Thesis Ref No: ________________

SEROEPIDEMIOLOGY AND ASSOCIATED RISK FACTORS OF *NEOSPORA CANINIUM* IN CATTLE OF PASTORAL PRODUCTION SYSTEM IN TELTELLE DISTRICT OF BORANA ZONE, SOUTHERN ETHIOPIA

MVSc THESIS

BY

KULA JILO TACHE

ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, DEPARTMENT OF CLINICAL STUDIES

MVSc PROGRAM IN VETERINARY EPIDEMIOLOGY

JUNE, 2021

BISHOFTU, ETHIOPIA

SEROEPIDEMIOLOGY AND ASSOCIATED RISK FACTORS OF *NEOSPORA CANINIUM* IN CATTLE OF PASTORAL PRODUCTION SYSTEM IN TELTELLE DISTRICT OF BORANA ZONE, SOUTHERN ETHIOPIA

A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Epidemiology

By

Kula Jilo Tache

June, 2021

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

Title: **Seroepidemiology and Associated Risk Factors of *Neospora Caninium* in Cattle of Pastoral Production System in Teltelle District of Borana Zone, Southern Ethiopia**

Submitted by: **Kula Jilo Tache** ____________ ____________

Name of Student Signature Date

Approved for submittal to a thesis assessment committee

1. Dr. Yitbarek Getachew(DVM, PhD, Asso. Prof) ____________ ____________

Major Advisor **Signature** **Date**

2. Dr. Haileleul Negussie(DVM, MSc, PhD,Asso.Prof) ____________ ____________

Department chairperson **Signature** **Date**

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Kula Jilo Tache** entitled **Seroepidemiology and Associated Risk Factors of *Neospora Caninium* in Cattle of Pastoral Production System in Teltelle District of Borana Zone, Southern Ethiopia** and recommended that it be accepted as fulfilling the thesis requirement for the degree of **Master of Veterinary Science in Veterinary Epidemiology.**

1. ________________________________ ______________ _________________

Chairman (title and name) **Signature** **Date**

2. ________________________________ ______________ _________________

External examiner (title and name) **Signature** **Date**

3. ________________________________ ______________ _________________

Internal examiner (title and name) **Signature** **Date**

DEDICATION

This piece of effort is dedicated to the victims of a covid-19 pandemic in the world.

STATEMENT OF AUTHOR

First, I declare that this thesis/dissertation is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under the rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

Name: **Kula Jilo Tache** Signature:_______________

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission:__________________________

TABLE OF CONTENTS

CONTENTS **PAGE**

TABLE OF CONTENTS(*Continued*)

TABLE OF CONTENTS(*Continued*)

ACKNOWLEDGEMENTS

First and foremost, the author praises God, the almighty, the author of knowledge and wisdom, for the provision of this opportunity and capability to proceed through. Above the level of simplicity, the author would like to express special gratitude to Teltelle District Administration and Teltelle District Livestock Resource Development Office for offering this golden opportunity. Next, the author would like to express deep and sincere gratitude to the research advisor for providing invaluable guidance throughout the research work and availing of test kits in such an unsmooth track and tough time of Covid-19. His humbleness, sympathy, and passion have deeply inspired and motivated the author.

The success of the author would have been not achieved without the contribution and support of collaborative institutions. In doing so, I would like to extend my gratitude to AAU-CVMA for offering me the basic facilities and wholehearted patience in this thesis work. I am very grateful to Dr. Golo Dabbasa the director of YRVL and their staff members for their unreserved material supports. Next, my sincere gratitude goes to Dr. Takele Abayneh director of research and development at NVI along with their highly cooperative staff for invaluable technical supports.

My wife, Haadha Hacaaluu your patience and unwavering stamina in those overlappings of life challenges have realized the fruitfulness of this effort. I am very very much thankful for you, for the love, care, and sacrifices you made for my education. The author feels exceptionally lucky to have such like wife and a wise family. Thank you very much! Long live.

In a challenging circumstance having a true friend maintains one’s morals and spirit. Last but not least, my heartfelt package of gratitude goes to my friends and relatives who voluntarily supported me during sample collection traveling all that many distances on foot at that tough and extremely hot sunny period viz; Gudeta Kanchu, Guyo Gandile, and Gollicha Roba. Your sacrifices are beyond my words of gratitude. Boldly, my friends and families of all time Gobu Jarso, Diba Boru, Tadhi, Boru, Guyo Guduro, Guyo Kuni, Iya Halake, Halake, Halake Elema, Firaol Worku, and many more unlisted you have a special place in my heart for eternity. Finally, I would like to acknowledge that this thesis work is financially supported by AAU Thematic Research Grant 2020 “Dairy Cattle Infectious Diseases”.

LIST OF ABBREVIATIONS

CNS Central Nervous System

CSA Central Statistical Agency

CSF Cerebro Spinal Fluid

CVMA College of Veterinary Medicine and Agriculture

DAT Direct Agglutination Test

DNA Deoxyribonucleic Acid

ELISA Enzyme Linked Immunosorbant Assay

GDP Gross Domestic Product

HIV Human Immuno Deficiency Virus

IFAT Indirect Florescent Antibody Test

IgG Immunoglobulin G

IHT Indirect Haemaglutination Test

ILCA International Livestock Center for Africa

LAT Latex Agglutination Test

MAT Modified Direct Agglutination Test

NVI National Veterinary Institution

OD Opacity Density

PA Peasant Association

PCR Polymerase Chain Reaction

QGIS Quantum Geographic Information System

rpm Revolution per Minutes

SFDT Sabin-Feldman Dye Test

SPNN Southern People Nation and Nationalities

USD United State Dollar

VIF Variance Inflation Factor

YRVL Yabello Regional Veterinary Laboratory

LIST OF TABLES

LIST OF FIGURES

LISTS OF APPENDICES

ABSTRACT

Neosporosis is a cosmopolitan disease that has recently emerged as a major cause of abortion in smallholder dairy farms in Ethiopia. However, the status and impact of Neosporosis in pastoral cattle production settings at large and Borana pastoral area, in particular, is uncovered. Therefore, a cross-sectional study was conducted from October 2020 to May 2021 to determine the seroprevalence and to elucidate potential associated factors for *N.caninium* infection in cattle in the Teltelle district of Borana zone. A total of 180 blood samples were collected from randomly selected herds(n=48) were examined to detect antibodies specific to *N.caninium* using indirect ELISA (ID.vet Innovative diagnostics, ID Screen®, and Montpellier, France). A semi-structured questionnaire was used to gather information about the potential risk factors of *N.caninium* from the owners (n=48) of sampled herds. Among the sampled animals 9 were positive on iELISA. The overall seroprevalence was 5.0% and 14.6% at animal and herd levels respectively. Animal level multivariate logistic regression analysis indicated animals with a history of abortion had significantly higher odds to be *N.caninium* seropositive (AOR=23, 95%CI: 2.354-188.702; P=0.006). Similarly, a significant association of prevalence was noted with the presence of open source of water (AOR=9, 95%CI: 1.599-47.568; P=0.012) and the presence of dogs (AOR=6, 95%CI: 11.213-27.222; P=0.028) respectively. At the herd level, the result of multivariate logistic regression analysis revealed a significantly higher likelihood of *N.caninium* seroraections in the herd with the history of abortion (AOR=16; CI=1.446-175.939; P=0.024) and dystocia (AOR=7; CI=1.008- 45.071; P=0.049) respectively. Therefore, the study revealed for the first time provided evidence for *N. caninium* infection that was significantly associated with history abortion in a pastoral cattle production system. Further confirmatory epidemiological studies and community sensitization to achieve separation of dogs from herds, to do not feeding dogs with raw animal products and provision of hygienic water to animals is recommended.

Key words: *Borana, Cattle, Neospora caninium; Seroepidemiology*

1 INTRODUCTION

Ethiopia possesses the largest livestock population in Africa and stands fifth from the world with a total cattle population estimated to be 59.5 million (ILCA, 2015). In the country, the livestock sub-sector covers 35.6% of total agricultural Gross Domestic Product (GDP) and 16.5% of national GDP respectively(Getabalew *et al.*, 2019). It contributes 30% to agricultural employment and 16% to national export earnings(ILCA, 2015). Down to this, the dairy sub-sector contributes 63% to the total value of ruminant output in Ethiopia(Getabalew *et al.*, 2019). However, the huge potential of livestock input to the national economy in Ethiopia is yet underutilized due to numerous impediments(Dinka, 2013; Hadush *et al.*, 2013; Benti and Zewdie, 2014; Haile *et al.*, 2014). Among them prevailing animal diseases, poor genetic performance, traditional production systems, malnutrition, and insufficient veterinary services are the major bottlenecks(Benti and Zewdie, 2014; Tilahun *et al.*, 2018). Reproductive performance is one of the most important factors impacting the profitability of the dairy cattle industry(Crowe *et al.*, 2018; Tilahun *et al.*, 2018; Montiel-Olguín *et al.*, 2019). However, reproductive failures are posing high economic loss in cow-calf operations and smallholder dairy cattle in Ethiopia(Dinka, 2013).

Neospora caninum is a cyst-forming apicomplexan protozoan parasite that causes reproductive disorders in dairy industries(Dubey *et al.*, 2007; Dubey and Schares, 2011). *N. caninum* causes reproductive pathologies and neurological conditions in dairy cattle and dogs (Innes *et al.*, 2007). Mainly, it induces abortion and stillbirth in cattle (Dubey *et al.*, 2007); whereas neonatal neuromuscular diseases are frequent in dogs(Dubey *et al.*, 2005 Innes *et al.*, 2007; Webster, 2010). Dogs and other members of felids are the definitive hosts of *N. caninum* that shed oocysts in their feces (Innes *et al.*, 2007). Cattle and other domestic animals are intermediate hosts and acquire the infections via ingestion of feed or water contaminated with oocysts of the parasite (Dubey *et al.*, 2007). *N. caninum* can be transmitted via horizontal, vertical, and lactogenic routes (Innes *et al.*, 2007; Al-qassab *et al.*, 2010).

Globally, *N.caninium* is the most serious cause of economic losses in dairy industries(Wouda, 2000). It’s highly abortifacient in cattle, where the risk of abortion is 3 to 7 times higher in infected cows and as high as 7.4 folds in congenitally infected heifers(Innes *et al.*, 2007). It also diminishes milk production by 84.7 Kg per lactation period in an infected cow (Zuniga, 2005). In the USA dairy industry, *N. caninum* attributes for 20% of overall abortions cases and 67% of economic losses incurred ($2.38 billion) by the industry per annum (Dubey *et al*., 2007; Reichel *et al.,* 2013). Similarly, *N. caninum* was found attributable for 12.5-16% proportions of all the annual bovine abortions in several countries(Haddad *et al.,* 2005*;* Moore *et al.,* 2013; Reichel *et al.,* 2013*).* Even though, there is no firm evidence about *N. caninum* infection in humans; detections of genetic materials and antibodies in humans and primates suggest potential zoonosis neosporosis(Lobato *et al.,* 2006; Pagmadulam *et al.,* 2018; Duarte *et al.*, 2020; Reichel *et al.*, 2020).

N. caninum is distributed worldwide; however, its more prevalent in warm climates and humid areas than in cold and dry regions(Pagmadulam *et al.*, 2018). Primarily the epidemiology of neosporosis is associated with the presence of definitive hosts(Dubey *et al*., 2007). A large number of serological surveys revealed that neosporosis is highly prevalent in dairy cattle worldwide(Dubey and Schares, 2011). Its prevalence in cattle has been estimated to be between 7.6% and 97.2% in America (Cerqueira *et al.,* 2017; Alexander *et al.,* 2018; Deaquino *et al.,* 2019),3.9% and 24.1% in Africa(Ghalmi *et al.,2012;* Asmare *et al.,* 2013b, Asmare, 2014; Fereig *et al.,* 2016; Mathew *et al.,* 2017; Olum *et al.,* 2020), 0.5% and 60% in Asia(Kul *et al.,* 2009; Dubey and Schares, 2011; Yadav *et al.,* 2016; Marzieh *et al.,* 2019), 0.7% and 76% in Europe(Bartels *et al.,* 2006; Imre *et al.,* 2012; Lefkaditis *et al.,* 2020) and 3.2% to 46.7% in Australia(Nasir *et al.,* 2012; Calarco and Ellis, 2020).

The aforementioned literatures imply increased attention to Neosporosis globally because of economic losses associated with abortion, stillbirth and reproductive inefficiency from infection in dairy cattle. In Ethiopia, the first attempt of *N.caninium* detection that has been made recently at urban, peri-urban and commercial dairy production systems implied that the agent is posing a deleterious effect on dairy and breeding cattle systems(Asmare *et al.(*2013a, 2013b); Asmare, 2014). Asmare and colleagues reported seroprevalence of dairy cattle neosporosis ranging from 13.3% to 23.8% in selected milk shed areas in Ethiopia(Asmare *et al.(*2013a, 2013b); Asmare,2014). Comparatively *N. caninum* was identified as the leading cause of abortion followed by Bovine Viral Diarrhoea Virus and Brucella species in dairy cattle urban and peri-urban smallholder dairy of Ethiopia(Asmare, 2014).

However data on the epidemiological status and the potential risk factors *N. caninium* in dairy cattle of Ethiopia is scarce, particularly nothing has been attempted in the pastoral area of Borana zone. In Borana pastoral area cattle play a pivotal role in the livelihoods as a source of milk, immediate cash income and for the breeding service. In Borana pastoral community, cats and dogs are also an integral part of livestock for protection against predators and rodents(Jilo *et al.*, 2021). Particularly, dogs are used as the second herdsman and kept with cattle at grazing land and watering points that could result in contamination of water and pasture. Moreover, surface water used for livestock that can openly accessible by wild felids may elicit the risk of neosporosis in cattle. Therefore, the present study attempted to provide the first insight into the epidemiology of *N. caninium* in cattle of pastoral production system specifically, cattle in Teltelle district of Borana zone. The following were the specific objectives of the study:

1.1 Specific Objectives

- To determine the seroprevalence of neosporosis in Borana cattle of the Teltelle district
- To assess associated risk factors of *N. caninium* infection in Borana cattle of pastoralists in the study district

2 LITERATURE REVIEW

2.1 Historical Background

Bjerkas *et al.*(1984) reported the first case of Neosporosis from Norway in a dog with encephalomyelitis and myositis under the name of Toxoplasma-like protozoan. Then, until 1988 *N. caninum* was misdiagnosed as *Toxoplasma gondii* because of close morphologically similarities. However, in 1988 it was isolated from the canine in the USA by cell culture and named as *N.caninum*(Dubey *et al.,* 1988). Later, in 1993 Californian researchers isolated Neospora organisms from bovine fetuses and classified Neospora in the family Sarcocystidae and phylum Apicomplexa(Dubey and Lindsay, 1996). Subsequently, Neospora was found to be a cause serious disease of cattle and dogs worldwide(Dubey and Lindsay, 2006). In addition, anti-bodies of *N.caninium* have been found in the sera of many species of domestic, wild mammals, marine mammals, avian species as well as human beings(Dubey and Lindsay, 2006; Dubey and Schares, 2011; Duarte *et al.*, 2020).

