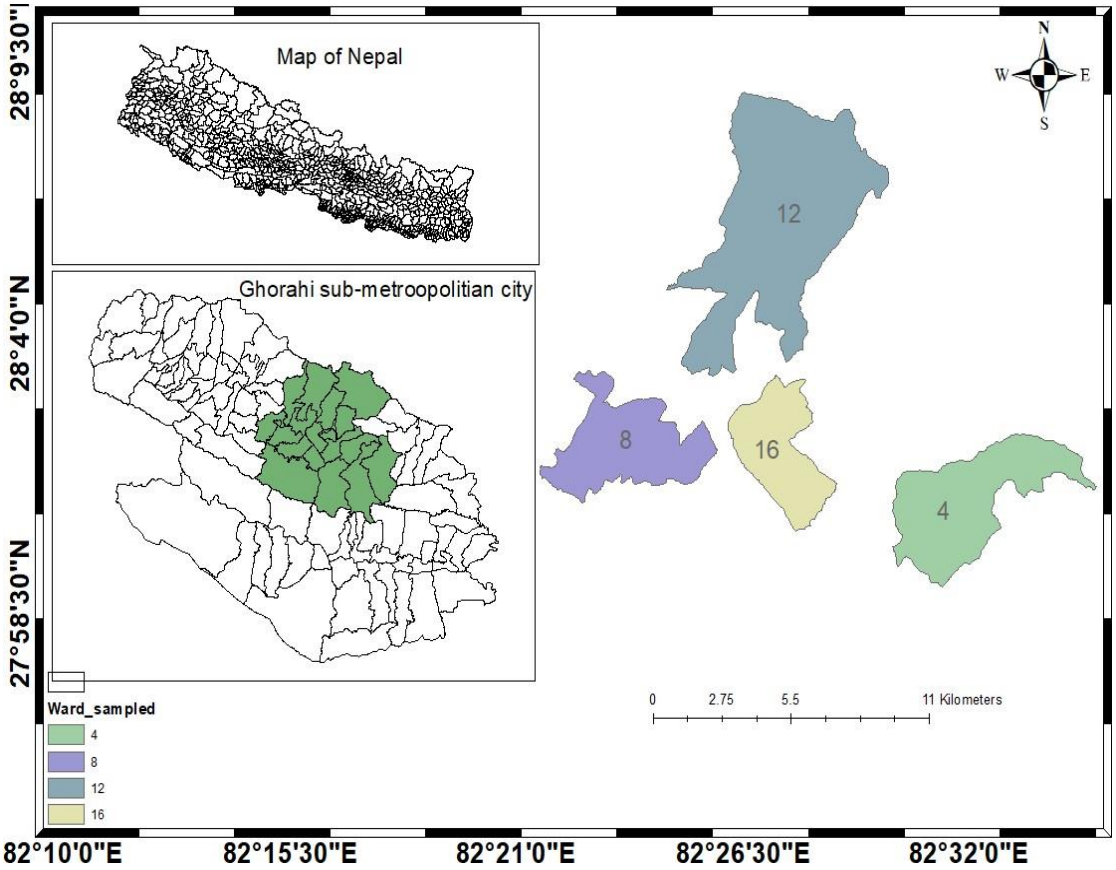


Figure 1: Map of Nepal showing study area including ward 4, ward 8, ward 12 and ward 16.

3.2 Blood sampling

This study was based on household and all the blood samples used for thesis work had been collected from four different wards of Ghorahi Sub Metropolitan City ward 4 (n=37); ward 8 (n=37); ward 12 (n=37); ward 16 (n=39) of different household cattle during october November 2024. Cattle of different house were selected for blood samples in alternate method. For blood collection first of all, cattle were placed in pole or a tree that support for easy puncturing jugular vein. With the assistance of a veterinarian, blood samples were taken from the jugular vein of 150 cattle. (Figure 2) Among them in age wise blood sampling cattle that is less 3 years was listed as young cattle and cattle that was equal or greater than 3 years were listed as adult cattle (Belal et al., 2015). The samples were collected using a sterile syringe and promptly transferred into an Ethylene Diamine Tetra Acetate (EDTA) vial. They were then stored in an icebox for preservation in the field. After completing samples collection immediately thin smear was prepared on glass slide and carried to Central Department of Zoology Laboratory, Kirtipur.

Figure 2: Collecting blood sample from study area

3.2.1 Preparation of thin blood smears

The glass slide was prior for the preparation of the thin smear. For thin smears, a drop of blood was placed at one end of a clean, dry glass slide. To create a smear with a tongue shape, a spreader slide, held at a 45˚ angle, was moved slowly along the slide from one end to the other. The thinner the film, the sharper the angle between the slides and the slower the movement of the spreader slide. The prepared film was then quickly dried by waving it in the air and fixed with methanol for 3-5 minutes. (Figure 3)

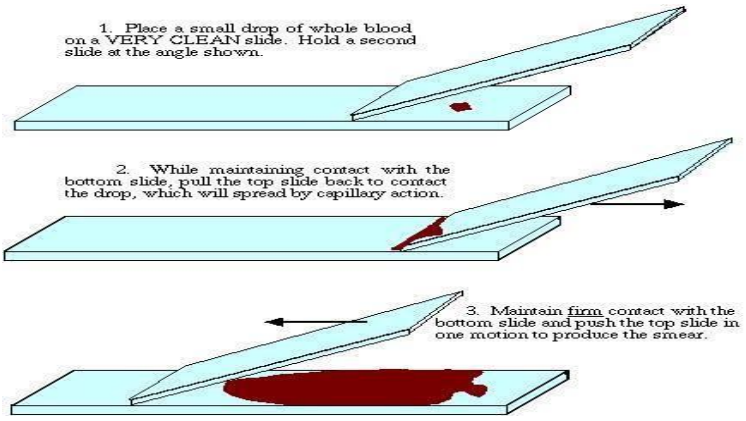


Figure 3: Steps of making thin blood smear.

3.2.2 Giemsa's stain solution

Giemsa stain is often used to prepare slides when studying protozoan parasites, especially to help identify them by looking at their internal structures. To make the stain, one part of standard Giemsa solution is mixed with nine parts of phosphate-buffered water at a pH of 7.2. This mixture is then filtered and stored in a dark (amber-colored) bottle with a stopper. Before using, the stain is diluted with distilled water. To keep the pH stable, the water can be buffered using 3.0 grams per liter of sodium phosphate (Na_2HPO_4) and 0.6 grams per liter of potassium phosphate (KH_2PO_4).

3.2.3 Giemsa staining

Air-dried and methanol-fixed blood smear slides were placed in a jar with prepared Giemsa stain solution and left to soak for 30 to 35 minutes. Giemsa stain solution containing coupling jar was used for 4 times then it was washed and again new solution was used for remaining slides to poured. The stained slides were gently rinsed with running tap water and then left to dry in the air. Then the stained slides became ready for microscopy. (Figure 4)

Figure 4: Preparing blood smear and Giemsa staining

3.2.5 Microscopic examination

The simplest method of diagnosis was to look at the peripheral blood smears. A high-power microscope (10x by 100x) with immersion oil was used to examine the slides. (Figure 5) Starting at the tail end of the slide, the inspection proceeded throughout the entire space, paying particular attention to any objects that seemed suspect so that they could be examined in further detail (Ibn Zubairu et al.,2013)

Figure 5: Microscopic examination of blood smear

3.2.6 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 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 (Sitotaw et al., 2014, 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)