2.2 Etiology and Morphology

Morphologically, by electron microscopy, *N. caninum* has a sub-cellular structure typical for family Sarcocystidae, subclass Coccidiasina of the phylum Apicomplexa(Dubey and Schares, 2011). It is an obligate intracellular coccidian parasite structurally very resembles *T. gondii* and genus Hammondia(Zuniga, 2005; Dubey *et al.,* 2007). There are two species of Neospora currently recognized: *N. caninum* and *N.hughesi*(Dubey and Lindsay, 2006) The former causes clinical disease in dogs, cattle, sheep, and equines and many wild animal species and *N.hughesi* is associated with reproductive losses and myoencephalitis in horses(Dubey and Schares, 2011). There are three infectious stages of *N.caninium* viz tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts). Tachyzoites and tissue cysts are the stages found in the intermediate hosts, and they occur intracellularly(Dubey and Lindsay,2006). Tachyzoites are approximately 6 µm ×2 µm long in size. Tissue cysts are oval up to 107 µm long and are primarily found in the central nervous system and muscles (Williams *et al.*, 2009). Oocysts are excreted unsporulated in feces of definitive hosts and measure 12 µm in diameter, and sporulation occurs outside the host(Mcallister, 2016)(Figure 1). Environmentally, unsporulated oocysts are resistant while nothing is known about the survival of unsporulated ones in the environment(Dubey and Schares, 2011).

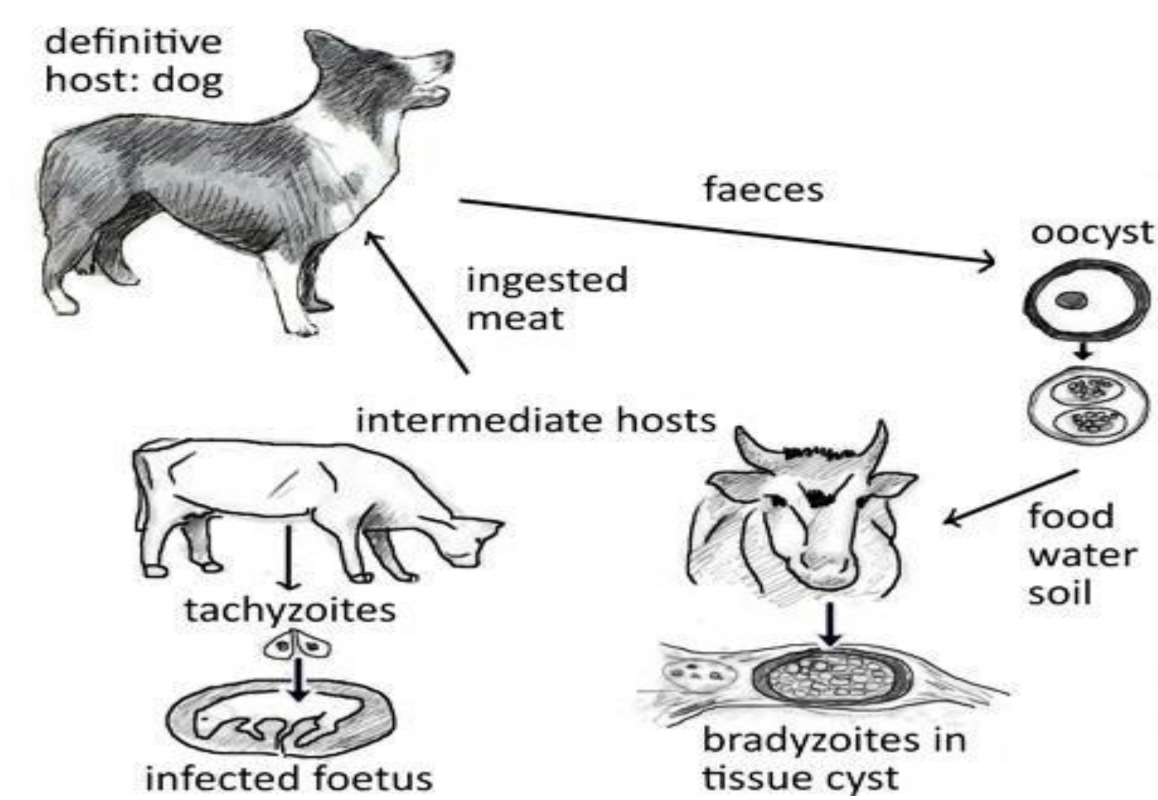


Figure 1: Schematic sketch of *N.caninium* structure in the intermediate and definitive hosts

Source: Dubey *et al.,* 2007

2.3 Life Cycle

N.caninum has heteroxenous life cycles. dogs, grey wolves, coyotes and dingoes are definitive hosts(Dubey and Lindsay(1996,2006); Innes *et al.,* 2007). Many species of domestic, wild and marine mammals are intermediate hosts (Mcallister, 2016). The life cycle of *N. caninium* consists of asexual and sexual phases of reproduction (Haddad *et al.,* 2005; Al-qassab *et al.,* 2010). In the intermediate hosts, only the asexual cycle takes place whereas both sexual and asexual cycles can occur in the definitive (Dubey and Lindsay, 2006; Razmi and Barati, 2017)(figure 1). After the ingestion by definitive hosts, the wall of tissue cysts is dissolved by proteolytic enzymes in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine and initiate the development of numerous generations of *N.caninium.* Besides systemic dissemination after conversion to the invasive tachyzoite stage, some organisms inside the epithelium of the definitive host undergo five different developmental stages that reproduce asexually by endodyogeny, where two daughter cells are created inside one and by schizogeny to differentiate into micro and macro gametocytes within 2 days of infection and involves the formation of multiple merozoite cells around a previously divided nucleus. hosts(Mcallister, 2016). The gametes fuse to form a zygote, which subsequently secretes a cyst wall to develop into oocysts. Oocysts rupture the intestinal epithelial cells to disseminate into the lumen and are excreted in feces 5 days after ingesting infected tissues but the total duration of oocyst shedding after primary infection can vary from 1 to several days(Dubey *et al.*, 1998).

Unsporulated oocysts shed by definitive hosts sporulate in the environment to form 2 sporocysts, each containing 4 sporozoites in both cases(Dubey and Lindsay, 2006; Innes *et al.*, 2007; Webster, 2010). Intermediate hosts ingest oocysts that are found in contaminated food and water then sporozoites are released in the intestinal tract where they penetrate cells and become tachyzoites (a rapidly dividing asexual phase). Tachyzoites divide and quickly spread to other host cells, which they invade and often destroy(Laura *et al.*, 2017)(figure 2).

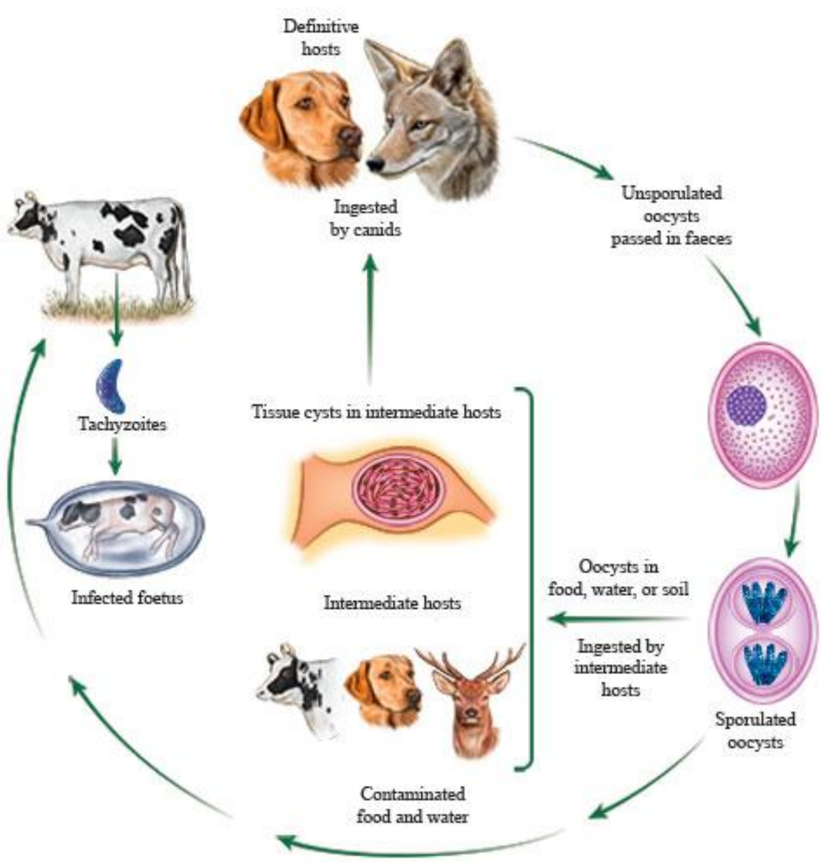


Figure 2: Life cycle of *Neospora caninum*,
Source:(Dubey and Lindsay, 2006).

2.4 Epidemiology of *N.caninium* in Dairy Cattle

2.4.1 Source of infections and transmissions

All three infectious stages of *N. caninum* (tachyzoites, bradyzoites, and oocysts) are involved in the transmission of the parasite routes(Silva and Machado, 2016). Horizontally, the definitive hosts acquire the infections following the consumption of bradyzoites within tissue cysts of intermediate host or rodents, or via ingestion of oocysts within their food or water(Dubey *et al.,* 2007; Laura *et al.,* 2017). In herbivores, it transmits via intake of water or foods contaminated by sporulated oocysts (Innes *et al.,* 2007; Al-qassab *et al*., 2010). Vertically, *N. caninum* can be transmitted from the dam to the fetus during pregnancy. Particularly, in cattle, *N. caninum* is one of the most efficiently transplacentally transmitted parasites among all known microbes with an estimated endogenous infection between 78 and 95% in successive pregnancies(Albuquerque *et al.,* 2011))(Figure 3). Moreover, postnatally, calves may become infected after ingestion of colostrum or milk contaminated with tachyzoites(Corbellini *et al*., 2006; Al-qassab *et al., 2010;* Laura *et al.,* 2017). Although, DNA of *N. caninum* has been detected in the semen of naturally infected bull venereal transmission of *N.caninium* has not been confirmed (Ferre *et al.,* 2005).

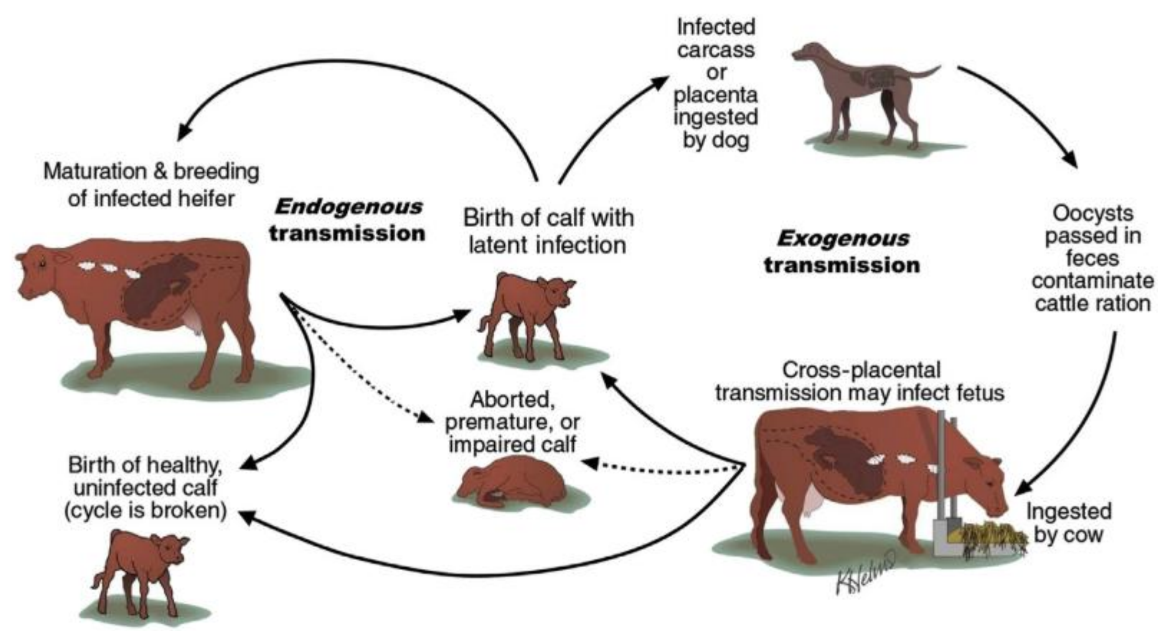


Figure 3: Transmissions of *N.caninium* in cattle
Source:(Mcallister, 2016)

2.4.2 Geographical distribution

The geographical distribution of *N. caninum* is worldwide; however, the infections are more prevalent in warm climates and humid areas than in cold and dry areas due to favorable conditions for sporulation and survival of oocyst(Pagmadulam, *et al.*, 2018; Aguado-mart *et al.,* 2016). Globaaly, neosporosis is highly prevalence in cattle with prevalence estimated to be between 7.6% and 97.2% in America (Cerqueira *et al.,* 2017; Alexander *et al.,* 2018; Deaquino *et al.,* 2019),3.9% and 24.1% in Africa(Ghalmi *et al.,2012;* Asmare *et al.*, 2013b, Asmare, 2014; Fereig *et al.*, 2016; Mathew *et al.*, 2017; Olum *et al.*, 2020), 0.5% and 60% in Asia(Kul *et al.,* 2009; Dubey and Schares, 2011; Yadav *et al.,* 2016; Marzieh *et al.,* 2019), 0.7% and 76% in Europe(Bartels *et al.*, 2006; Imre *et al.*, 2012; Lefkaditis *et al.*, 2020) and 3.2% to 46.7% in Australia(Nasir *et al.,* 2012; Calarco and Ellis, 2020)(Table 1).

Table 1: Prevalence of *N.caninium* in cattle in some countries of the world

Country	No. Examined	Prevalence (%)	Test	Reference
Portugal	119	49	ELISA	(Thompson *et al.*, 2001)
Uruguay	4444	13.9	ELISA	(Banales *et al.*, 2006)
Kenya	552	24.1	ELISA	(Olum *et al.*, 2020)
Egypt	301	18.9	ELISA	(Fereig *et al.*, 2016)
Sudan	262	10.7	ELISA	(Ibrahim *et al.*, 2012)
Senegal	196	17.9	ELISA	(Kamga-waladjo *et al.*, 2010)
Algeria	102	3.9	ELISA	(Ghalmi *et al.*, 2009)
Colombia	294	76.9	ELISA	(Peña *et al.*, 2012)
Brazil	575	97.2	IFAT	(Cerqueira *et al.*, 2017)
Norway	1657	0.7	ELISA	(Klevar *et al.*, 2010)
Sweden	100	0.5	ELISA	(Bartels *et al.*, 2006)
Iran	184	3.8	ELISA	(Marzieh *et al.*, 2019)
Mongolia	1438	26.2	ELISA	(Pagmadulam *et al.*, 2018)
Tanzania	658	4.5	ELISA	(Mathew *et al.*, 2017)
Spain	7577	76	ELISA	(Bartels *et al.*, 2006)
Nepal	186	4.8	ELISA	(Yadav *et al.*, 2016)
Australia	133	2.7	ELISA	(Nasir *et al.*, 2012)
China	262	17.2	ELISA	(Yu *et al.*, 2007)
Pakistan	240	43	ELISA	(Nazir *et al.*, 2013)
Turkey	25	60	ELISA	(Kul *et al.*, 2009)
Korea	108	4.1	ELISA	(Kim *et al.*, 2002)
Japan	2420	5.7	ELISA	(Koiwai *et al.*, 2006)
Czech Republic	463	5.8	IFAT	(Vaclavek *et al.*, 2003)

2.4.3 Host range

The anti-bodies of *N.caninium* have been found in sera of a wide range of hosts including domestic, free-range land mammals, marine mammals as well as human beings(Dubey and Lindsay, 2006; Dubey and Schares, 2011; Duarte *et al.,* 2020). However, it primarily affects cattle and dogs(Duarte *et al.,* 2020). The oocysts of *N. caninum* have been isolated from cattle, sheep, dogs, horses, bison, white-tailed deer, and water buffaloes by bioassays techniques(Dubey and Schares, 2011; Almeria, 2013; Mcallister, 2016; Duarte *et al.,* 2020).

2.4.4 Risk factors

Primarily the prevalence rate of neosporosis is associated with the presence of definitive hosts that excrete oocysts(Dubey *et al.,* 2007). Domestic fowls also found as mechanical vectors of oocysts for dairy cattle infections(Wouda, 2000) epidemiological studies revealed that the presence of farm dogs, either currently or within the past 10 years, the number of farm dogs was a risk factor for seropositivity of neosporosis in cattle (Williams *et al.*, 2009). Generally, the prevalence of bovine neosporosis is determined by the presence/absence of dogs/other felids, geographical area, management system, water sources, stocking density, herd size, immune status, number of parity, and age of the animal(Dubey *et al.,* 2007; Robayo-Sanchez *et al.,* 2017; Schneider, 2018). Large herd sizes have a higher risk of the disease as a higher number of animals are usually kept under the extensive or semi-intensive system(Yu *et al.,* 2007).

The risk of neosporosis in the dairy herd may increase with age but the risk of abortion decreases with the number of parity(Dubey *et al.,* 2007). The stage and number of gestation are important factors in the outcome of bovine neosporosis(Luká *et al.*, 2018). As pregnancy progresses the immunological maturity of the fetus progresses to defend itself against the invading pathogen (Innes *et al.*, 2007). Immunosuppression may reactivate a chronic Neospora infection in dairy cattle(Pagmadulam *et al.,* 2018). Particularly, in endogenous transplacental transmission, the occurrence of abortions influenced by the immune status of the dam whereas the abortion risk is influenced by the number of oocysts ingested by the dam and the gestational stage in exogenous transplacental transmission(hazardous(Mcallister, 2016; Dubey *et al.,* 2007)(Figure 4).

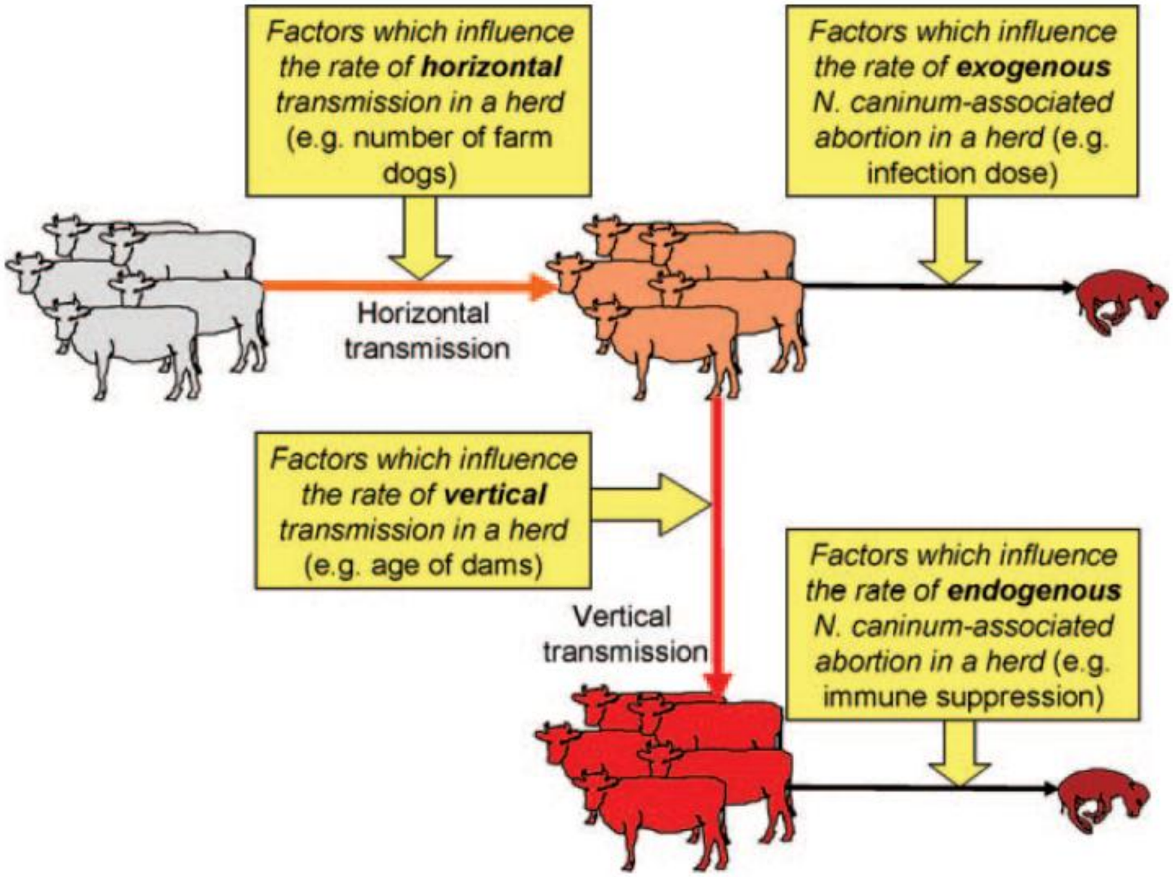


Figure 4: Pictorial diagram of risk factors influencing the transmission of *N.caninum* in cattle
Source:(Dubey *et al.*, 2007)

2.5 Pathogenesis

N.caninium is an obligate intracellular parasite that can infect all nucleated cells of susceptible hosts(Innes *et al.,* 2007). Particularly they induce abortion and stillbirth by multiplying in the placenta and fetal infections(Dubey and Lindsay, 2006). Tachyzoites cause necrosis of cotyledons that can result in the abortion or the birth of weak lambs/kids, which may be accompanied by a mummified fetus(Ahmed *et al.*, 2017).

2.6 Clinical Manifestations

Neosporosis causes reproductive pathologies and neurological conditions in dairy cattle and dogs (Innes *et al.*, 2007). *N. caninum* causes abortion in dairy cattle from 3 months of gestation period to term but mostly abortions occur during mid-gestation from 5 to 6 months of the gestation period(Dubey *et al.,* 2007). By quantitative studies, in many countries indicate that 12% to 42% of aborted fetuses from dairy cattle are infected with *N. caninum*(Dubey and Lindsay, 2006). Other signs such as foetal resorption, mummification, autolysis and stillbirth can be present(Schneider, 2018). Clinically *N.caninium* is asymptomatic in cattle but neurologic signs, hydrocephalus, underweight, ataxia and flexion of limbs may occur in calves less than 2 months of age(Wouda, 2000).

2.7 Diagnosis

The diagnosis of *N.caninum* infections can be made by the isolation parasites, detecting deoxyribonucleic acid (DNA) or their antibodies using serologic, histological and molecular methods(Mcallister, 2016).

2.7.1 Detection of the parasites

A definitive diagnosis rests on the demonstration of the active form of the organism in tissues taken at post mortem examination or in biopsy samples from acute patients by directly visualizing the parasite in the fluid or tissue, but this is a difficult and low yield process(Ahmed *et al.,* 2017). Antigen detection is not 100% accurate due to interspecies antigen cross-reactivity(Luká *et al.,* 2018).

2.7.2 Isolation of the parasites

Neosporosis can be diagnosed by isolation of the parasites from inoculated mice with suspected body fluids (blood, CSF, bronchoalveolar lavage fluid) and subsequent demonstration of tachyzoites or bradyzoites in smears of organs or serous cavities(Dubey and Lindsay, 2006). But the isolation studies may not be helpful for a rapid diagnosis since it takes time(Duarte *et al.,* 2020).

2.7.3 Serological examination

The serological examination is used to indicate the presence of infection by detecting neospora specific antibodies or parasitic antigens in the body fluid of infected individuals. An infected animal develops *N. caninum* IgG antibodies after 2 weeks of inoculation and higher titers 3 weeks after inoculation. Different serological tests such as Sabin-Feldman dye test (SFDT), indirect haemaglutination test (IHT), indirect florescent antibody test (IFAT), latex agglutination test (LAT), direct agglutination test (DAT), modified direct agglutination test (MAT). Enzyme-linked immunosorbent Assay (ELISA), is popularly used to detect the circulating antigens or antibodies for the diagnosis of toxoplasmosis in animals and humans(Dubey and Lindsay, 2006).

2.7.4 Molecular techniques

Polymerase chain reaction (PCR) can be used to amplify extracted from fresh, frozen and formalin or fixed paraffin-embedded tissues of aborted fetuses (Dubey and Lindsay, 2006). Fetal brain tissues appear to be the best sample for PCR detection of *N.caninium* but fetal heart, lung, kidneys and placenta can also yield positive results. *N.caninium* be demonstrated from whole blood, serum and amniotic fluid of infected cow(Dubey and Lindsay, 2006; Mcallister, 2016).

2.8 Economic Importance of *N. Caninium* in Dairy Cattle Industry

N.caninum is a major cause of economic loss in the dairy industry worldwide. The main economic losses incurred by neosporosis infections arise from abortion, stillbirths, neonatal mortality, delays in conception, reductions in milk production, and elevated veterinary services(Reichel *et al.,* 2013). Globally, *N. caninumin* is a major cause of abortion in cattle and the risk of abortion is 3 to 7 times higher in infected cows (Innes *et al.*, 2007). This risk can be as high as 7.4 folds in congenitally infected heifers(Wouda, 2000). On other hand, milk production reduces by 84.7 Kg per lactation period in *N. caninum* infected cow (Zuniga, 2005).

In the USA, two-third of economic losses incurred by the dairy industry are caused by *N. caninum*; it attributes for 20% of abortions in the country (Dubey *et al.,* 2007). As a result, the country losses over 2.380USD billion per annum (Reichel *et al.,* 2013). Epidemiological studies in Scotland showed that 16% of aborted fetuses were seropositive for *N. caninum*(Haddad *et al.,* 2005). Similarly, in UK *N. caninum* is the most frequently diagnosed cause of bovine abortion where about 13% of all bovine abortions are attributable to it. Further study in Wales indicated that the national seroprevalence of the proportion of abortions attributable to *N. caninum* was 12.5%. In Latin America, the annual losses for the dairy industry were estimated to be USD 43.6 million (range, 15.62-194.41 million USD) in Argentina and USD 51.3 million (range, 35.8–111.3 million USD) in Brazil(Haddad *et al.*, 2005; Zuniga, 2005; Moore *et al.*, 2013; Reichel *et al.*, 2013).

2.9 Zoonotic Potential of *N. caninium*

At present, there is no firm evidence about neosporosis in humans since viable *N. caninum* has not been isolated from human tissues so far(Dubey and Lindsay, 2006). However, detections of *N. caninum* genetic materials and antibodies in humans and primates suggest the potential zoonosis aspect of *N.caninium*(Lobato *et al.*, 2006; Pagmadulam *et al.*, 2018; Duarte *et al.*, 2020). For instance, a study from Brazil demonstrated seroprevalence of 38, 18 and 5% in samples from HIV infected individuals, people with neurological symptoms and newborns respectively(Duarte *et al.*, 2020) Moreover, this study also suggested that an association existed between positive serostatus and exposure to dogs(Reichel *et al.*, 2020). Most recently, again in Brazil, the genetic material of *N. caninum* was detected in the umbilical cord blood of the fetus. Furthermore, a quarter of the cord sample was tested by also showed IgG antibodies to *N. caninum* (Duarte *et al.*, 2020). On other hand, primates like rhesus monkeys have been successfully infected with *N. caninum* and thus, there is a concern about the zoonotic potential of *N. caninum*(Haddad *et al.*, 2005).

2.10 Prevention and Control Measures

Currently, there is no safe and effective chemotherapy for bovine neosporosis. However, experimental studies have shown that toltrazuril and its derivative ponazuril are effective on tachyzoites of *N. caninum*(Dubey *et al.*, 2007). Avoiding the introduction of the infection through standard biosecurity measures is the primary goal in *N.caninium* free herds. In the infected herd, the reduction of seropositive cattle and principally by control of definitive host population as a source of oocyst contamination are important. Improvement of biosecurity and maintaining farm hygiene, quarantine and testing of replacement and purchased cattle. Provisions of hygienic water and control of rodents reduce the potential risk of infections in dairy cattle(Dubey *et al.*, 2007; Mcallister, 2016). A more recent effort to prevent Neospora-induced abortion is vaccination. The knowledge to develop an effective vaccine and vaccination strategy against it is increasing(Horcajo, 2016). So far, There is only one live-attenuated vaccines that commercially available have shown good efficacy against exogenous transplacental transmission; however, they have relevant disadvantages and associated risks, which render inactivated or subunit vaccines the best way forward.

2.11 Status of Neosporosis in Dairy Cattle in Ethiopia

In Ethiopia, even though limited studies have been conducted on dairy cattle seroprevalence neosporosis ranging from 13.3 to 23.8% is reported(Asmare *et al.(*2013a, 2013b); Asmare, 2014).Accordingly, the seroprevalence of 13.3% was reported from a comprehensive serosurvey of *N.caninum* in intensive or semi-intensively dairy cattle of Ethiopia*(Asmare et al.,* 2013b). In the same year, the assessment *N.caninum* in breeding and dairy cattle with reproductive disorders revealed seroprevalence of 17.2% from central and southern Ethiopia(Asmare *et al.*, 2013a). Similarly, the seroprevalence of 23.8 % was recorded in urban and periurban smallholder farms of Ethiopia(Asmare, 2014)(Table 2).

Table 2: Seroprevalence of neosporosis in cattle in Ethiopia

Area	No. Tested	Prevalence (%)	Test	Reference
SE	731	23.8	ELISA	(Asmare, 2014)
CSE	402	17·2	ELISA	(Asmare *et al.*, 2013a)
SE	2334	13.3	ELISA	(Asmare *et al.*, 2013b)

SE=Southern Ethiopia; CSE=Central and Southern Ethiopia

3 MATERIALS AND METHODS

3.1 Descriptions of the Study Area

Teltelle district is one of 13 districts of the Borana zone located at the most southern tip of Ethiopia. Milammi, the capital town of the district is 668km far from the Ethiopian capital city Addis Ababa in the southern direction. The district shares border with South Omo in West, Elwayye district in East, Konso zone in the North, Dillo district in North East and the Republic of Kenya in Southern direction. Teltelle has 23 administrative peasant associations, of which 12 are inhabited by pure pastoralists and the remaining 11 are dominated by agro-pastoralists. 'Kolla'is agro-climatic is dominant with latitude ranging from 500 to 1420 meters above sea level. Distinct bimodal rainfall varying from 400 to 650mm is received from September–November (short rainy season) and March-May (long rainy season) annually whereas annual temperature range from 17 to 34^{o}C. Teltelle district is sparsely populated with 72,476 human populations and their livelihoods almost rely on livestock husbandry and to some extent crop production. Due to the scarcity of water and pasture during recurrent droughts in the district and the surroundings, the tension of animal mobility across the Kenyan and SPNN border is high. The livestock components in the area are cattle, goats, sheep, camel and equines. The numbers of livestock of the district are 215, 918 cattle, 170, 055 goats, 76, 785 sheep, 2, 646 camels, 43, 174 chicken, and 9, 201 donkeys(CSA, 2016).