3.2.7 Study design

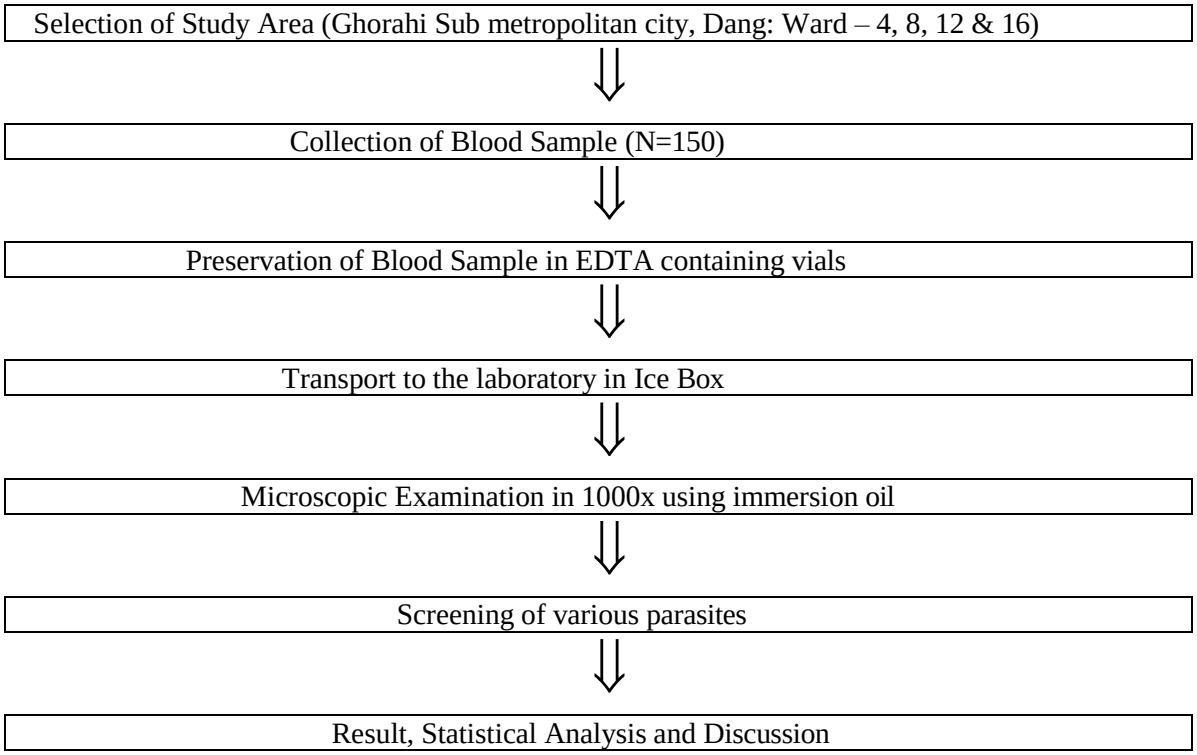


Figure 6: Flow chart showing study design

3.2.8 Questionnaire survey

To collect data, a questionnaire was created and used to gather information about possible risk factors like age, sex, and body condition. The selected farmers were interviewed one by one, focusing on the heads of households or their representatives. The questionnaire included both open and closed-ended questions to get details about the household's social and economic background, such as their knowledge of tick infections and whether they used anti-parasitic drugs.

3.2.9 Statistical analysis

The collected data were analyzed to determine how common hemoparasites were in cattle and how this was related to risk factors like age, sex, species, and location (by ward). The results were shown using tables and graphs. The prevalence was calculated using the statistical software R version 3.4.1, with descriptive statistics presented as proportions along with a 95% confidence interval (CI). The Chi-Square Test was used to compare groups, and results were shown as percentages with p-values. A result was considered significant if the p-value was less than 0.05.

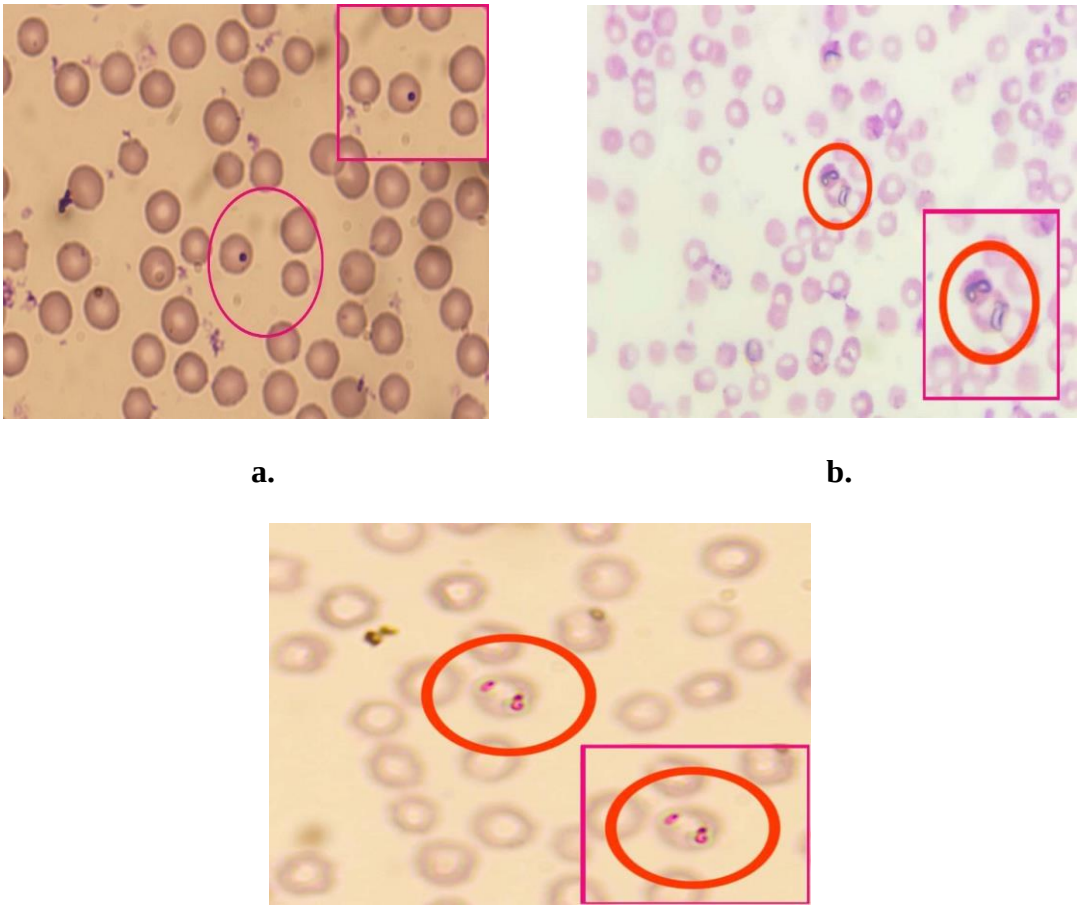
4. Results

4.1 Prevalence of hemoparasites in cattle of Ghorahi sub metropolitan city, Dang

A total of 150 cattle from different wards of Ghorahi Sub-Metropolitan City in Dang were tested for hemoparasites. Blood samples were taken from the jugular vein of cattle and brought to the Central Department of Zoology lab at TU Kirtipur. There, the samples were examined under a microscope using the Giemsa staining method. Out of total 150 samples collected 35 blood samples were positive showing 23.33% overall prevalence of parasitic infection.

4.2 Microscopic observation of hemoparasites.

Out of 35 positive samples, 9 samples were *Anaplasma* spp., 18 samples were *Babesia* spp., and 8 samples were *Theileria* spp. that are seen under the microscopy with immersion oil. Among all *Babesia* spp. shows higher prevalence. These parasites are identified by their shapes and size that are found in blood smear. (Figure 7)



a. **b.**

c.

Figure 7: a) *Anaplasma* spp., b) *Babesia* spp., c) *Theileria* spp.

4.3 Prevalence of hemoparasites in cattle

Among the hemoparasites, *Anaplasma* spp., *Babesia* spp., and *Theileria* spp., were found with prevalence 9(6%), 18(12%) and 8(5.33%) respectively (Figure 8). Although the prevalence of *Babesia* spp. was higher as compared to *Anaplasma* spp. and *Anaplasma* spp was higher as compared to *Theileria* spp. showing statistically insignificant association ($\chi 2$= 5.65, p-value=0.0596).

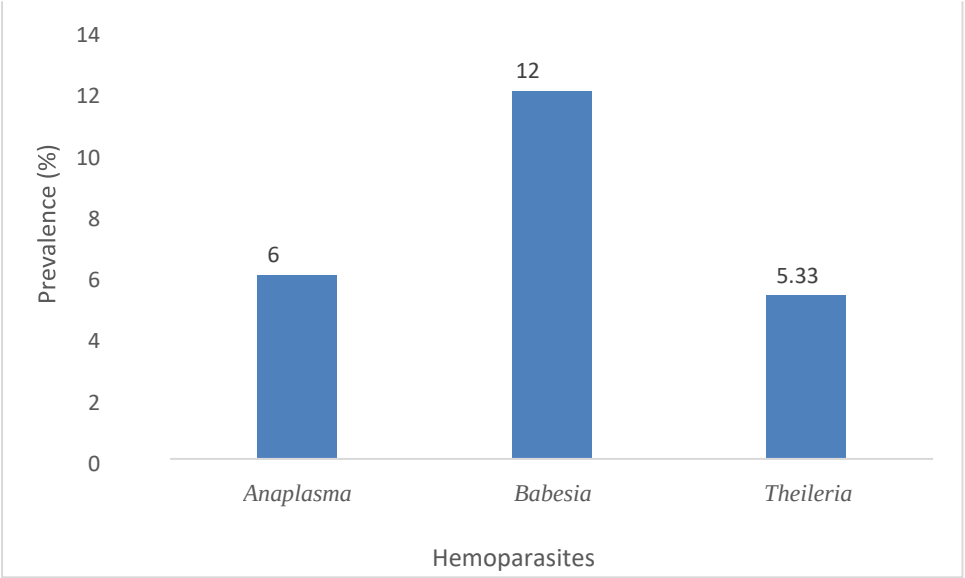


Figure 8: Prevalence of hemoparasites in cattle

4.4 Overall prevalence of hemoparasites on different demographic factors

Hemoparasites infections were studied based on different demographic factors. Sex wise infection of hemoparasites showed that 16(19.75%) of male cattle and 19(27.53%) of female cattle were infected with blood parasites showing insignificant differences. Age-wise prevalence revealed a higher infection rate in cattle aged ≤ 3 years 14(25.45%) compared to cattle > 3 years 21(22.10%) showing statistically significant differences between two age group. Ward-wise prevalence analysis showed that Ward-4 had the lowest prevalence at 7(18.91%), while Ward-16 had higher prevalence rates of 11(28.20%) showing statistically insignificant association. (Table 1)

Table 1. Overall prevalence of hemoparasites in cattle of Ghorahi, Dang.

Parameter of cattle	Category	Total sample N=150	Prevalence of blood parasite	P value	Chi square
Sex-wise	Male	81	16(19.75%)	0.3526	0.86416
	Female	69	19(27.53%)		
Age-wise	young	55	14(25.45%)	0.7894	0.071325
	Adult	95	21(22.10%)		
Ward-wise	Ward 4	37	7(18.91%)	0.7268	1.3097
	Ward 8	37	8(21.62%)		
	Ward 12	37	9(24.32%)		
	Ward 16	39	11(28.20%)		

4.5 Comparative prevalence of hemoparasites in Ghorahi sub metropolitan city, Dang

The prevalence of each of the three genera of hemoparasites found in cattle was described demographically. Prevalence of hemoparasites by age, sex, and ward were given priority.

4.5.1 Age wise prevalence of hemoparasites

The prevalence of *Anaplasma* species was found to be relatively higher in young cattle, but the difference was not statistically significant for that age group ($\chi2$=1.0606e-29, df=1, pvalue=1). *Babesia* species were higher than the other blood parasites in both age groups. The infection rate was higher in young cattle, but the difference between age groups was not statistically significant ($\chi2$=0.073818, df=1, p-value= 0.7859). On the other hand, *Theileria* species were found to be less common compared to the other blood protozoan parasites and among two age group *Theileria* spp. was higher in adult cattle showing statistically not significant ($\chi2$= 2.1231e-31, df=1, p-value=1). (Figure 9)

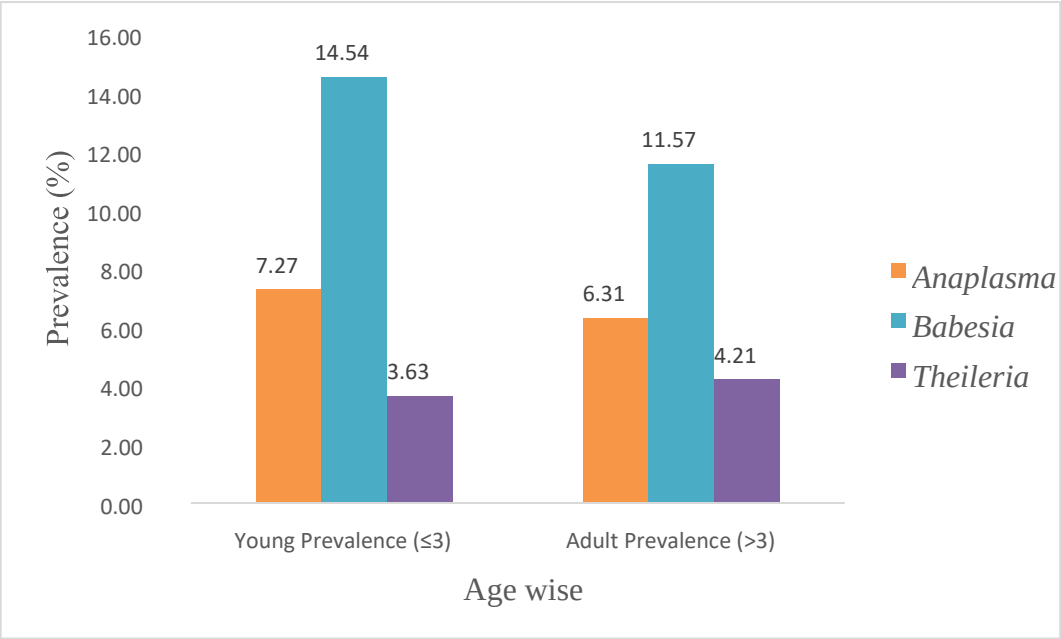


Figure 9: Age wise prevalence of hemoparasites

4.5.2 Sex wise prevalence of hemoparasites

According to sex-wise prevalence, *Anaplasma* spp. was comparatively higher in female cattle showing insignificant association ($\chi 2$=0.35021, df= 1, p-value= 0.554). In this case, the prevalence of *Babesia* species was also higher in female cattle compared to males showing insignificant association ($\chi 2$=0.36604, df= 1, p-value= 0.5452). Whereas prevalence of *Theileria* spp. was less as compared to other hemoparasites in both the sex group other hemoparasites. The prevalence of *Theileria* spp. had lowest prevalence in both male and female compared to other blood parasites. While the prevalence of *Theileria* spp. was comparatively high in male cattle than female cattle showing statistically insignificant ($\chi 2$= 1.0208e-30, df= 1, p-value= 1). (Figure 10)