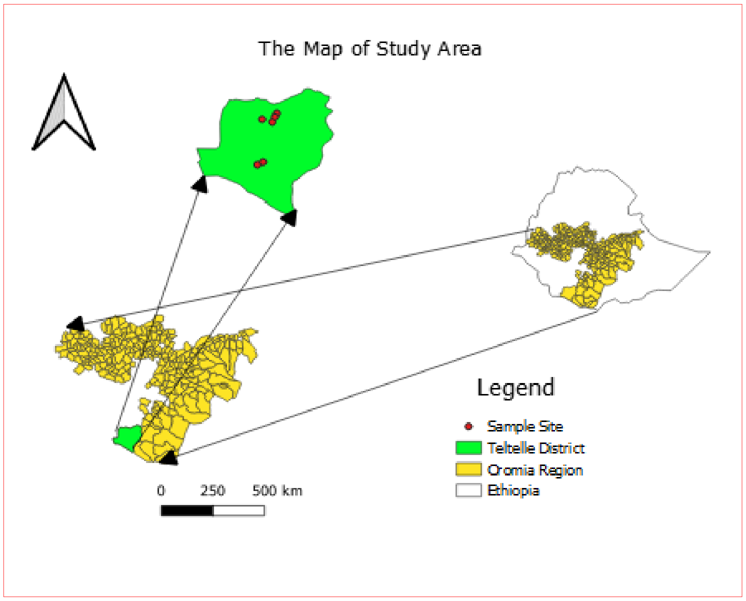


Figure 5: The map of the study area
Source: (Quantum Geographic Information System (QGIS) software 3.14 version).

3.2 Study Population

The population of interest was Borana cattle aged greater than 3 years in the Teltelle district of Borana zone that was managed under a pastoral production system for dual purposes (milk and meat).

3.3 Study Design and Procedures

A cross-sectional study was conducted from October 2020 to May 2021 to detect *N.caninium*, determine the seroepidemiology and potential risk factors of *N.caninium* in cattle in the Teltelle district of the Borana zone.

3.4 Sample size determination

The optimal sample size to establish the seroepidemiology of *N. caninium* in the cattle population was computed using the formula designated for diagnostic kits with predetermined sensitivity and specificity(Thrusfield, 2005).

$$\mathbf{n} = \frac{(\mathbf{1.96}/\boldsymbol{d})^{\mathbf{2}}\,[(\boldsymbol{Se}\times\boldsymbol{Pexp})+(\mathbf{1}-\boldsymbol{Sp})(\mathbf{1}-\boldsymbol{Pexp})][(\mathbf{1}-\boldsymbol{Se}\times\boldsymbol{Pexp})-(\mathbf{1}-\boldsymbol{Sp})-(\mathbf{1}-\boldsymbol{Pexp})]}{(\boldsymbol{Se}+\boldsymbol{Sp}-\mathbf{1})^{\mathbf{2}}}$$

Where n is the required sample size, Pexp is expected prevalence, Se= test sensitivity, Sp= test specificity, d= desired absolute precision. Accordingly, 188 sample size was determined optimum to detect the minimum expected seroprevalence of 13.3% reported by Asmare *et al.*(2013b) with 5% precision by considering predetermined sensitivity (98.7%) and specificity (99.5%) described for ID Screen® *Neospora caninum* Indirect ELISA kit(Alvarado-Esquivel *et al.,* 2014).

3.4.1 Sampling strategies

Multistage sampling method was applied in purposively selected sampling frame of 6 Peasant Associations (PA) namely; Bila, Bule Korma, Gandile, Jerersa, Mekkanisa and Milammi) due to road accessibility. This was followed by the random selection of 8 herds having a minimum of 4 female animals that aged ≥3 years. The age of the animals was determined by dentition as per Parish and Brandi(2013); whereas body condition scores were categorized based on the Ferguson *et al.*(1994) principle. The herd size was conveniently classified as low (<30 heads), medium (30-50) and large (>50) respectively. Then from each selected herd blood sample was randomly collected from 4 female animals with a preference of reproductive problems if available. As result, a total of 188 blood samples were collected from 48 herds. From each sampled herd physiological, history of reproductive problems, environmental conditions and management of the sampled animals and herds were collected from the herd owners (n=48) using a semi-structured questionnaire survey.

3.5 Sample Collection

3.5.1 Blood sample collection

About 10ml of blood sample was collected from the jugular vein of dairy cattle by using plain vacutainer tubes, vein puncture needle and needle holder after disinfecting the skin with alcohol. Each sample was labeled with a unique identification number using a waterproof marker, transferred in the icebox and transported to Yabello Regional Veterinary Laboratory (YRVL) Yabello, Ethiopia and kept at room temperature until serum decantation. Aliquots of sera were obtained by centrifugation at 3000 rpm for 10 min, decanted into cryovial tubes and transported under a cold chain using an ice box to Bishoftu, College of Veterinary Medicine(CVMA) and Agriculture and stored at -20°C until serological examination.

3.5.2 Questionnaires

A semi-structured questionnaire was used to gather information about the potential risk factors of *N.caninium*. This was conducted by face to interviewing the owners of herds during blood sample collection. Accordingly, data about the history of reproductive disorders, putative management and environment-wise risk factors of *N.caninium* in cattle managed under pastoral rearing conditions were collected both at animal and herd levels (Appendix 1).

3.6 Laboratory analysis

3.6.1 Serology procedure

Serological analysis was carried out at National Veterinary Institution (NVI) Bishoftu, Ethiopia. The qualitative serological test was undertaken for the presence of anti *N.caninium* antibodies by indirect ELISA (ID.vet Innovative diagnostics, ID Screen®, and Montpellier, France) according to manufactures instructions. Antigen and sera required for serological testing were taken out from the cold storage and brought to room temperature for 30 minutes before testing. Preparation of the reagents and the ELISA test was performed as per the protocol described by the manufacturer [Appendix 2] at the NVI. Positive and negative controls were included in each test and an animal was considered infected if the serum was presented an OD% > 50% with ELISA. Lastly, the results of 180 sera were produced whereas the remaining 8 sera were used for positive and negative controls.

3.6.2 Methods of Data Analysis

Data generated from laboratory investigation and questionnaire survey was recorded in a Microsoft Excel spreadsheet (Microsoft Corporation), coded and analyzed using R software version 3.6.2. Seroprevalence was calculated by dividing the number of serologically positive samples by the total number of tested samples. Descriptive analysis was applied to describe the outcome to risk factors and variables. In the present study, the percentage of the outcomes was less than 10%(rare event) of the total sample size; thus, the ordinary logistic regression model was not suitable at the animal level (Puhr *et al.*, 2017). Thus, the association of the risk factors or variables for seropositivity of *N.caninium* at the animal level was analyzed using firth's bias reduced logistic regression analysis.

An ordinary logistic regression model was applied to measure the association of the risk factors or variables for seropositivity of *N.caninium* at the herd level. A Pearson's Chi-square ($\chi 2$).was used to measure the significance of serostatus variance between PAs and herds respectively. The association of the assumed risk factors and variables was screened out by univariate logistic regression analysis and risk factors or variables with P-value <0.25(maximum likelihood ratio test) were offered to the final multivariable model. Risk factors or variables having P-value <0.05 were considered significant. Multicollinearity and goodness fit of the models were checked using variance inflation factor (VIF) and Hosmer and Lemeshow tests.

3.6.3 Quality control

Proper animal straining, aseptic blood collection, proper sample handling during transportation and cold chain storage standards were strictly followed. For laboratory investigations, standard operating procedures and manufacturer's instructions were strictly followed. The validity of serological tests was checked by the mean value of positive control opacity density (> 0.350) and the ratio of mean values of positive and negative controls opacity density (> 3) (Appendix 4).

3.6.4 Ethical consideration

The protocol for field studies and collection of animal materials strictly followed the ethical procedures as recommended by the committee of the CVMA. For the questionnaire survey, pastoralists were informed of the aim of the study and their verbal consent was sought before the commencement of data collection (Appendix 1).

3.7 Limitation of the study

Since *N.caninium* investigations are not yet adopted in our country molecular and serological test kit suppliers are not available in Ethiopia; which was the major challenge in the current study. The effort for molecular characterization of *N.caninium* from dead fetal tissues and whole blood of respective dams was not successful due to difficulties encountered to import *N.caninium* molecular kits. As, a result, dead fetal tissues and whole blood samples that were collected for molecular characterization were simply stored at YRVL for future investigation if possible. In addition, the period of blood sample collection was met up with severe drought in the study district. Thus high mobility of pastoralists in search for water and pasture made the study effortful and limited the accessibility of some selected herds in the sampling frame.

4 RESULTS

4.1 Overall Seroprevalence at PAs and herds

The result of the present study revealed that out of 180 sera examined 9 were found positive for anti-*N.caninium* antibodies with an overall seroprevalence of 5.00 %(95%CI: 1.816-8.184). Seroprevalence ranging from 0 to 10% was recorded at the PA level (Table 3). The highest seroprevalence(10%) was found from Milammi and the lowest(0%) encountered from Bule Korma PA. However, the rate of *N.caninium* infection between assessed PAs was statistically insignificant ($\chi2$=3.86; P=0.570). At the herd level, Out of 48 herds examined 7 herds had at least one seropositive animal with an overall herd-level prevalence of 14.60 %(95%CI: 4.598-24.567). Within the herd, seroprevalence ranges from 0 %(0/8) to 25 %(2/8), however, the rate of *N.caninium* infection between the herds was found not significant towards seropositivity of *N.caninium* ($\chi2$=6.85; P=0.231). Overall and herd-level prevalence data are summarized below in Table 3.

Table 3: Summary of seroprevalence of *N.caninium* at PA and herd level in study animals

	Animal level				**Herd level**			
PA	NE	Prevalence(%)	$\chi2$	P-value	NE	Prevalence(%)	$\chi2$	P-value
Bila	30	6.67(2/30)			8	25(2/8)		
Bule Korma	30	0(0/30)			8	0(0/8)		
Gandile	30	6.67(2/30)			8	12.5(1/8)		
Jerersa	30	3.33(1/30)	3.86	0.570	8	12.5(1/8)	6.85	0.231
Makkanisa	30	3.33(1/30)			8	12.5(1/8)		
Milammi	30	10(3/30)			8	25(2/8)		
Total	**180**	**5.00**			**48**	**14.60**		

PA=Peasant Associations; NE=Number Examined; $\chi2$=Pearson Chi-Square

4.2 Animal level seroprevalence and associated risk factors

4.2.1 Physiological risk factors

Age, body condition score, gestation status and the number of parity were considered as physiological risk factors. As a result, seroreactions of 5.36 %(3/56), 4.58 %(5/109) and 6.67 %(1/15) were obtained from animals aged 3-5 years, 6-7 years and ≥ 8 years respectively.

Regarding body condition score, an equal seroprevalence of 5% was recorded in animals with poor (2/40) and good (7/140) body conditions whereas 19.35 %(6/31) and 2.01 %(3/149) were recorded in pregnant and nonpregnant animals respectively. Concerning parity, seroreactions of 5.66 %(3/53), 4.46 %(5/112) and 6.67 %(1/15) were found in animals with number parity 0-2, 3-4 and ≥ 5 respectively. By univariate logistic regression analysis body condition score, gestation status and the number of parity were dropped out (P>0.25) whereas, only the age of the animal remained in the analysis after variables reductions (P < 0.25). However, by the last multivariable logistic regression analysis age was not found significantly associated with seropositivity of *N.caninium* in cattle (P > 0.05). Univariable analysis of physiological factors associated with N.caninium infection is summarized below in table 4.

4.2.2 Clinical reproductive disorders

In this study, the history of clinical reproductive disorders such as abortion, dystocia, retained placenta, stillbirth, infertility, repeated breeding and neonatal mortality in the last year were considered. Accordingly, the proportion of presence was 33.33% in abortion, 22.5% in dystocia, 20% in infertility, 16.9% in both retained placenta and neonatal mortality, 13.2% in repeated breeding and 12.5% in stillbirth respectively. Regarding serostatus, all the 9 seroreactors animals had at least one or more history of reproductive problems (100%). As a result, the seroprevalence of *N. caninium* in cattle with the respective clinical disorder was 77.8% in abortion, 66.7% in infertility,33.3% in dystocia, 22.2% in the retained placenta, 22.2% stillbirth,11.1% repeated breeding and 11.1% neonatal mortality respectively.

Concerning seropositivity, retained placenta, stillbirth, repeated breeding and neonatal mortality were screened out by univariate model (P > 0.25) but abortion, dystocia and infertility were fitted to multivariable model (P< 0.25)(Table 4). By the last model abortion and dystocia were found significantly associated with seropositivity of *N.caninium*(P < 0.05). Accordingly, the odds of acquiring *N.caninium* infection were 23 and 11 times higher in a dairy cattle with the history of abortion(AOR= 23; CI: 2.354-188.702; P=0.006) and dystocia(AOR=11;CI=22.275-55.860; P=0.003) respectively(Table 6).

Table 4: Univariable logistic regression analysis for physiological factors and clinical disorders associated with *N. caninium* infection

Variable	Category	NE(n=180)	NP (%)	COR (95% CI)	P-Value
age	3-5	56	3(5.36)	Ref*	Ref*
	6-7	109	5(4.58)	1.23(0.230 - 6.534)	0.244
	≥ 8	15	1(6.67)	4(0.513 - 31.13)	
body condition	good	40	2(5.00)	1(0.199 - 5.014)	1.00
	poor	140	7(5.00)	Ref*	Ref*
pregnancy	absent	149	3(2.01)	2.3(0.550-9.394)	0.260
	present	31	6(19.35)	Ref*	Ref*
parity	0-2	53	3(5.66)	Ref*	Ref*
	3-4	112	55 (4.46)	0.6(0.134-2.890)	0.531
	≥ 5	15	1(6.67)	3(0.460-20.751)	
abortion history	present	45	7(15.55)	30 (2.516-365.098)	0.007
	absent	135	2(1.48)	Ref*	Ref*
retained placenta	present	26	2(7.69)	Ref*	Ref*
	absent	154	7(4.54)	0.6 (0.070-5.647)	0.681
stillbirth	absent	160	7(4.37)	1.8(0.096- 6.117)	0.804
	present	20	2(10.00)	Ref*	Ref*
neonatal mortality	absent	154	8(5.19)	0.9(0.158-.4.986)	0.894
	present	26	1(3.84)	Ref*	Ref*
repeated breeding	absent	159	8(5.03)	0.9(0.112-7.945)	0.952
	present	21	1(4.76)	Ref*	Ref*
infertility	absent	150	6(4.00)	2.4(0.556-9.926)	0.743
	present	30	3(10.00)	Ref*	Ref*
dystocia	absent	147	6(4.08)	Ref*	Ref*
	present	33	3(9.09)	11(2.623- 47.426)	0.001

NE=Number examined; NP=Number positive; COR=Crude Odd Ratio; CI=Confience Interval

4.2.3 Environmental factors associated with N.caninium infection

Ecology, source of water, presence of dog and wild felid contacts with the animal were considered as environmental factors for *N.caninium* infection. As presented in table 5, the seroprevalence of animals managed in lowland and midland was 6.79 %(6/103) and 3.89 %(3/77) respectively. In source water, seroreactors of 10.34 %(6/58) and 2.45 %(3/122) were found from animals managed to drink wells and pipe water respectively. Regarding dog presence, the seroprevalence of 6.38 %(6/94) and 3.48 %(3/86) recorded in animals with dog contact and those in no similar condition respectively. additionally, the seroprevalence of 5.80 %(7/120) and 3.33 %(2/60) were obtained from animals where wild felids are present and absent respectively.