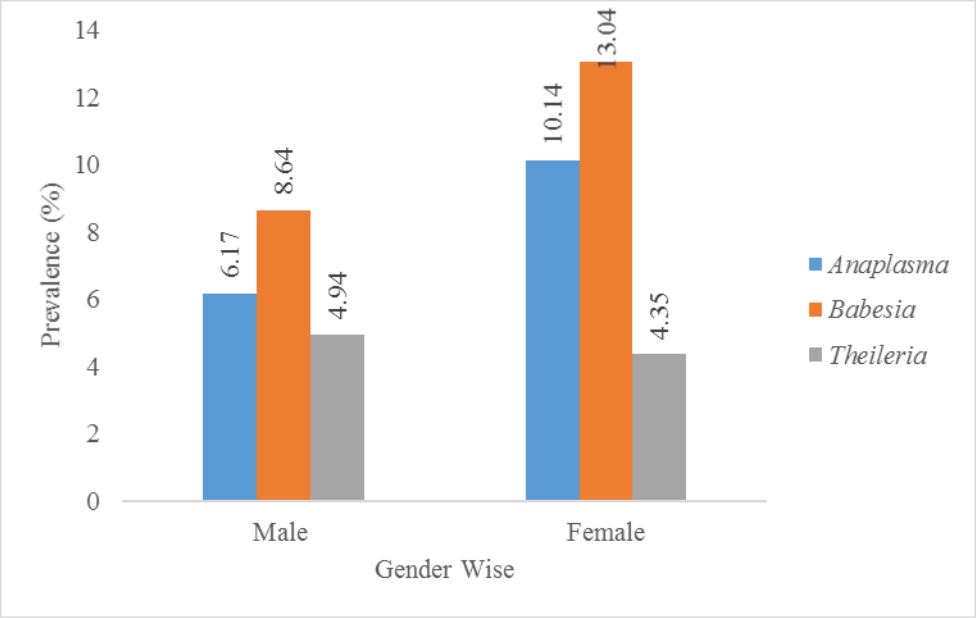


Figure 10: Gender wise prevalence of hemoparasites

4.5.3 Ward wise prevalence of hemoparasites

A total 4 ward were selected for sample collection. I collected 37, 37, 37and 39 blood samples from ward 4, ward 8, ward 12 and ward 16 respectively. Out of the total samples, 35 tested positives for different types of hemoparasites. Out of them ward number 16 was highly prevalent than another ward. While ward number 4 had the lowest prevalence rate as compared to another ward. In ward wise prevalence of BPP the prevalence of *Anaplasma* spp was higher in ward 16 and similar in other remaining wards showing insignificant association ($\chi 2$= 0.26762, df= 3, p-value= 0.966). Whereas *Babesia* spp. was found to be highly prevalent in every ward compared to other parasites. Among different wards *Babesia spp.* was higher in ward 16 showing statistically insignificant association ($\chi 2$= 1.0836, df= 3, p-value= 0.781). While the prevalence of *Theileria* spp. was found to be higher in ward 12 showing statistically insignificant association ($\chi 2$= 1.075, df= 3, p-value= 0.7831). (Figure 11)

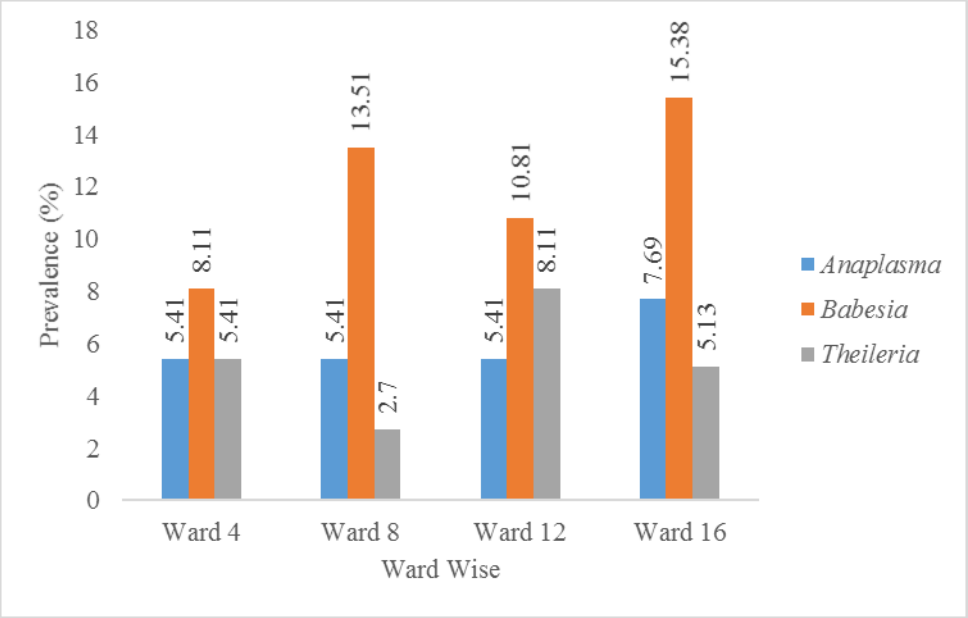


Figure 11: Ward-wise prevalence of hemoparasites

5. Discussion

Livestock farming plays an important role in Nepal's agricultural sector. As reported by the Central Bureau of Statistics (Gibbs, 2004), it contributes roughly 24 percent to the agricultural Gross Domestic Product (GDP). Tick-borne protozoan infections in cattle and buffaloes cause blood protozoan diseases such anaplasmosis, theileriosis, and babesiosis. When blood-feeding flies such as *Tabanus* and *Stomoxys* bite a person, they can mechanically transmit trypanosomiasis. This disease can lead to symptoms like loss of appetite, weakness, weight loss, decreased milk and meat production, reduced working ability, miscarriage, and even death. The dairy business is severely impacted financially by these illnesses, which result in countless losses and significant obstacles for the sector globally (Krishna murthy et al., 2016). The presence and impact of blood parasites highlight the complex relationships between the pathogens, vectors, vertebrate hosts, and their environment. In general, blood parasites are known to destroy red blood cells, resulting in anemia, jaundice, loss of appetite, weight loss, and infertility (Akande et al., 2010). Blood parasite-related illnesses are widespread, particularly in tropical and subtropical areas of the world. Blood parasite disease transmission is highly influenced by the health of the cattle (host), disease agents, vectors, and the surrounding environment, which includes the region's climate, geography, sociocultural, and socioeconomic aspects (Ritonga et al., 2020). Vector-borne hemoprotozoan infections, including babesiosis, trypanosomosis, and theileriosis, are known to cause severe clinical sickness in cattle, among other economically significant bovine diseases (Singh et al., 2012).

In this study, hemoparasites were detected in 23.33% of cattle in Ghorahi Sub Metropolitan City, Dang. The present prevalence rate hemoparasites was similar to 23% reported by (Vahora et al., 2012) and less than 32.91% of Makawanpur district (Mishra 2003). The present prevalence rate was higher by 6.67%, 16.25%, 17.33%and 19.23% reported in Makwanpur, Gorkha, Nawalparasi and Siraha district respectively (Maharjan & Mishra, 2006) (Khankhawash, 2018) (Phuyal, 2018) (Yadav, 2015). Haemo-protozoans have been reported in various regions of Nepal, including the Terai areas such as Sunsari, Morang, and Jhapa (Gupta et al., 2013). The prevalence rate found in this study was higher compared to the 9%, 6.67%, and 9.92% reported by the (Bhatnagar & Bhardawaj, 2015), (Velusamy et al., 2015) and (Okorafor & Nzeako, 2014) However, it was similar to the findings of (Maharana et al., 2016), (Gökalp, 1981), (Velusamy et al., 2015) and (Ananda et al., 2016) they reported prevalence rates of 25.7%, 27.88%, 37%, and 39.9%

from different regions of world. The prevalence report suspects that the BPPs are common throughout the country in perspective to the geographical regions whether in terai or hilly regions. Similarly in other parts of the world. The differences in parasitic prevalence may be attributed to climatic factors, which directly affect the distribution of the vectors. Regarding the prevalence of hemoparasites based on age, young cattle were found higher with the prevalence rate 25.45% than adult with the prevalence rate 22.10% which is similar with the prevalence rate reported in Southern Thailand (Bohman et al., 2024) with 4.16% in young and 2.82% in adult. It may be due to immature immune system in young cattle and young cattle may be more to vectors like ticks due to behavior and grazing habits. In this study, the prevalence of hemoparasites was higher in females (27.53%) compared to males (19.75%). It may be due to hormonal and physiological differences. Female cattle, particularly during pregnancy and lactation, may experience changes in their immune system, making them more susceptible to infections. Additionally, females may be more exposed to vectors like ticks, which transmit these parasites, due to their increased time spent in grazing areas or specific farming practices. Hormonal fluctuations, especially during estrus, can also make females more attractive to ticks, further increasing their chances of infection. (Okorafor & Nzeako, 2014) also reported similar the higher infection in female. The disease has been reported in cattle, buffalo, and goats in Makawanpur district (Maharjan & Mishra, 2006). Blood protozoan parasites affect various tropical and subtropical regions around the world. In Saudi Arabia, camels, cattle, sheep, and goats have been infected by these parasites (Al-Khalifa et al., 2009). Similarly, blood protozoan parasites have been reported in cattle in Sudan (Salim et al., 2011). Blood protozoan parasites have been reported in deer, cattle, buffaloes, and pigs in Malaysia (Nurulaini et al., 2013).

The results of this study highlight the prevalence of hemoparasites in cattle, with *Babesia* spp. showing the highest prevalence at 12%, followed by *Anaplasma* spp. at 6%, showing similar prevalence rate in Ethiopia 11.7% and 6.1% respectively (Abdela et al., 2018) *Theileria* spp. at 5.33%, that show similar result 5.14% reported in Turkey (Altay et al., 2008). and *Babesia spp.* was notably more prevalent compared to *Anaplasma* spp., and *Anaplasma* spp. was found to be more prevalent than *Theileria* spp. indicating that it may not be as widely distributed or may have lower transmission rates in the area. This result was similar to findings of (Siddique et al., 2020) from Pakistan. Through the questionnaire survey in my research, most of the cattle are not vaccinated and they are

taken for open grazing which directly show the exposure to the vectors due to which there is high chance of infection of various parasites. Result also shows expected prevalence from the questionnaire survey.

In the current study, the sex-wise prevalence showed that *Babesia* species had a higher prevalence in both male and female cattle compared to other protozoan parasites. Whereas, *Anaplasma* spp. was higher in female and *Theileria* spp. was higher in males, which could be attributed to factors such as hormonal difference reproductive status, or differences in immune responses between the sexes. In this study female prevalence rate was higher than male that is 19.75%, 27.53% respectively which is similar with the findings of (Abdela et al., 2018).

Finally, the ward-wise prevalence analysis showed that Ward 16 had the highest prevalence (28.20%) of hemoparasites, followed by ward 12 (24.32%) and ward 8 (21.62%) while Ward 4 (18.91%) had the lowest prevalence. Where *Babesia* had the higher prevalence rate in every ward followed by *Anaplasma* and *Theileria* had lowest prevalence rate in every ward. This could be due to varying environmental or management conditions across different wards, such as differences in vector density, animal husbandry practices, or geographical factors that influence the exposure to vectors like ticks. This variation highlights the importance of local factors in parasite transmission and underscores the need for targeted interventions to control parasitic infections in specific areas.

6. Conclusion and recommendation

6.1 Conclusion

In conclusion, the study found a 35(23.33%) overall prevalence of blood protozoan parasites in cattle from Ghorahi, Dang out of 150 samples. The prevalence did not show significant differences based on sex female cattle had higher infection rates than male cattle. However, age-wise analysis revealed higher prevalence rate in younger cattle (≤3 years) than older cattle (>3 years). Among the different hemoparasites, *Babesia* spp. had the highest prevalence (12%), followed by *Anaplasma* spp. (6%), *Theileria* spp. (5.33%). In terms of geographical variation, there was no significant difference in prevalence across the selected wards, although Ward-16 showed the highest prevalence suggesting that other factors may be influencing the infection rates in cattle in this region.

Regarding the knowledge, attitude, and practices (KAP) of cattle owners, it was found that all of them owned only local cattle under the traditional system, and none had received livestock farming training. Pond water was the primary source for drinking, and most cattle owners did not use any parasitic treatments. Cattle were allowed to graze in open fields, and deworming practices were not followed by the owners during management.

6.2 Recommendations

Based on the results of this study, the following recommendations have been suggested to minimize the risk of hemoparasitic diseases in cattle.

- Regular monitoring of cattle for parasitic infections should be conducted periodically.
- Cattle farmers should be informed about the negative effects of parasites and the advantages of deworming because it appears that they lack knowledge about haemoprotozoan parasites.
- Additional research at the molecular level should be conducted.

7. References

Abdela, N., Ibrahim, N., & Begna, F. (2018). Prevalence, risk factors and vectors identification of bovine anaplasmosis and babesiosis in and around Jimma town, Southwestern Ethiopia. *Acta Tropica*, *177*, 9–18. https://doi.org/10.1016/j.actatropica.2017.09.010

Abdullah, D. A., Ali, M. S., Omer, S. G., Ola-Fadunsin, S. D., Ali, F. F., & Gimba, F. I. (2019). Prevalence and climatic influence on hemoparasites of cattle and sheep in Mosul, Iraq. *Journal of Advanced Veterinary and Animal Research*, *6*(4), 492–498. https://doi.org/10.5455/javar.2019.f373

Akande, F. A., Takeet, M. I., & Makanju, O. A. (2010). Haemoparasites of Cattle in Abeokuta, South West Nigeria. *World Science Journal*, *5*(4), 19–21. www.scienceworldjournal.org

Al-Khalifa, M. S., Hussein, H. S., Diab, F. M., & Khalil, G. M. (2009). Blood parasites of livestock in certain Regions in Saudi Arabia. *Saudi Journal of Biological Sciences*, *16*(2), 63–67. https://doi.org/10.1016/j.sjbs.2009.10.002

Altay, K., Aydin, M. F., Dumanli, N., & Aktas, M. (2008). Molecular detection of Theileria and Babesia infections in cattle. *Veterinary Parasitology*, *158*(4), 295–301. https://doi.org/10.1016/j.vetpar.2008.09.025

Aregawi, W. G., Agga, G. E., Abdi, R. D., & Büscher, P. (2019). Systematic review and meta-analysis on the global distribution, host range, and prevalence of Trypanosoma evansi 07 Agricultural and Veterinary Sciences 0707 Veterinary Sciences. *Parasites and Vectors*, *22*(3), 1–25.

Article, O. (2021). *Sahel Journal of Veterinary Sciences*. *18*(3), 8–18. Barros, J. H. S., Xavier, S. C. C., Bilac, D., Lima, V. S., Dario, M. A., & Jansen, A. M. (2017). Identification of novel mammalian hosts and Brazilian biome geographic distribution of Trypanosoma cruzi TcIII and TcIV. *Acta Tropica*, *172*(April), 173–179. https://doi.org/10.1016/j.actatropica.2017.05.003

Batista, J. S., Riet-Correa, F., Teixeira, M. M. G., Madruga, C. R., Simões, S.D. V, & Maia, T. F. (2007). Trypanosomiasis by Trypanosoma vivax in cattle in the Brazilian semiarid: Description of an outbreak and lesions in the nervous system.