Regarding measures of association, ecology and dog contact were dropped by univariable logistic regression analysis (P>0.25). In contrast, a source of water and the presence of felid in the environment were fitted into multivariate logistic regression analysis (P<0.25)(Table 5). Lastly, by multivariable model the presence of wild felid in surrounding was found insignificantly associated with *N.caninium* infection in dairy cattle (P>0.05) while a source of water found significantly associated with *N.caninium* seropositivity((P<0.05). As a result, the odds of acquiring *N.caninium* infection was 7 times higher in the cow that drunk wells water than that drunk a piped water (AOR=9; CI=1.599-47.568; P=0.012) (Table 6).

4.2.4 Management and community practices associated with N.caninium infection

Herd size, a type of dog feeding, type of dog housing, disposal way of fetal membrane and the status of barn hygiene were the hypnotized risk factors for *N.caninium* infection in this study. Accordingly, the proportion of 40(22.22%), 76(42.22%) and 64(35.55%) were a sample from small, medium and large herd sizes respectively. Regarding a type of dog feed, 110(61.10%) owners of sampled animals feed their dogs raw animal products whereas that the reaming 70(38.90%) feed on house leftover. Regarding type dog housing, 52(28.89%) owners had a separate house for their dogs while 128(71.11%) had not. Concerning the way of fetal membrane disposal, 116(64.44%) owners threw it away but the reaming 64(35.56%) give it to their pets. as far as barn hygiene is concerned, 112(62.22%) of the barns are poor whereas 68(37.78%) clean their barn daily.

Regarding seroreaction status, the seroprevalence of 5.00 %(2/40), 5.26 %(4/76) and 4.69%(3/64) was recorded in small, medium and large herds respectively. With a type of dog feeding, dogs that feed on raw animal products was contributed 4.54 %(5/110) while that feed household leftover reacted 5.71 %(4/70). For the type housing, 5.76 %(3/52) and 4.68 %(6/128) proportions were obtained from animals managed with dogs of separate and non-separate houses respectively. On other hand, the seroprevalence of 4.31 %(5/116) and 6.25 %(4/64) resulted from animals owned by individuals who threw away fetal membrane and gave to their pets respectively. Moreover, seroprevalences of 5.35 %(6/112) and 4.41 %(3/68) were revealed in animals managed in poor and good hygiene barns respectively. On analysis, herd size, the type of dog housing and status of barn hygiene were ruled out by initial univariable logistic analysis (P>0.25)(Table 5). On other hand, the type of dog feeding and disposal way of the fetal membrane were found fit for multivariate logistic regression analysis (P<0.25). Lastly, multivariate logistic regression analysis revealed that the type of dog feeding was a significant indicator of *N.caninium* seropositivity in cattle (P<0.05). As a result, dairy cattle that owned by the owner who fed his/her dog a raw animal product had 6 times higher risk of acquiring *N.caninium* than that managed by the owner who fed his/her dog a human leftover(AOR=6; CI=1.213-27.222; P=0.028)(Table 6).

Table 5: Univariable analysis of environmental factors and community practices associated with *N. caninium* infection at animal level

Variable	Category	NE(n=180)	NP (%)	COR (95% CI)	P-Value
ecology	lowland	103	6(6.79)	Ref*	Ref*
	midland	77	3(3.89)	0.4 (0.081-1.994)	0.265
herd size	small	40	2(5.00)	Ref*	Ref*
	medium	76	4(5.26)	1(0.184-6.027)	0.952
	large	64	3(4.69)	0.9(0.149- 5.851)	0.942
dog contact	present	94	6(6.38)	Ref*	Ref*
	absent	86	3(3.48)	1.5(0.360-6.155)	0.580
wild felid	present	120	7(5.80)	Ref*	Ref*
	absent	60	2(3.33)	2.7(0.628-11.318)	0.181
water	wells	58	6(10.34)	6.9(1.340- 35.150)	0.020
	pipe	122	3(2.45)	Ref*	Ref*
dog feeding	raw	110	5(4.54)	5.3(1.267-21.961)	0.021
	leftover	70	4(5.71)	Ref*	Ref*
dog housing	separate	52	3(5.76)	0.6(0.114-2.839)	0.493
	non-separate	128	6(4.68)	Ref*	Ref*
fetal membrane	threw aw	116	5(4.31)	Ref*	Ref*
	give to pet	64	4(6.25)	0.3(0.068- 1.177)	0.080
barn hygiene	poor	112	6(5.35)	Ref*	Ref*
	good	68	3(4.41)	0.6(1.153-2.293)	0.449

NE=Number Examined; NP=Number Positive; COR=Crude Odd Ratio; CI=Confidence Interval

Table 6: Summary of multivariate logistic regression analysis of *N. caninium* seropositivity at animal level

Variable	Category	NE	NP	Prevalence (%)	AOR(95%CI)	P-value
water	wells	58	6	10.34	9(1.599-47.568)	0.012*
	pipe	122	3	2.45	Ref*	Ref*
age	3-5	56	3	5.36	Ref*	Ref*
	6-7	109	5	4.58	0.6 (0.027-12.112)	0.73
	≥ 8	15	1	6.67	78 (0.657-9293.418)	0.07
abortion	present	45	7	15.55	23(2.354-188.702)	0.006*
	absent	135	2	1.48	Ref*	Ref*
Fm disposal	threw away	116	5	4.31	Ref*	Ref*
	give to pet	64	4	6.25	0.6(0.063-1.150)	0.077
dog feed	raw	110	5	4.54	6(1.213-27.222)	0.028*
	leftover	70	4	5.71	Ref*	Ref*
wild felid	present	120	7	5.80	3(0.116-15.567)	0.126
	absent	60	2	3.33	Ref*	Ref*
infertility	absent	150	6	4.00	Ref*	Ref*
	present	30	3	10.00	1.3 (0.2187- 7.817)	0.768
dystocia	absent	147	6	4.08	Ref*	Ref*
	present	33	3	9.09	11(22.275-55.860)	0.003*

$\chi 2$= 1.53, P-value =0.99, ROC = 0.84

AOR=Crude Odd Ratio; CI=Confidence Interval, $\chi 2$=Hosmer-Lemeshow Chi-Square,ROC=Area Under Curve

4.3 Herd level seroprevalence and associated risk factors

4.3.1 Clinical reproductive disorders

At the herd level, abortion, dystocia, retained placenta, stillbirth, infertility, repeated breeding and neonatal mortality in the last year were assessed. The result revealed every herd had experienced at least one or more reproductive disorders in the last year. Accordingly, the proportion of occurrence were 58.55% (stillbirth), 58.33 %(infertility), 58.33 %(repeated breeding), 50.00 %(abortion), 41.67 %(dystocia), 33.33% (retained placenta) and 8.33 %(neonatal mortality), respectively. Regarding seroprevalence, the highest seroreaction was recorded in the herds with the history of retained placenta(31.25%(5/16), abortion(25%(6/24)), presence of dystocia(25%(5/20) absence of neonatal mortality(25%(1/4) followed by the presence of repeated breading and stillbirth with an equal proportion of 17.85%(5/28).

As depicted in table 7, by univariable logistic analysis, dystocia, abortion and retained placenta were found fit for multivariable logistic regression analysis (P<0.25). Accordingly, dystocia and abortion were found significantly associated with seropositivity of *N.caninium* in the dairy herd (P<0.05). As a result, the herd with the history of abortion had 16 times higher risk of *N.caninium* infection than herd without the history of abortion (AOR=16; CI=1.446-175.939; P=0.024); whereas the odd of acquiring *N.caninium* infection was 7 times higher in the herd with a history of dystocia that the herd without the history of dystocia(AOR=7; CI=1.008- 45.071; P=0.049)(Table 8).

4.3.2 Environmental risk factors

Ecology, source of water, presence of dog and wild felid contacts with the animal were considered as environmental factors associated with *N.caninium* infection in the herds. Likewise, among assessed herds, 58.33 %(28/48) and 41.67 %(20/48) were from lowland and midland respectively; whereas, 83.33% proportion of total sampled herds were managed to drink open water. On other hand, the herds with a history of dog contact and wild felid contact were 58.33 %(28/48) and 52.08 %(25/48) respectively. As presented in table 7, among environmental factors only the presence of wild felid was found fit for further analysis of multivariable logistic regression(P<0.25)(Table 7). However, by the last model, it was found an insignificant predictor of *N.caninium* seropositivity at herd level (P>0.05) (Table 8).

4.3.3 Management wise risk factors

The herd size, the type of dog feeding, type of dog housing, disposal way of fetal membrane and the status of barn hygiene of the herd were assessed as management and community practice factors associated with *N.caninium* infection. Accordingly, the proportion of 12(25%), 20(41.67%) and 16(33.33%) were a sample from small, medium and large herd sizes respectively.

Concerning dog feeding, 35(72.91%) owners of sampled herds feed their dogs raw animal products whereas that the reaming 13(27.09%) feed on house leftover. Regarding type dog housing, 34(70.83%) owners had no separate house for their dogs while 14(29.17%) had. Concerning the way of fetal membrane disposal, 40(83.33%) owners threw it away but the reaming 8(16.67%) give it to their pets. Similarly, in barn hygiene 40(83.33%) of the barns of the owners were poor whereas 8(16.67%) owners clean their barns on daily basis.

Regarding serostatus, the seroprevalence of 16.67 %(2/12), 20 %(4/20) and 6.25 %(1/16) was obtained from small, medium and large herd sizes respectively. By type of dog feeding, the herds of individuals who fed their dogs raw animal products were contributed 17.14 %(6/35) while that feed household leftover reacted 7.69 %(1/13). On other hand, 14.28 %(2/14) and 14.70 %(5/34) proportions were obtained from animals managed with dogs of separate and non-separate houses respectively. Additionally, the seroprevalence of 15.00 %(6/40) and 12.50 %(1/8) resulted from animals owned by individuals who threw away fetal membrane and gave to their pets respectively. Equivalent to disposal of fetal membrane, seroprevalences of 15.00 %(6/40) and 12.50 %(1/8) were obtained from the animals managed in poor and good hygiene barns respectively. Later on, all the hypothesized risk factors were subjected to univariate logistic regression analysis. However, none of them were found fit for the multivariable model ($P>0.25$) (Table 7 and 8).

Table 7: Univariate logistic regression analysis of *N. caninium* seropositivity at herd level

Variable	Category	HT(n=48)	NP (%)	COR(95%CI)	P-Value
ecology	lowland	28	5(17.85)	Ref*	Ref*
	midland	20	2(10.00)	0.6(0.141- 2.969)	0.57
herd size	small	12	2(16.67)	Ref*	Ref*
	medium	20	4(20.00)	1.3(0.192-8.128)	0.815
	large	16	1(6.25)	0.3(0.026-4.185)	0.395
retained placenta	absent	32	2(6.25)	Ref*	Ref*
	present	16	5(31.25)	5.8(1.217- 27.634)	0.02
abortion history	absent	24	1(4.17)	Ref*	Ref*
	present	24	6(25.00)	11.5 (1.307-101.181)	0.028
repeated breed	absent	20	2(10.00)	Ref*	Ref*
	present	28	5(17.85)	0.6(0.141- 2.969)	0.57
stillbirth	absent	20	2(10.00)	0.6(0.141- 2.969)	0.57
	present	28	5(17.43)	Ref*	Ref*
neonatal mortality	absent	44	6(13.63)	Ref*	Ref*
	present	4	1(25.00)	0.7(0.061- 7.270)	0.739
fetal membrane	threw away	40	6(15.00)	Ref*	Ref*
	give to pet	8	1(12.50)	1.5(0.152-13.848)	0.745
barn hygiene	poor	40	6(15.00)	1.8(0.187-16.338)	0.623
	good	8	1(12.50)	Ref*	Ref*
dog feeding	raw	35	6(17.14)	Ref*	Ref*
	leftover	13	1(7.69)	3.6(0.399- 31.682)	0.256
dog housing	separate	14	2(14.28)	Ref*	Ref*
	no separate	34	5(14.70)	0.8(0.166- 3.707)	0.761
Wild felid	absent	23	1(4.34)	Ref*	Ref*
	present	25	6(24.00)	4. (0.751- 22.193)	0.103
infertility	absent	20	3(15.00)	Ref*	Ref*
	present	28	4(14.28)	1.5(0.336- 7.091)	0.575
dystocia	absent	28	2(7.14)	Ref*	Ref*
	present	20	5(25.00)	3.6(0.771- 16.535)	0.104
dog contact	absent	20	5(25.00)	Ref*	Ref*
	present	28	2(7.14)	1.8(0.187- 16.338)	0.623
water	wells	40	6(15.00)	Ref*	Ref*
	pipe	8	1(12.50)	0.9 (0.156- 5.214)	0.909

HT=Herd Tested; NP=Number Positive; COR=Crude Odd Ratio; CI=Confidence Interval

Table 8: Multivariate logistic regression analysis of *N. caninium* seropositivity at herd level

Variable	Category	Herd Tested	Positive (%)	AOR(95%CI)	P-value
wild felid	absent	23	1(4.34)	Ref*	Ref*
	present	25	6(24.00)	0.9(0.102- 9.311)	0.983
dystocia	absent	28	2(7.14)	Ref*	Ref*
	present	20	5(25.00)	7(1.008- 45.071)	0.049*
abortion history	absent	24	1(4.17)	Ref*	Ref*
	present	24	6(25.00)	16(1.446-175.939)	0.024*
retained placenta	absent	32	2(6.25)	Ref*	Ref*
	present	16	5(31.25)	4(0.695- 26.558)	0.117

$\chi 2$= 1.83, P-value =0.606, ROC = 0.78

AOR=Crude Odd Ratio; CI=Confidence Interval, $\chi 2$=Hosmer-Lemeshow Chi-Square,ROC=Area Under Curve

5 DISCUSSIONS

Bovine neosporosis is a cosmopolitan disease that has recently emerged as a major cause of abortion in cattle. Globally, its prevalence in cattle has been estimated to be between 7.6% and 97.2% in America (Cerqueira *et al.,* 2017; Alexander *et al.,* 2018; Deaquino *et al.,* 2019),3.9% and 24.1% in Africa(Ghalmi *et al.,2012;* Asmare *et al.,* 2013b, Asmare, 2014; Fereig *et al.,* 2016; Mathew *et al.,* 2017; Olum *et al.,* 2020), 0.5% and 60% in Asia(Kul *et al.,* 2009; Dubey and Schares, 2011; Yadav *et al.,* 2016; Marzieh *et al.,* 2019), 0.7% and 76% in Europe(Bartels *et al.,* 2006; Imre *et al.,* 2012; Lefkaditis *et al.,* 2020) and 3.2% to 46.7% in Australia(Nasir *et al.,* 2012; Calarco and Ellis, 2020).

In Ethiopia, seroprevalence ranging from 13.3 to 23.8% have been reported from cattle managed under intensive and semi-intensive management systems(Asmare *et al.,* 2013a; Asmare, 2014). In the present study, the overall seroprevalence was 5%. This finding was comparable with findings from Korea (4.1%)(Kim *et al.,* 2002), Tanzania (4.5%)(Mathew *et al.,* 2017), Nepal(4.84%)(Yadav *et al.,* 2016), and Japan (5.7%)(Koiwai *et al.,* 2006) and Czech Republic (5.83%)(Vaclavek *et al.,* 2003) However, substantially larger seroprevalence ranging from 13.33 to 97.2% have been reported from different parts of the world including Ethiopia(Peña *et al.,* 2012; Asmare *et al.,* 2013b; Asmare, 2014; Cerqueira *et al.,* 2017; Alexander *et al.,* 2018). In cattle seroprevalence of *N.caninium* varies based on the country, region, type of serologic test used, age, the abundance of canids, study design, the sample size used, gender, management, and breed of the animals (Dubey and Lindsay, 2006; Bártová *et al.,* 2015). So far, it has been stated that animals with access to pasture might have a greater opportunity of horizontal *N.caninnnium* infections when compared to those raised in the semi and semi-intensive systems(Venturoso *et al.,* 2021). However, our current finding, the lower seroreaction in the free-range rearing system was quite contradicting with several previous findings(Dubey and Schares, 2011; Almeria, 2013; Yadav *et al.,* 2016; Venturoso *et al.,* 2021).