Veterinary Parasitology, *143*(2), 174–181. https://doi.org/https://doi.org/10.1016/j.vetpar.2006.08.017

Belal, S. S. H., Mahmud, M. A. Al, & Ferdous, M. J. (2015). Prevalence of Anaplasmosis in cattle in Sirajganj district of Bangladesh. *Research in Agriculture Livestock and Fisheries*, *1*(1), 97–103. https://doi.org/10.3329/ralf.v1i1.22370

Bhatnagar, C. S., & Bhardawaj, B. (2015). *Original Research Article Incidence* of Haemoprotozoan diseases in Cattle in Southern Rajasthan , India. 4(3), 509–514.

Bock, R., Jackson, L., De Vos, A., & Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology*, *129*(SUPPL.). https://doi.org/10.1017/S0031182004005190

Bock, R., Jackson, L., De Vos, A., & Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology*, *129*(SUPPL.). https://doi.org/10.1017/S0031182004005190

Bohman, W., Chooruang, N., Sakuna, K., Jarujareet, W., Areekit, K., & Chantip, D. (2024). Prevalence of hemoparasitic infections and influencing factors among fighting bulls in Southern Thailand: A retrospective analysis. *Open Veterinary Journal*, *14*(10), 2587–2598. https://doi.org/10.5455/OVJ.2024.v14.i10.8

Cayla, M., Rojas, F., Silvester, E., Venter, F., & Matthews, K. R. (2019). African trypanosomes. *Parasites and Vectors*, *12*(1), 1–8. https://doi.org/10.1186/s13071-019-3355-5

Darghouth, M., Bouattour, A., Miled, L. Ben, & Sassi, L. (1996). Diagnosis of Theileria annulata infection of cattle in Tunisia: Comparison of serology and blood smears. *Veterinary Research*, *27*(6), 613–621.

Desquesnes, M., Holzmuller, P., Lai, D.-H., Dargantes, A., Lun, Z.-R., & Jittaplapong, S. (2013). Trypanosoma evansi and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *BioMed Research International*, *2013*, 194176. https://doi.org/10.1155/2013/194176

Dhakal, M., Gompo, T. R., Devkota, P., Kafle, S. C., Subedi, J. R., Gong, H., Arima, H., Culleton, R., Asada, M., & Pandey, K. (2023). Molecular Detection and Identification of Piroplasm in Cattle from Kathmandu Valley, Nepal. *Pathogens*, *12*(8). https://doi.org/10.3390/pathogens12081045

Dumler, J. S., Barbet, A. F., Bekker, C. P. J., Dasch, G. A., Palmer, G. H., Ray, S. C.,

Rikihisa, Y., & Rurangirwa, F. R. (2001). Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: *International Journal of Systematic and Evolutionary Microbiology*, *51*(6), 2145–2165. https://doi.org/10.1099/00207713-51-6-2145

Ellis, J. T., Morrison, D. A., & Reichel, M. P. (2003). Genomics and its impact on parasitology and the potential for development of new parasite control methods. *DNA and Cell Biology*, *22*(6), 395–403. https://doi.org/10.1089/104454903767650667

Farooqi, S. H., Ijaz, M., Rashid, M. I., Aqib, A. I., Ahmad, Z., Saleem, M. H., Hussain, K., Islam, S., Naeem, H., & Khan, A. (2017). Molecular epidemiology of Babesia bovis in bovine of Khyber Pakhtunkhwa, Pakistan. *Pakistan Veterinary Journal*, *37*(3), 275–280.

Ferris, N. P., Donaldson, A. I., Shrestha, R. M., & Kitching, R. P. (1992). A review of foot and mouth disease in Nepal. *Revue Scientifique et Technique (International Office of Epizootics)*, *11*(3), 685–698. https://doi.org/10.20506/rst.11.3.625

Gebrekidan, H., Nelson, L., Smith, G., Gasser, R. B., & Jabbar, A. (2017). An outbreak of oriental theileriosis in dairy cattle imported to Vietnam from Australia. *Parasitology*, *144*(6), 738–746. https://doi.org/10.1017/S0031182016002328

Ghosh, S., Bansal, G. C., Gupta, S. C., Ray, D., Khan, M. Q., Irshad, H., Shahiduzzaman, M., Seitzer, U., & Ahmed, J. S. (2007). Status of tick distribution in Bangladesh, India and Pakistan. *Parasitology Research*, *101*(SUPPLEMENT 2). https://doi.org/10.1007/s00436-007-0684

Gibbs, W. W. (2004). From finish to start. Was the grand challenge robot race in March the fiasco it appeared to be? Hardly, argues William “Red” Whittaker. The annual event is pushing mobile robotics to get real. *Scientific American*, *291*(2), 33–34. http://www.ncbi.nlm.nih.gov/pubmed/15298117

Githaka, N. W., Bishop, R. P., Šlapeta, J., Emery, D., Nguu, E. K., & Kanduma, E. G. (2022). Molecular survey of Babesia parasites in Kenya: first detailed report on occurrence of Babesia bovis in cattle. *Parasites and Vectors*, *15*(1), 1–11. https://doi.org/10.1186/s13071-022-05279-7

Gohil, S., Herrmann, S., Günther, S., & Cooke, B. M. (2013). Bovine babesiosis in the 21st century: Advances in biology and functional genomics. *International Journal for Parasitology*, *43*(2), 125–132. https://doi.org/10.1016/j.ijpara.2012.09.008

Gökalp. (1981). Short communication. *Combustion Science and Technology*, *25*(5–6), 241–242. https://doi.org/10.1080/00102208108547506

Gupta, V., Gupta, R., & Shrestha, S. P. (2013). Infectivity of Theileria annulata in Hyalomma ticks of Eastern Terai districts, Nepal. *Nepalese Journal of Zoology Online*, *1*(1), 15–23.

Haan, D. (n.d.). I C0P. In *World Petroleum.*

Holland, W. G., Claes, F., My, L. N., Thanh, N. G., Tam, P. T., Verloo, D., Büscher, P., Goddeeris, B., & Vercruysse, J. (2001). A comparative evaluation of parasitological tests and a PCR for Trypanosoma evansi diagnosis in experimentally infected water buffaloes. *Veterinary Parasitology*, *97*(1), 23–33. https://doi.org/10.1016/S0304-4017(01)00381-8

Ibn Zubairu, A., Midau, A., Dazala, I. U., Yahya, M. M., & Buba, Z. M. (2013). The prevalence of bovine trypanosomiasis in song local government area of Adamawa state, Nigeria. *Global Veterinaria*, *11*(3), 310–313. https://doi.org/10.5829/idosi.gv.2013.11.3.7547

Irshad, N., Qayyum, M., Hussain, M., & Khan, M. Q. (2010). Prevalence of tick infestation and theileriosis in sheep and goats. *Pakistan Veterinary Journal*, *30*(3), 178–180.

Khanal, P., Dhakal, R., Khanal, T., Pandey, D., Devkota, N. R., & Nielsen, M. O. (2022). Sustainable Livestock Production in Nepal: A Focus on Animal Nutrition Strategies. *Agriculture (Switzerland)*, *12*(5), 1–20. https://doi.org/10.3390/agriculture12050679

Khankhawash, G. (2018). *Haemo-Protozoan Parasites of Cattle in Gorkha*.