The difference in prevalence that observed in the current study could arise from numerous impact factors such as difference in sample size, serological tests applied and the time lapse between sampling and recall of reproductive disorders by pastoralists. However, It is quite unsound to establish with high confidence that the seroprevalence of *N.caninium* in the current study district is relatively lower as compared to other parts of Ethiopia. Presumably, the prevalence would have been higher if the study would have done during the rainy season rather than at the time of harsh climate conditions and severe drought.

Biologically, warm temperature and humid climate conditions are favorable for the maintenance of *N.caninium* oocysts and subsequent sporulation(Razmi *et al.,* 2006). On other hand, the oocysts of *N.caninium* readily desiccate in warm and dry climate conditions (Marzieh *et al.,* 2019). Recently, the world's highest prevalence of *N. caninum*(97.2%) that was recorded in Brazil around the Amazon region was claimed as due to favorable hot, humid and high-level precipitation of the Amazon region for *N. caninum* oocysts(Cerqueira *et al.*, 2017). Similarly, in Canada, the highest number of seroprevalence that was obtained during winter than summer season was justified as mild temperatures and humidity favor for the sporulation and survival of *N.caninium* oocysts in winter than summer season(Dubey and Schares, 2007).

The current study was conducted in arid and semi-arid climate condition of distinct bimodal rainfall "Ganna" and "Hagayya" extends from March to May and September to November respectively. However, unfortunately, the study was conducted from late February to early March during harsh climate conditions and severe drought that may hinder the maintenance and sporulation of *N.caninium* oocysts. In addition, during severe droughts due to the unavailability of pasture cattle shift to browse on drought-tolerant forage trees and shrubs which may reduce the chance of pasture-related infections. Moreover, In Borana pastoral area, during severe droughts, the herd of cattle temporarily separated from the household and all other domestic animals and settle nearby water sources until the commencement of the rainy season. This segregation reduces the dog contacts and subsequently may reduce the chance of *N.caninium* infection in cattle during drought periods. It also reported that antibody titer of the animals is also determinant of *N. caninum* seropositivity in cattle(Garcı, 2009). Thus, a diminished cell-mediated immune response may occur in low body conditions of animals as a result of drought.

In the current study, out of 20 hypothesized risk factors, 8 potential predictors of *N.caninium* were screened out by univariable logistic analysis at the animal level($P<0.25$). However, following multivariable logistic analysis, only 4 of them were found significantly associated with seropositivity of *N.caninium* in study animals (P<0.05). Thus, in the current study the history of abortion, a source of water, a type of dog feeding and dystocia were the independent predictors of *N.caninium* infection at the animal level(P<0.05). Likewise, at the herd level, a total of 16 putative risk factors were considered of which 4 of them found potential indicators of animal serostatus at herd level(P<0.25). However, by multivariable logistic analysis, only the history of abortion and dystocia were found independent predictors of *N.caninium* infection at herd level (P<0.05).

N. caninum is a main cause of reproductive disorders in bovine particularly; it induces abortion storms in cattle(Dubey and Schares, 2007; Asmare, 2014). In the present study abortion and dystocia were the clinical reproductive disorders that were found significant predictors of seropositivity of *N.caninium* in cattle both at the animal level and herd level. Accordingly, out of 9 seroreactors 7(78%) were suffered from abortion. Statistically, it was found the strong predictor *N.caninium* infection; depicting that a cow with a history of abortion is 23 times more likely seropositive for *N.caninium* infection than a cow with no similar history(P=0.006). In the same manner, at herd level, the odd of *N.caninium* seropositivity is 16 times higher in the herd with a history of abortion than that without an abortion record (P=0.024). This strong association might be due to the pathogenic nature of the agent; placental and foetal tissue infections.

Comparably, the strong association of abortion with seropositivity for *N. caninum* supported many scholars(Macaldowie *et al.,* 2004; Woodbine *et al.,* 2008; Asmare *et al.,* 2013a; Asmare, 2014). In line with our finding, it has been reported that a cow infected with *N. caninumin* is 3 to 7 times more likely to abort than an uninfected one(Innes *et al.,* 2007). Another study also revealed that the risk of abortion is 7.4 fold higher in congenitally infected heifers than seronegative ones(Wouda, 2000). Additionally, it has been confirmed that 12 to 42% of aborted fetuses from dairy cattle were found seropositive *N caninum*(Dubey and Schares, 2007).

Regarding dystocia, out of 33 dams with a history of dystocia 3(9.09%) of them found seroreactors for *N.caninium* infection. With the presence of dystocia, significantly, the odds of *N.caninium* seropositivity were 11 times higher at the animal level and 7 folds higher at herd level respectively. So far, studies that have related the occurrence of dystocia and *N.caninium* infection in bovine are rare. However, it has been stated that *N.caninium* can cause grossly visible lesions and deformities in calves that may pave the way for the occurrence of dystocia during parturition(Dubey and Lindsay, 2006). In addition to that, a more recent study revealed that *N. caninum* is capable of destroying a variety of nerve cells including those of cranial and spinal nerves(Venturoso *et al.,* 2021). Thus, destructions of cranial and spinal nerves may cause paralysis of abdominal musculatures that might diminish the expulsion power of the dam during parturition.

The management system is a crucial factor in determining disease occurrence in animal husbandry worldwide(McDougall *et al.,* 2014). In sophisticated dairy farm industries the likelihood of disease occurrence is mainly determined by housing type, the hygiene status of watering and feeding troughs, humidity and air circulation, condition of biosecurity standards(Salobir *et al.,* 2012; Crowe *et al.,* 2018). However, in pastoral settings disease occurrence largely arise from environmental contamination (water and pasture contamination), high contact rate with different species of animals, malnutrition and practices. In the current study, among investigated environmental factors and management practices, a source of water and a type of dog feeding were found statistically significant predictors of *N.caninium* serostatus in cattle ($P<0.05$).

At the time of this study, surface water sources such as lakes and rivers were dried up due to severe drought; so that public shallow wells and rarely available motorized piped water were assessed. As a result, multivariable logistic regression analysis revealed that a cow that drunk open water(wells) is 7 times fold higher at risk of *N.caninium* infection than that drunk pipe water($P=0.012$). This finding was inconsistent with the findings of (Corbellini *et al.,* 2006; Alexander *et al.,* 2018; Venturoso *et al.,* 2021). However, it has been addressed horizontal transmission of oocysts of *N.caninium* in intermediate hosts are via ingestions of ingestion of infected food and water (Dubey and Schares, 2007; Gharekhani, 2020).

In the current study, variation of serostatus between animals managed to drink two different sources of water could be due to the difference in hygienic status, accessibility of canids and the practices of the local community. The watering troughs of investigated wells were made up of muddy soil as compared to concrete watering troughs of motorized pipe waters. So that soil-born water contamination with *N.caninium* oocysts would be higher in wells water of the study area. By default, water contamination is higher in open water due to the easiest accessibility by wild and domestic canids. Meant to serve the wildlife in surroundings and the lost group of domestic animals that would come to wells during the night; often some sort of water made remain in troughs as a norm of Borana pastoral community! Nevertheless, it is very interesting; water reserve for nocturnal watering service in wells might have a higher risk of water contamination with oocyst from wild canids.

Dog a definitive host of *N.caninium* and acquires the parasite by ingestion of contaminated material, aborted fetuses, or placentas(Dubey and Lindsay, 2006; Dubey *et al.*, 2007). So far, *N.caninium* has been found in naturally-infected placentas and dogs that were fed placentas of naturally infected cows shed *N. caninum* oocysts(Bergeron *et al.*, 2000; Dijkstra *et al.*, 2001). Moreover, *N. caninum* oocysts have been identified by bioassay and PCR in faeces of naturally infected dogs(Basso *et al.,* 2001). In Borana pastoralists, pet animals are reared as an integral part of livestock for the protection against rodents and predators(Jilo *et al.,* 2021). In the current study, the feeding habit of dogs was assessed and found a determinant factor of *N.caninium* seropositivity in cattle. As a result, cattle owned by the individuals who fed their dogs a raw animal product had a 6 times higher risk of acquiring *N.caninium* infection than that managed by the individuals who fed their dogs a household leftover (P=0.028). In line with our finding, in Canada, it has been reported that the risk of infection increases by 3 folds in farms that have dogs access to the placentas and fetuses (Vanleeuwen *et al.,* 2010).

6 CONCLUSIONS AND RECOMMENDATIONS

The current study was the first attempt of *N.caninium* investigation in cattle managed under a pastoral production system in Ethiopia. Thus the finding of this study showed *N.caninium* infection in cattle reared under pastoral production system with overall seroprevalence of 5% and 14.6% at individual and herd level in Teltelle district respectively. It was underpinned that, the history of abortion, a source of water, a type of dog feeding and dystocia were the independent predictors of *N.caninium* infection in cattle at the individual level. On another way, the history of abortion and dystocia were found independent predictors of *N.caninium* infection at the herd level in the study area. Therefore, based on the above conclusion remarks, the following recommendations are worthwhile:

- Community practice of feeding pets raw animal product has to avoided to break the life cycle and reproduction of *N.caninium*
- Provision of hygienic water animals and keeping sanitary measures of water sources are of paramount importance to tackle the economic consequences of *N.caninium* infection in animals
- A comprehensive assessment and surveillance studies encompassing dogs and other ruminants are required to understand the risk factors, distribution of *N.caninium* and its transmission dynamics on seasonal interfaces.
- Further confirmatory studies like isolation and molecular characterization should be conducted to obtain a real figure of neosporosis in the study area.

7 REFERENCES

Aguado-mart, A., Gonza, E., Pereira-bueno, J., Mercedes, G., Rubio, P., Ortega-, L.M., Collantes-ferna, E.(2016): Effect of Different Ecosystems and Management Practices on *Toxoplasma gondii* and *Neospora Caninum*. *J. Wildl. Dis.* **52:** 293–300.

Ahmed, N.E., Mohamed, L., Akabway, A., Ramadan, M.Y., Mohamed, S.(2017): Serological and PCR-sequencing assays for diagnosis of *Toxoplasma gondii* and *Neospora caninum* infecting camels in Egypt.*Ben.Vet. Med.J.* **33(2):** 200-210.

Al-qassab, S.E., Reichel, M.P., Ellis, J.T.(2010): On the Biological and Genetic Diversity in *Neospora caninum*. *Diver***.3:** 411-438.

Albuquerque, G.R., Munhoz, Alexandre D, Teixeira, Marcel, Medeiros, Simoni M De, Lopes, C.W.G., R, A.A.G., Munhoz, A D, Teixeira, M, Flausino, W., Medeiros, S M.(2011): Risk factors associated with *Toxoplasma gondii* infection in dairy cattle. *Stat. Rio de Jan*. **31:** 287–290.

Alexander, H., Llano, B., Sales, M., Martins, R., Polo, G., Caetano, A.(2018): Seroprevalence and risk factors for *Neospora caninum* infection in cattle from the eastern Antioquia , Colombia. *Vet. Anim. Sci.* **6:** 69–74.

Almeria. S.(2013): *Neospora caninum* and Wildlife. *Parasitol.* **2013:** 1-23.

Alvarado-Esquivel, C., Romero-Salas, D., García-Vázquez, Z., Crivelli-Diaz, M., Barrientos-Morales, M., Lopez-de-Buen, L., Dubey, J.P.(2014): Seroprevalence and correlates of *Toxoplasma gondii* infection in domestic pigs in Veracruz State, Mexico. *Trop. Anim. Health Prod.* **46:** 705–709.

Asmare, K.(2014): *Neospora caninum* versus *Brucella spp*. exposure among dairy cattle in Ethiopia: A case control study. *Trop. Anim. Health Prod*. **46:** 961–966.

Asmare, K., Regassa, F., Robertson, L.J., Martin, A.D., Skjerve, E.(2013a): Reproductive disorders in relation to *Neospora caninum, Brucella spp*. and *Bovine Viral Diarrhoea virus* serostatus in breeding and dairy farms of central and southern Ethiopia. *Epidemiol. Infect.* **141:**1772–1780.

Asmare, K., Regassa, F., Robertson, L.J., Skjerve, E.(2013b): Seroprevalence of *Neospora caninum* and associated risk factors in intensive or semi-intensively managed dairy and breeding cattle of Ethiopia. Vet. Parasitol. **193:**85–94.

Banales, P., Fernandez, L., Repiso, M. V, Gil, A., Dargatz, D.A., Osawa, T.(2006): A nationwide survey on seroprevalence of *Neospora caninum* infection in beef cattle in Uruguay. *Vet. Parasitol.* **139:**15–20.

Bartels, C.J.M., Arnaiz-Seco, J.I., Ruiz-Santa-Quitera, A., Björkman, C., Frössling, J., Von Blumröder, D., Conraths, F.J., Schares, G., Van Maanen, C., Wouda, W.(2006): Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. *Vet. Parasitol.* **137:**17–27.

Bártová, E., Sedlák, K., Budíková, M.(2015): A study of *Neospora caninum* and *Toxoplasma gondii* antibody seroprevalence in healthy cattle in the Czech Republic. *Ann. Agri.Environ Med.* **22(1):** 3-7.

Basso, W., Venturini, L., Venturini, M.C., Moore, P., Rambeau, M., Unzaga, J.M., Campero, C., Bacigalupe, D., Dubey, J.P.(2001): Prevalence of *Neospora caninum* infection in dogs from beef-cattle farms, dairy farms, and from urban areas of Argentina. *J. Parasitol*. **87:** 906–907.

Benti, A.D., Zewdie, W.(2014): Major reproductive health problems of indigenous Borena cows in Ethiopia. *J. Adv. Vet. Anim. Res.* **1:**182–188.

Bergeron, N., Fecteau, G., Pare, J., Martineau, R., Villeneuve, A.(2000): Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Québec. *Can. Vet. J.* 41: 464-466.

Bjerkas, I., Mohn, F., Prestus, J.(1984): Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. *Z. Parasitenkd* **70:**271–274.

Calarco, L., Ellis, J.(2020): Species diversity and genome evolution of the pathogenic protozoan parasite, *Neospora caninum. Infect. Genet. Evol.* **30:**10–44.

Cerqueira, C., Calero, B., Prakash, D., Maria, G.(2017): All about neosporosis in Brazil. Brazilian *J. Vet. Parasitol.* **2961:**253–279.

Corbellini, L.G., Smith, D.R., Pescador, C.A., Schmitz, M., Correa, A., Steffen, D.J., Driemeier, D.(2006): Herd-level risk factors for *Neospora caninum* seroprevalence in dairy farms in southern Brazil. *Prev. Vet. Med.* 74: 130–141.

Crowe, M.A., Hostens, M., Opsomer, G.(2018): Reproductive management in dairy cows - The future. Ir. Vet. J. **71(1):**1-13.

CSA, (2016): Federal democratic republic of Ethiopia. Central statistical agency. Agricultural sample survey, Volume II, Report on livestock and livestock characteristics. Statistical bulletin. Addis Ababa.

Deaquino, L.V., Minutti, A.F., de Souza Lima Nino, B., Costa, L.R., Bosculo, M.R.M., de Almeida, B.F.M., Garcia, J.L., de Barros, L.D.(2019): Vertical transmission of *Neospora caninum* in bovine fetuses from a slaughterhouse in Brazil. *Trop. Anim. Health Prod.* **51:**1751–1755.

Dijkstra, T., Eysker, M., Schares, G., Conraths, F.J., Wouda, W., Barkema, H.W.(2001): Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with Neospora caninum tachyzoites. *Int. J. Parasitol.* **31:**747–752.

Dinka, H.(2013):Major reproductive disorders of dairy cows in and around Asella town, Central Ethiopia. *J.Vet.Med.Anim.Health*.**5:**113–117.

Duarte, P.O., Oshiro, L.M., Zimmermann, N.P., Csordas, B.G., Dourado, D.M., Barros, J.C., Andreotti, R.(2020): Serological and molecular detection of *Neospora caninum* and *Toxoplasma gondii* in human umbilical cord blood and placental tissue samples. *Sci. Rep.* **25:**1–8.

Dubey, J.P., Carpenter, J.L., Speer, C.A., Topper, M.J., Uggla, A.(1988): Newly recognized fatal protozoan disease of dogs. *J. Am. Vet. Med. Assoc*. **192:**1269–1285.

Dubey, J.P., Knickman, E., Greene, C.E.(2005): Neonatal *Neospora caninum* infections in dogs. *Acta Parasitol.* **50:**176–179.

Dubey, J. P. and Lindsay, D. S. (2006): Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. *Vet. Clin.Food Anim.Pract.***22(3):** 645-671.

Dubey, J.P. and Lindsay, D.S.(1996): A review of Neospora caninum and neosporosis. *Vet. Parasitol.* **67:**1–59.

Dubey, J.P. and Schares, G.(2011): Neosporosis in animals — The last five years. *Vet. Parasitol.* **180:** 90–108.

Dubey, J.P., Schares, G., Ortega-Mora, L.(2007): Epidemiology and Control of Neosporosis and *Neospora caninum*. Clin. Microbiol. Rev. **20:**323–367.

Fereig, R.M., AbouLaila, M.R., Mohamed, S.G., Mahmoud, H.Y., Ali, A.O., Ali, A.F., Hilali, M., Zaid, A., Mohamed, A. A., Nishikawa, Y.(2016): Serological detection and epidemiology of *Neospora caninum* and *Cryptosporidium parvum* antibodies in cattle in southern Egypt. *Acta Trop.* **162:**206–211.

Ferguson, J.D., Galligan, D.T., Thomsen, N.(1994): Principal Descriptors of Body Condition Score in Holstein Cows. *J. Dairy Sci.* **77:**2695–2703.

Garcı, I.(2009):Effects of crossbreed pregnancies on the abortion risk of *Neospora caninum* -infected dairy cows. *Vet. Parasitol.* **263:**323–329.

Getabalew, M., Alemneh, T., Akeberegn, D.(2019): Dairy Production in Ethiopia - Existing Scenario and Constraints. *Biomed. J. Sci. Tech. Res*. **16:**12304–12309.

Ghalmi, F., China, B., Ghalmi, A., Hammitouche, D., Losson, B.(2012): Study of the risk factors associated with *Neospora caninum* seroprevalence in Algerian cattle populations. *Res. Vet. Sci.* **93:**655–661.

Ghalmi, F., Kaidi, R., Losson, B.(2009): First epidemiological study on exposure to *Neospora caninum* in different canine populations in the Algiers district, Algeria. *Parasitol. Int.* **58:** 444–450.

Gharekhani, J.(2020): *Neospora caninum* infection in Iran (2004 – 2020): A review. *J. Parasit. Dis.* **44:**671–686.

Haddad, J.P.A., Dohoo, I.R., Vanleewen, J.A.(2005): A review of *Neospora caninum* in dairy and beef cattle — a Canadian perspective. *Can. Vet. J.* **46:**230–243.

Hadush, A., Abdella, A., Regassa, F.(2013): Major prepartum and postpartum reproductive problems of dairy cattle in Central Ethiopia. *J. Vet. Med. Anim. Heal.* **5:**118–123.

Haile, A., Tsegaye, Y., Tesfaye, N.(2014): Assessment of major reproductive disorders of dairy cattle in urban and per urban area of Hosanna, Southern Ethiopia. *Environ.* **8:** 2225.

Horcajo, P.(2016): Vaccines for bovine neosporosis : current status and key aspects for development. *Parasite Immunol*. **38:**709–723.

Ibrahim, A.M.E., Elfahal, A.M., El Hussein, A.R.M.(2012):First report of *Neospora caninum* infection in cattle in Sudan. *Trop. Anim. Health Prod.* **44:**769–772.

ILCA, (2015): International livestock center for Africa annual report, 2015. Addis Ababa, Ethiopia.

Imre, K., Marius, I., Mirela, I., Nicola, F., Claudio, G., Gheorghe, D.(2012): Serological Survey of *Neospora caninum* Infection in Cattle Herds From Western Romania. *J. Parasitol.* **98:**683–685.

Innes, E.A., Bartley, P.M., Maley, S.W., Wright, S.E., Buxton, D.(2007). Comparative host–parasite relationships in ovine toxoplasmosis and bovine neosporosis and strategies for vaccination. *Vacc*. **25:**5495–5503.

Jilo, K., Tegegne, D., Kasim, S., Dabasa, G., Zewdei, W.(2021): Seroprevalence and Public Health Significance of Toxoplasmosis in Small Ruminants of Pastoral Community in Yabello District , Borana Zone , Southern Ethiopia. *Vet. Med. Internat.* **2021:**1–11.

Kamga-waladjo, A.R., Gbati, O.B., Kone, P., Lapo, R.A., Chatagnon, G., Bakou, S.N., Pangui, L.J., El, P., Diop, H., Akakpo, J.A., Tainturier, D.(2010): Seroprevalence of *Neospora caninum* antibodies and its consequences for reproductive parameters in dairy cows from Dakar – Senegal , West Africa. *Trop. Anim. Health Prod.* **42:** 953–959.

Kim, J.-H., Lee, J.-K., Lee, B.-C., PARK, B.-K., Yoo, H.-S., Hwang, W.-S., Shin, N.-R., Kang, M.-S., Jean, Y.-H., Yoon, H.(2002): Diagnostic survey of bovine abortion in Korea: with special emphasis on *Neospora caninum. J. Vet. Med. Sci.* **64:**1123–1127.

Klevar, S., Norström, M., Tharaldsen, J., Clausen, T., Björkman, C.(2010.): The prevalence and spatial clustering of *Neospora caninum* in dairy herds in Norway. *Vet. Parasitol*. **170:** 153–157.

Koiwai, M., Hamaoka, T., Haritani, M., Shimizu, S., Zeniya, Y., Eto, M., Yokoyama, R., Tsutsui, T., Kimura, K., Yamane, I.(2006): Nationwide seroprevalence of *Neospora caninum* among dairy cattle in Japan. *Vet. Parasitol*. **135:**175–179.

Kul, O., Kabakci, N., Yildiz, K., Öcal, N., Kalender, H., İlkme, N.(2009): *Neospora caninum* associated with epidemic abortions in dairy cattle: The first clinical neosporosis report in Turkey. *Vet. Parasitol.* **159:**69–72.

Lefkaditis, M., Mpairamoglou, R., Sossidou, A., Spanoudis, K., Tsakiroglou, M.(2020): *Neospora caninum*, A potential cause of reproductive failure in dairy cows from Northern Greece. *Vet. Parasitol. Reg. Stud. reports* 19: 100365-70.

Lobato, J., Silva, D.A.O., Mineo, T.W.P., Amaral, J.D.H.F., Segundo, G.R.S., Costa-Cruz, J.M., Ferreira, M.S., Borges, A.S., Mineo, J.R.(2006): Detection of immunoglobulin G antibodies to Neospora caninum in humans: high seropositivity rates in patients who are infected by human immunodeficiency virus or have neurological disorders. *Clin. Vaccine Immunol.* **13:** 84–89.

Luká, R., Halajian, A., Bártová, E., Murat, J., Michael, K., Luus-powell, W.J.(2018):

Molecular detection of *Toxoplasma gondii* and *Neospora caninum* in birds from South Africa. *Acta Tropica*. **178**: 93–96.

Luo, H., Li, K., Zhang, H., Gan, P., Shahzad, M., Wu, X., Lan, Y., Wang, J.(2017): Seroprevalence of *Toxoplasma gondii* infection in zoo and domestic animals in Jiangxi Province , China. *Parasit.* **24**:1-3.

Macaldowie, C., Maley, S.W., Wright, S., Bartley, P., Esteban-Redondo, I., Buxton, D., Innes, E.A.(2004): Placental pathology associated with fetal death in cattle inoculated with *Neospora caninum* by two different routes in early pregnancy. *J. Comp. Pathol*. **131:**142–156.

Marzieh, N., Mehdi, R., Maryam, G., Reza, S., Reza, F.(2019): Seroprevalence of *Neospora caninum* Infection and Associated Risk Factors in Cattle of Sistan Areas, Southeastern Iran. *Iran J Parasitol* **14:** 340–346.

Mathew, C., Klevar, S., Løken, T., Mdegela, R.H., Mwamengele, G., Skjerve, E., Godfroid, J.(2017): Reproductive Infections in Cattle in Tanzania – Lessons for Control Priorities. *SOJ Microbiol. Infect. Dis*. **5:**1–9.

Mcallister, M.(2016): Diagnosis and control of bovine neosporosis.*Vet. Clin. Food Anim. Prac.* **32:**443-463.

McDougall, S., Heuer, C., Morton, J., Brownlie, T.(2014): Use of herd management programmes to improve the reproductive performance of dairy cattle. *Animal*. **8:** 199–210.

Montiel-Olguín, L.J., Estrada-Cortés, E., Espinosa-Martínez, M.A., Mellado, M., Hernández-Vélez, J.O., Martínez-Trejo, G., Ruiz-López, F.J., Vera-Avila, H.R.(2019): Risk factors associated with reproductive performance in small-scale dairy farms in Mexico. *Trop. Anim. Health Prod.* **51:**229–236.

Moore, D., Reichel, M., Spath, E., Campero, C.(2013): *Neospora caninum* causes severe economic losses in cattle in the humid pampa region of Argentina. *Trop. Anim. Health Prod.* **45:**1237–1241.

Nasir, A., Lanyon, S.R., Schares, G., Anderson, M.L., Reichel, M.P.(2012): Sero-prevalence of *Neospora caninum* and *Besnoitia besnoiti* in South Australian beef and dairy cattle. *Vet. Parasitol.* **186:**480–485.

Nazir, M.M., Maqbool, A., Khan, M.S., Sajjid, A., Lindsay, D.S.(2013): Effects of age and breed on the prevalence of *Neospora caninum* in commercial dairy cattle from Pakistan. *J. Parasitol.* **99:** 368–370.

Olum, M., Mungube, E., Njanja, J., Kidali, J., Njenga, E., Maichomo, M., Tsuma, V.T., Mugambi, J.(2020): Seroprevalence of canine neosporosis and bovine viral diarrhoea in dairy cattle in selected regions of Kenya. *Transbound. Emerg. Dis.* ***67:*** 154–158.

Pagmadulam, B., Myagmarsuren, P., Fereig, R.M., Igarashi, M., Yokoyama, N., Battsetseg, B., Nishikawa, Y.(2018): Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in cattle in Mongolia. *Vet. Parasitol. Reg. Stud. Reports* **14:**11–17.

Parish, J., and Brandi. B.(2013): Estimating cattle age using dentition. 1st edition, Mississippi State University. Extension Service. Pp.2779

Peña, L., Araujo, A. V, Rubio, D.E., Mojica, C.P., Falquez, J.J., Avendaño, K.E.(2012):Estudio serológico de DVB, IBR y Neospora en bovinos de la Microrregión del Valle del Cesar. *Rev Colomb Microbiol Trop.* **2:**35–40.

Razmi, G. and Barati, M.(2017): Prevalence of *Neospora caninum* and *Toxoplasma gondii* Antibodies in Bulk Milk of Dairy Cattle, Mashhad, Iran. Arch. *Razi Inst.* **72:** 265–269.

Razmi, G.R., Mohammadi, G.R., Garrosi, T., Farzaneh, N.(2006): Seroepidemiology of *Neospora caninum* infection in dairy cattle herds in Mashhad area. *Iran. Vet. Parasitol.* **135:**187–189.

Reichel, M.P., Wahl, L.C., Ellis, J.T.(2020): Research into Neospora caninum — What Have We Learnt in the Last Thirty Years .*Pathogen.* **9:**1–17.

Reichel, P., Alejandra, M., Pita, L.F., John, T.(2013): The global economic impact of *Neospora caninum* in cattle - the billion dollar question. *Int. J. Parasitol.* **43:** 133–142.

Robayo-Sánchez, L.,, Jorge Gómez-Marín,D., and Jesús Cortés-Vecino, P.(2017): Neospora caninum : Biological Relationship with Toxoplasma gondii and its Potential as Zoonosis Neospora caninum. Rev. Córd.22:6355–6365.

Salobir, J., Frankič, T., Rezar, V.(2012): Animal nutrition for the health of animals, human and environment. *Acta Agric. Slov.* **100:**41–49.

Schneider, A.,(2018): Seroprevalence estimate and associated risk factors for neosporosis in dairy cattle in the northwest region of Rio Grande do Sul State , Brazil.*Ciênc. Rural.* ***48*****(7):**8-12.

Silva, R. amd Machado, P.(2016): Canine neosporosis : perspectives on pathogenesis and management.*Vet. Med. Res. Report.7***:**59-65.

Stelzer, S., Basso, W., Silván, J.B., Ortega-mora, L.M., Maksimov, P., Gethmann, J., Conraths, F.J., Schares, G.(2019): Toxoplasma gondii infection and toxoplasmosis in farm animals : Risk factors and economic impact. *Food. Waterborne Parasitol*. **12:**1–28.

Thompson, G., Canada, N., Topa, M. do C., Silva, E., Vaz, F., Rocha, A.(2001): First Confirmed Case of *Neospora caninum*-associated Abortion Outbreak in Portugal. *Reprod. Domest. Anim.* **36:** 309–312.

Thrusfield, M.(2005): Veterinary epidemiology. 3rd ed, Cambridgck, USA: Blak Well Science Ltd.

Tilahun, B., Tolossa, Y.H., Tilahun, G., Ashenafi, H., Shimelis, S.(2018): Seroprevalence and risk factors of *Toxoplasma gondi*i infection among domestic ruminants in East Hararghe zone of Oromia Region, Ethiopia. *Vet. Med. Int.* **2018:**1-7.

Vaclavek, P., Koudela, B., Modrý, D., Sedlák, K.(2003): Seroprevalence of *Neospora caninum* in aborting dairy cattle in the Czech Republic. *Vet. Parasitol.* **115:** 239–245.

Vanleeuwen, J.A., Haddad, J.P., Dohoo, I.R., Keefe, G.P., Tiwari, A., Scott, H.M.(2010): Risk factors associated with *Neospora caninum* seropositivity in randomly sampled Canadian dairy cows and herds. *Prev. Vet. Med.* **93:**129–138.

Venturoso, S., Venturoso, J., Silva, G., Maia, O.(2021): Risk factor analysis associated with *Neospora caninum* in dairy cattle in Western Brazilian Amazon. *J.Vet. Parasitol.* **30:**1–11.

Webster, J.P.(2010): Review of Toxoplasmosis of Animals and Humans, Second edi. ed. CRC Press. Pp 356.