Kispotta, S., Islam, M. F., Hoque, M. F., Rahman, M. S., Borman, A., Haque, M. A., & Rahman, M. R. (2017). Study of prevalence and associated risk factors of anaplasmosis and theileriasis in cattle. *Asian Journal of Medical and Biological Research*, *2*(4), 567–576. https://doi.org/10.3329/ajmbr.v2i4.30998

Kocan, K. M., De la Fuente, J., Guglielmone, A. A., & Meléndez, R. D. (2003). Antigens and Alternatives for Control of Anaplasma marginale Infection in Cattle. *Clinical Microbiology Reviews*, *16*(4), 698–712. https://doi.org/10.1128/CMR.16.4.698-712.2003

Kohli, S., At, U. K., & Thapliyal, A. (2014). Prevalence of theileriosis in cross- bred cattle : Its detection through blood smear examination and polymerase chain reaction in Dehradun district, Uttarakhand, India. 7, 168–171.

Krishna murthy, C. M., Ananda, K. J., & Adeppa, J. (2016). Prevalence of Haemoprotozoan infections in bovines of Shimoga region of Karnataka state. *Journal of Parasitic Diseases*, *40*(3), 890–892. https://doi.org/10.1007/s12639-014-0599-z

Krishnamoorthy, P., Akshata, L. G., Jacob, S. S., Suresh, K. P., & Roy, P. (2021). Theileriosis prevalence status in cattle and buffaloes in India established by systematic review and meta-analysis. *Indian Journal of Animal Sciences*, *91*(4), 269–279. https://doi.org/10.56093/ijans.v91i4.114331

Lama, D. (2023). *Analysis of Heavy Metals in Mushroom Produced Using Water Hyacinth*. *March*. https://www.researchgate.net/publication/369439717

Levine, N. D. (1971). Taxonomy of the Piroplasms. *Transactions of the American Microscopical Society*, *90*(1), 2. https://doi.org/10.2307/3224894

Maharana, B. R., Tewari, A. K., Saravanan, B. C., & Sudhakar, N. R. (2016). Important hemoprotozoan diseases of livestock: Challenges in current diagnostics and therapeutics: An update. *Veterinary World*, *9*(5), 487–495. https://doi.org/10.14202/vetworld.2016.487-495

Maharjan, M., & Mishra, D. R. (2006). Trypanosomiasis in domestic animals of Makwanpur district, Nepal. *Annals of the New York Academy of Sciences*, *1081*(November 2006), 320–321. https://doi.org/10.1196/annals.1373.042

Mbaya, A., Ibrahim, U., & Apagu, S. (2011). Trypanosomosis of The Dromedary Camel (*Camelus dromedarius*) and its Vectors in The Tsetse- free Arid Zone of North Eastern, Nigeria. *Nigerian Veterinary Journal*, *31*(3), 195–200. https://doi.org/10.4314/nvj.v31i3.68975

MoALD. (2023). Statistical Information on Nepalese Agriculture 2078/79 (2021/22). *MoALD*, 269. https://medium.com/@arifwicaksanaa/pengertian- use-case-a7e576e1b6bf

Nurulaini, R., Premaalatha, B., Zaini, C. M., Adnan, M., & Chandrawathani, P. (2013). Trypanosomiasis Outbreak in Deer , Cattle , Buffaloes and Pigs in Perak. *Malaysian Journal of Veterinary Research*, *4*(1), 55–58.

Okorafor, U. P., & Nzeako, S. O. (2014). Prevalence of Haemoparasites of Cattle from Three Abattoirs in Ibadan Metropolis, Oyo State, Nigeria. *International Journal of Scientific Research in Environmental Sciences*, *June*, 244–249. https://doi.org/10.12983/ijsres-2014-p0244-0249

Ooshiro, M., Zakimi, S., Matsukawa, Y., Katagiri, Y., & Inokuma, H. (2008). Detection of Anaplasma bovis and Anaplasma phagocytophilum from cattle on Yonaguni Island, Okinawa, Japan. *Veterinary Parasitology*, *154*(3–4), 360–364. https://doi.org/10.1016/j.vetpar.2008.03.028

Otte, J., Hinrichs, J., Rushton, J., Roland-Holst, D., & Zilberman, D. (2008). Impacts of avian influenza virus on animal production in developing countries. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *3*(080), 1–18. https://doi.org/10.1079/PAVSNNR20083080

Phuyal, G. (2018). Prevalence and Risk Factors Associated with Occurrence of Blood Protozoan Disease in Cattle of Ramgram municipality, Nawalparasi District.

Plantharayil, A. B. (2023). *Advanced training in poultry production with special emphasis on recent techniques in antimicrobial resistance and food safety* (Issue July 2023). https://www.researchgate.net/publication/382597740

Rajput, Z. I., Hu, S. H., Arijo, A. G., Habib, M., & Khalid, M. (2005). Comparative study of Anaplasma parasites in tick carrying buffaloes and cattle. *Journal of Zhejiang University: Science*, *6 B* (11), 1057–1062. https://doi.org/10.1631/jzus.2005.B1057

Ritonga, M. Z., Putra, A., Prastia, A., Nasution, F., & Br Ginting, R. (2020). Detection Of Blood Parasites In Cattle In Kutalimbaru Subdistrict, Deli Serdang Regency, North Sumatera. *E3S Web of Conferences*, *151*, 2019– 2021. https://doi.org/10.1051/e3sconf/202015101040

Sahoo, N., Behera, B. K., Khuntia, H. K., & Dash, M. (2017). Prevalence of carrier state theileriosis in lactating cows. *Veterinary World*, *10*(12), 1471– 1474. https://doi.org/10.14202/vetworld.2017.1471-1474

Sajid, M. S., Siddique, R. M., Khan, S. A., Iqbal, Z., & Khan, M. N. (2014). Prevalence and risk factors of anaplasmosis in cattle and buffalo populations of district Khanewal, Punjab, Pakistan. *Global Veterinaria*, *12*(1), 146–153. https://doi.org/10.5829/idosi.gv.2014.12.01.8252

Salim, B., Bakheit, M. A., Salih, S. E., Kamau, J., Nakamura, I., Nakao, R., & Sugimoto, C. (2011). An outbreak of bovine trypanosomiasis in the Blue Nile State, Sudan. *Parasites and Vectors*, *4*(1), 2–6. https://doi.org/10.1186/1756-3305-4-74

Schnittger, L., Rodriguez, A. E., Florin-christensen, M., & Morrison, D. A. (2012). Infection , Genetics and Evolution Babesia : A world emerging. *Infection, Genetics and Evolution*, *12*(8), 1788–1809. http://dx.doi.org/10.1016/j.meegid.2012.07.004

Seangseerattanakulchai, K., & Piratae, S. (2021). Drug resistance in blood parasitic infections in cattle: a review. *Annals of Parasitology*, *67*(4), 583–590. https://doi.org/10.17420/ap6704.374

Selim, A., Weir, W., & Khater, H. (2022). Prevalence and risk factors associated with tropical theileriosis in Egyptian dairy cattle. *Veterinary World*, *15*(4), 919–924. https://doi.org/10.14202/vetworld.2022.919-924

Silva, M. G., Henriques, G., Sánchez, C., Marques, P. X., Suarez, C. E., & Oliva, A. (2009). First survey for Babesia bovis and Babesia bigemina infection in cattle from Central and Southern regions of Portugal using serological and DNA detection methods. *Veterinary Parasitology*, *166*(1–2), 66–72. https://doi.org/10.1016/j.vetpar.2009.07.031

Sharpe, D. V, Fan, L., Hansen, L. T., Doucette, C., Lisa, M., & Fillmore, S. (2013). *Ph ton*. *128*(February), 257–268.

Sitotaw, T., Regassa, F., Zeru, F., & Kahsay, A. G. (2014). Journal of Parasitology and Vector Biology Epidemiological significance of major hemoparasites of ruminants in and around Debre-Zeit, Central Ethiopia. *Journal of Parasitology and Vector Biology*, *6*(2), 16–22. https://doi.org/10.5897/JPVB2014.

Shrestha, S. P., Prajapati, M., Shrestha, R., Kunwar, B. L., & Shrestha, S. P. (2017). Study on Piroplasmosis and Anaplasmosis: Killer Diseases of Diary Cattle. *Proceeding of the 10th National Workshop on Livestock and Fisheries Research in Nepal*, *5*(March 2017), 7.

Siddique, R. M., Sajid, M. S., Iqbal, Z., & Saqib, M. (2020). Association of different risk factors with the prevalence of babesiosis in cattle and buffalos. *Pakistan Journal of Agricultural Sciences*, *57*(2), 517–524. https://doi.org/10.21162/PAKJAS/19.8626

Singh, N. K., Singh, H., Jyoti, Haque, M., & Rath, S. S. (2012). Prevalence of parasitic infections in cattle of Ludhiana district, Punjab. *Journal of Parasitic Diseases*, *36*(2), 256–259. https://doi.org/10.1007/s12639-012- 0119-y

Solano, P., De La Rocque, S., Cuisance, D., Geoffroy, B., De Meeus, T., Cuny, G., & Duvallet, G. (1999). Intraspecific variability in natural populations of Glossina palpalis gambiensis from West Africa, revealed by genetic and morphometric analyses. *Medical and Veterinary Entomology*, *13*(4), 401–407. https://doi.org/10.1046/j.1365-2915.1999.00189.

Tasew, S., & Duguma, R. (2012). Cattle anaemia and trypanosomiasis in western Oromia State, Ethiopia. *Revue de Medecine Veterinaire*, *163*(12), 581–588.

Tay, S. T., Koh, F. X., Kho, K. L., & Ong, B. L. (2014). Molecular survey and sequence analysis of Anaplasma spp. in cattle and ticks in a Malaysian farm. *Tropical Biomedicine*, *31*(4), 769–776.

Thapa, P., Mishra, D., Magar, S. R., Lamsal, D., & Karna, A. K. (2025). Epidemiological Study of Bovine Babesiosis in Siyari Rural Municipality , Rupandehi , Epidemiological Study of Bovine Babesiosis in Siyari Rural Municipality , Rupandehi , Nepal Introduction : January. https://doi.org/10.62418/ijvph.10.2.2024.41-46

Vahora, S. P., Patel, J. V., Patel, B. B., Patel, S. B., & Umale, R. H. (2012). Seasonal incidence of haemoprotozoal diseases in crossbred cattle and buffalo in kaira and anand districts of Gujarat, India. *Veterinary World*, *5*(4), 223–225. https://doi.org/10.5455/vetworld.2012.223-225

Velusamy, R., Rani, N., Ponnudurai, G., & Anbarasi, P. (2015a). Prevalence of intestinal and haemoprotozoan parasites of small ruminants in Tamil Nadu, India. *Veterinary World*, *8*(10), 1205–1209. https://doi.org/10.14202/vetworld.2015.1205-1209

Velusamy, R., Rani, N., Ponnudurai, G., & Anbarasi, P. (2015b). Prevalence of intestinal and haemoprotozoan parasites of small ruminants in Tamil Nadu, India. *Veterinary World*, *8*(10), 1205–1209. https://doi.org/10.14202/vetworld.2015.1205-1209

Wang, C. T., Kubota, S., Kakuda, T., Kuo, C. C., Hsu, T. L., & Onuma, M. (1998). Survey of Theileria Parasite Infection in Cattle in Taiwan. *Journal of Veterinary Medical Science*, *60*(2), 253–255. https://doi.org/10.1292/jvms.60.253

Weerasooriya, G., Sivakumar, T., Lan, D. T. B., Long, P. T., Takemae, H., Igarashi, I., Inoue, N., & Yokoyama, N. (2016). Epidemiology of bovine hemoprotozoa parasites in cattle and water buffalo in Vietnam. *Journal of Veterinary Medical Science*, *78*(8), 1361–1367. https://doi.org/10.1292/jvms.16-0099

Wen, Y. Z., Lun, Z. R., Zhu, X. Q., Hide, G., & Lai, D. H. (2016). Further evidence from SSCP and ITS DNA sequencing support Trypanosoma evansi and Trypanosoma equiperdum as subspecies or even strains of Trypanosoma brucei. *Infection, Genetics and Evolution*, *41*, 56–62. https://doi.org/10.1016/j.meegid.2016.03.022

Yadav, S. K. (2015). PREVALENCE OF BLOOD PROTOZOAN PARASITES OF CATTLE , BUFFALOES AND GOATS OF THREE VDCs OF SIRAHA DISTRICT , NEPAL SUJIT KUMAR YADAV T . U . Registration No . : 5-3-28-98-2010 T . U . Examination Roll No . : 18306.

Zhou, M., Cao, S., Sevinc, F., Sevinc, M., Ceylan, O., Moumouni, P. F. A., Jirapattharasate, C., Liu, M., Wang, G., Iguchi, A., Vudriko, P., Suzuki, H., & Xuan, X. (2016). Molecular detection and genetic identification of Babesia bigemina, Theileria annulata, Theileria orientalis and Anaplasma marginale in Turkey. *Ticks and Tick-Borne Diseases*, *7*(1), 126–134. https://doi.org/10.1016/j.ttbdis.2015.09.008

Zaman, M. A. (2020). Brief Account of Bovine Theileriosis Prevalence in Some South Asian Countries. *Agrobiological Records*, *2*, 38–48. https://doi.org/10.47278/journal.abr/2020.010

8. Appendix:

8.1 Questionnaire Survey:

Prevalence of Blood Protozoans in cattle of Ghorahi Sub Metropolitan City.

(A Questionnaire by Binita Dangi)

- **Owner's Information**
- Name: Contact Information:
- Location : Date of Survey:

Animal Information

SN	Code	Species	age	sex
1				

- **General Health**
  - Has the animal shown any symptoms such as fever, lethargy, or weight loss in the past 6 months? (Yes/No)
  - Has the animal been treated for any illnesses in the past 6 months? (Yes/No) • If yes, specify the illness and treatment:
- **Vaccination and Deworming**
  - Is the animal regularly vaccinated? (Yes/No)
  - If yes, list vaccinations received:
  - Is the animal dewormed regularly? (Yes/No)
  - If yes, how often is deworming performed?
- **Tick or Insect Control**
  - Is the animal treated for ticks or other insects regularly? (Yes/No)
  - If yes, specify the method of control (e.g., topical treatment, sprays, etc.):
- **Housing Conditions**
  - Where is the animal housed? (Indoor/Outdoor/Mixed)
  - How often is the housing cleaned? (Daily/Weekly/Rarely/Never)

- Is the housing shared with other animals? (Yes/No)

- **Water Source**
  - What is the primary source of drinking water for the animal?

    (River/Lake/Well/Pond/Tap/Other)
  - Is the water treated before use? (Yes/No)
- **Feeding Practices**
  - What is the main type of feed given to the animal? (Grass/Fodder/Commercial Feed/Mixed)
  - What is the predominant grazing area for the animal ?
  - (Open pasture, Forested area, Enclosed fields, No grazing)
- **Tick and Insect Exposure**
  - Has the animal been exposed to ticks or other biting insects in the last 6 months?

    (Yes/No)
  - If yes, describe the type of exposure:
- **Travel History**
  - Has the animal traveled to other areas in the past 6 months? (Yes/No) • If yes, specify location and duration of stay:
- **Diagnostic Tests**
  - Have any blood tests or diagnostic tests been performed on the animal recently?

    (Yes/No)
  - If yes, provide details of the test(s) and result(s):
- **Previous Protozoan Infections**
  - Has the animal been diagnosed with any blood protozoan infections before? (Yes/No)
  - If yes, specify the protozoan species and test(s).