Williams, D.J.L., Hartley, C.S., Rkman, C.B., Trees, A.J.(2009): Endogenous and exogenous transplacental transmission of *Neospora caninum* – how the route of transmission impacts on epidemiology and control of disease, 1st ed. Cambridge University Press.Pp 150.

Woodbine, K.A., Medley, G.F., Moore, S.J., Ramirez-, A., Mason, S., Green, L.E.(2008): A four year longitudinal sero-epidemiology study of *Neospora caninum* in adult cattle from cattle herds in south west England : *Vet. Res.* **12:**1–12.

Wouda, W.(2000): Diagnosis and epidemiology of bovine neosporosis : A review. *Vet. Q.* **22:**71–74.

Yadav, G.P., Manandhar, S., Karna, A.K., Acharya, K.P., Maharjan, D., Singh, D.K.(2016): Seroprevalence and risk factors associated with *Neospora caninum* in dairy cattle of western dairy pocket area in chitwan district of nepal. Bangladesh *J. Vet. Med.* **14:**215–220.

Yu, J., Xia, Z., Liu, Q., Liu, J., Ding, J., Zhang, W.(2007): Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes (Bubalus bubalis) in the People's Republic of China. *Vet. Parasitol.* **143:**79–85.

Zuniga, R.(2005):Appraisal of the epidemiology of Neospora caninum infection in Costa Rican dairy cattle, 1st ed. Wageningen.

8 APPENDICES

Appendix 1: Questionnaire survey to investigate potential risk factors of N.caninium infection in cattle in Teltelle district of Borana zone, southern Ethiopia

PART ONE: General information of herd owner:

i. District ________ Kebele _______ Village_________GPS ______________________
ii. Age: a. 15 -24 b. 25-46 c. 37-48 d. >48
iii. Residential place: a. Urban b. Semi-urban c. rural

PART TWO: General information about the herd:

i. Herd code(if sampled only)_____________
ii. Total number of dairy cattle in the herd_______
iii. Herd size a) small b) medium c) large
iv. Indicate the water sources for your herd.
   a) Pipe water b) Wells c) Pond d) River
v. Sanitary system of the barn: a) Poor b) Fair c) Good
vi. Presence of reproductive problems in the herd in the last one year a) yes b) no
vii. The history of reproductive problems in the herd for the last one year: a) delayed conception b) abortion c) stillbirth d) dystocia e) retained placenta f) neonatal mortality h) infertility
viii. Duration of abortion a) < 3 months b) 3-6 months c) > 6 months
ix. Disposal of the fetal membrane a) burying b) throwing away c) giving to pets
x. The history of dog contacts in the herd: a) yes b) no
xi. Access of dogs to pasture and water source used for herd a) yes b) no
xii. The presence of wild felids in the localities: a) yes b) no
xiii. Access of wild felids to pasture and water source used for herd a) yes b) no

PART THREE: General information about sampled dairy cattle:

i. Animal code______________
ii. Biodata of sampled animal: a) Age_______ b) body condition__________ c) number of parity_______d) Pregnancy status_____________
iii. The history of reproductive problems in the herd over the last one year: a) delayed conception b) abortion c) stillbirth d) dystocia e) retained placenta f) infertility

iv. Duration of abortion a) < 3 months b) 3-6 months c) > 6 months

PART FOUR: General management system for pets:

i. Do you have dogs? a) yes b) no if yes, how many______

ii. Do you have a separate house (kennel) for your dogs?
   a. a) yes b) no

iii. Are there stray dogs in your localities?
   a. a) yes b) no

iv. Are there wild felids in your localities?
   a. a) yes b) no

v. Do wild felids have close contact with herds?
   a. a) yes b) no

vi. Do your dogs have access to dead animal's carcasses and fetal membranes?
   a. a) yes b) no

vii. Pet feeding practices your pet?
   a. a) raw animal products b) cooked animal products
   b. c) Household left over d. Others, please specify_________________

viii. Purpose of rearing dogs: a. to protect predators from animal b. guarding compound c. Accompanying service for human d. specify if any___________________________

Appendix 2: Kit components, Sample preparation, Testing Procedures, Validation and Interpretations

Kit components

Reagents
Microplates coated with purified *Neospora Caniniumm* extract
Concentrated Conjugate(10×)
Positive Control
Negative Control
Dilution Buffer 2
Dilution Buffer 3
Wash Concentrate(20×)
Substrate Solution
Stop Solution(0.5M)

Samples Preparation

96-wells plates containing the test and control specimens were prepared before transferring them into an ELISA microplate using multichannel pipette.

Wash Solution Preparation

The Wash Concentrate (20×) was brought to room temperature and mixed thoroughly to ensure that Wash Concentrate is completely solubilized.

The Wash Concentrate (1×) was prepared by diluting the Wash Concentrate (20×) in distilled water.

Testing Procedures

All the reagents were brought to room temperature (21 $\pm$ 5^{o}C) before use and homogenized by inversions.

1. 90µl of Dilution Buffer 2 was added to each microwell
2. 10µl of Negative Control was added to wells A1 and B1. 10µl of Positive Control was added to wells C1 and D1. 10µl of each testing sample was added to the remaining wells.
3. The plates were incubated for 45 minutes at 21^{o}C.
4. Each well was emptied and washed three times with 300µl of the Wash Solution avoiding the drying of the wells between washings.
5. The Conjugate 1× was prepared by diluting Concentrated Conjugate 10× to 1/10 in Dilution Buffer 3.
6. 100µl of the Conjugate 1× was added to each well
7. The plates were incubated for 30 minutes at 21^{o}C.
8. Each well was emptied and washed three times with 300µl of the Wash Solution avoiding the drying of the wells between washings.
9. 100 µl of the Substrate Solution was added to each well
10. The plates were incubated for 15 minutes at 21^{o}C in dark.
11. 100 µl of the Stop Solution was added to each well to stop the reaction.
12. The microplates were read at 450 nm by the OD reader.

Test Validation

The test is valid if:

- The mean value of Positive Control O.D (OD_{PC}) is greater than 0.350

  $$\mathbf{OD_{PC} > 0.350}$$

- The ratio of mean values of Positive and Negative Controls(OD_{PC} and OD_{NC}) is greater than 3

  $$\mathbf{OD_{PC} / OD_{NC} > 3}$$

Interpretation

- For each sample calculate S/P percentage(S/P%) sample value(OD_{sample})divided by the mean Positive Control value(OD_{PC}) multiplied by 100

  $$\mathbf{S/P\% = (OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC}) * 100}$$

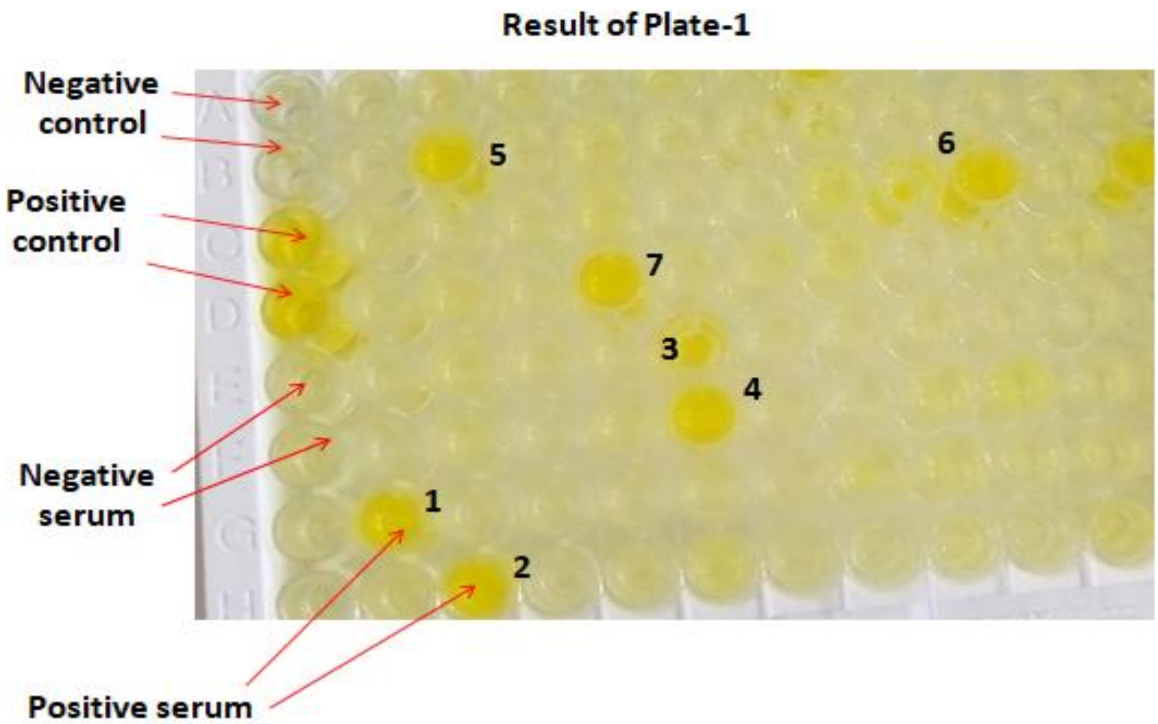
- Sample with a S/P%

Result	Status
S/P% ≤ 40%	Negative
40% < S/P% < 50%	Doubtful
S/P% ≥ 50%	Positive

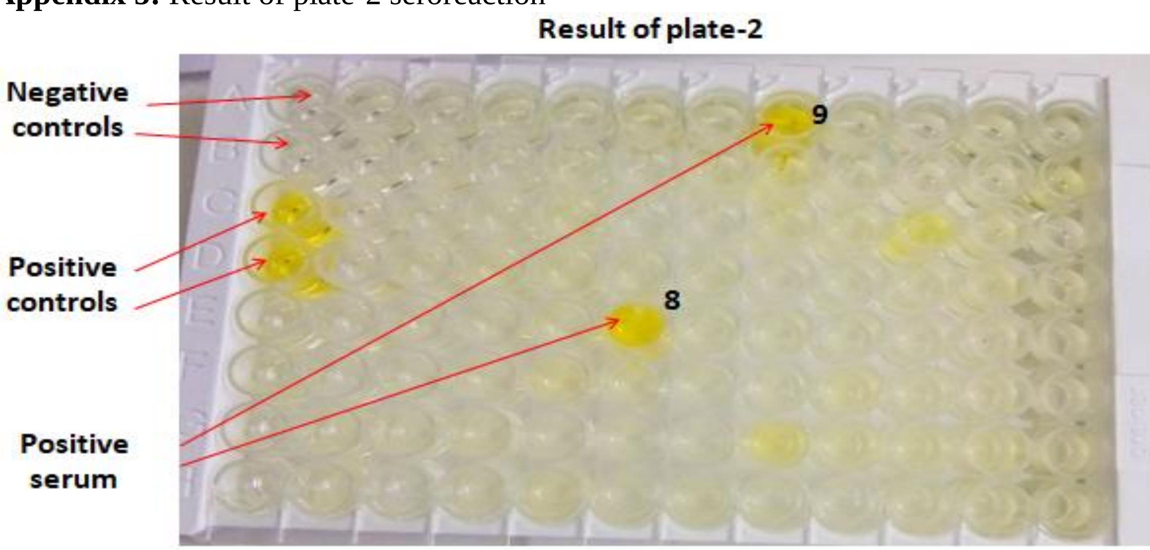
Appendix 3: Data record format for serological survey

S.N	District	PA	Reproductive disorder				Environmental factor				Management factor			Lab result

Appendix 4: The result of plate-1 seroreaction



Appendix 5: Result of plate-2 seroreaction



Appendix 6: OD reader out puts of N.caninium for test microplates

A27 | fx | mean OD Pos /OD of neg >3

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1				p1	Neospora caninum for bovine sera				19/04/21					
2		1	2	3	4	5	6	7	8	9	10	11	12	
3	A		3.02	2.82	4.32	3.02	6.98	3.47	121.71	3.67	1.91	4.64	2.89	
4	B	0.05	2.69	4.96	3.34	5.03	2.56	6.39	10.93	4.32	2.82	4.64	9.51	
5	C		3.34	3.73	4.70	7.62	2.24	2.43	6.52	5.94	23.91	6.65	1.33	
6	D	1.59	2.69	5.03	7.43	7.17	4.64	4.38	2.37	2.37	4.25	6.98	3.99	
7	E	4.25	3.41	3.47	3.47	4.64	115.87	3.99	5.16	4.12	3.54	4.38	3.21	
8	F	5.55	4.06	3.67	3.73	8.34	5.68	3.28	2.50	7.43	4.83	4.64	2.76	
9	G	3.21	5.09	4.12	4.57	2.89	2.56	2.17	15.41	6.07	6.72	7.56	5.81	
10	H	1.85	4.90	0.88	3.02	5.16	2.43	3.80	2.11	1.20	3.54	5.48	5.55	
11														
12		29.80												
13														
14				p2	Neospora caninum for bovine sera				19/04/21					
15		1	2	3	4	5	6	7	8	9	10	11	12	
16	A		3.23	6.49	6.76	3.70	6.49	2.62	10.16	5.47	1.94	2.96	3.91	
17	B	0.047	2.35	104.32	5.06	10.91	6.15	3.23	4.04	3.43	2.28	3.84	5.06	
18	C		5.81	4.86	3.70	9.08	2.07	3.16	15.40	7.38	131.65	2.89	93.10	
19	D	1.518	4.66	9.82	1.67	147.42	3.98	6.56	12.34	2.69	3.03	7.44	2.55	
20	E	2.617	3.43	8.74	4.66	1.33	2.89	2.07	3.70	5.06	2.82	3.43	3.43	
21	F	4.521	2.28	5.95	0.85	2.41	133.34	2.82	2.62	7.65	14.04	3.77	12.54	
22	G	3.365	109.42	3.23	3.03	3.30	5.40	4.59	2.14	7.24	2.62	4.18	2.62	cointrols
23	H	3.025	5.68	104.32	2.35	3.91	12.41	3.91	4.18	7.58	3.98	10.71	4.18	pc/nc
24														
25		32.63												

Appendix 7:Photo galleries of the field, transportation and lab duties

Sample labeling At Sample Site

Dogs at Watering Points

Sample transportations

Appendix 8: Body condition score estimation

Description	BCS	Back Fat Thickness(mm)	Total Fat Content(kg)
Emaciated	1.0	<5	<50
Very poor	1.5	5	50
Poor	2.0	10	70
Moderate	2.5	15	98
Good	3.0	20	122
Very good	3.5	25	146
Fat	4.0	30	170
Adipose	4.5	35	194
Obese	5.0	35	194

Source:(Parish *et al.*, 2013)

Appendix 9: Cattle age estimation by dentation

Teeth		Eruption	Full development	Wear
Incisors	Pinchers	18 to 24 months	24 months	Leveled at 5-6 years, noticeable wear at 7-8 years
	1st intermediate pair	30 months	36 months	Leveled at 6-7 years, noticeable wear at 8-9 years
	2nd intermediate pair	36 months	48 months	Leveled at 7-8 years, noticeable wear at 9-10 years
	Corners	42-48 months	60 months	Leveled at 9 years, noticeable wear at 10 years
Premolars	1st Cheek tooth pair	24-30 months	-	-
	2st Cheek tooth pair	18-30 months	-	-
	3st Cheek tooth pair	30-36 months	-	-
Molars	4st Cheek tooth pair	5-6 months	-	-
	5st Cheek tooth pair	12-18 months	-	-
	6st Cheek tooth pair	24-30 months	-	-

Source:(Ferguson *et al.*, 1994)