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SMALL RUMINANT REPRODUCTIVE HEALTH PROBLEMS: RISK FACTORS AND PUBLIC HEALTH RISK OF INFECTIOUS CAUSES OF ABORTION IN ETHIOPIA

PhD Dissertation

By

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As members of the Examining Board of the final Ph.D. open defense, we certify that we have read and evaluated the Dissertation prepared by Gezahegn Alemayehu titled: SMALL RUMINANT REPRODUCTIVE HEALTH PROBLEMS: RISK FACTORS AND PUBLIC HEALTH RISK OF INFECTIOUS CAUSES OF ABORTION IN ETHIOPIA and recommend that it be accepted as fulfilling the dissertation requirement for the degree of Philosophy in Veterinary Public Health

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Final approval and acceptance of the dissertation are contingent upon the submission of its corrected copy to the CGC through the concerned departmental graduate committee. I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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DEDICATION

To my mother, ZEWDE BEDHANE who raised me and five of my sisters and brothers alone, as mother and father with high discipline and dedication.

STATEMENT OF AUTHOR

First, I declare that this thesis/dissertation is my Bonafede 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 a Ph.D. 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.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AR: Abortion rate
ARO: Reproductive output
ARW: Annual reproductive wastage
BDR: Birth defect rate
CGIAR: Consultative Group for International Agricultural Research
CRP Livestock: CGIAR research program on Livestock
Df: Degree of freedom
DIF: Differential item functioning
ELISA: enzyme-linked immunosorbent assay
FAT: Fluorescent antibody test
G-ARPI: Goat annual reproductive performance index
HP: Histopathology
ICCs: Item characteristic curves
IHC: Immunohistochemistry
IRT: Item Response theory
KAP: Knowledge, Attitude and Practice
KI: Kidding interval
KMR: Kid mortality rate
KR: Kidding rate
PC: Principal component analysis
PCA: Principal component analysis
PCR: Polymerase chain reaction
PR: Pregnancy rate
RP: Reproductive performance
RW: Weaning rate
SC: Stomach contents
SNNP: Southern Nation, Nationality and People

SUMMARY

Reproductive performance is a key determinant for the efficiency of small ruminant production. However, low productivity per animal and flock limits the potential contribution of sheep and goats for rural households in Ethiopia. The overall objective of this Ph.D. thesis is to generate information on the cause, magnitude, and risk factor of reproductive health problems, and public health risk of infectious causes of small ruminant abortion. A cross-sectional study design was used to collect data in three production systems of Ethiopia between July 2018 to February 2019. Two districts from lowland mixed crop-livestock production system, two districts from lowland pastoral production system, and one district from highland mixed crop-livestock production system were selected from Amhara, Oromia, and Southern Nation, Nationality and People (SNNP). Abergelle and Zequala districts in the Wagihimira zone of the northern part of the Amhara region were selected to represent the lowland mixed crop-livestock production system. Yabello and Elwaya districts in the Borena zone from the southern part of the Oromia region were selected to represent the lowland pastoral production system. Doyogana district in Kembata Tembaro Zone from the SNNP region represented a highland mixed crop-livestock production system. The structured questionnaire and laboratory analyses of serum samples were used to generate information for this Ph.D. thesis. Information was collected form the total of 327 households where data on pregnancy outcomes and management risk factors were collected for 299 goat and 242 sheep flocks, serum samples were collected from a total of 1,402 animal (980 goats and 422 sheep). To develop a novel quantitative tool to determine goat annual reproductive performance index at flock level (Chapter 3), the reproductive performance of the flock was estimated based on the annual reproductive output, kidding interval and annual reproductive wastage, principal component analysis was used both as reproductive measures dimensional reduction technique and to develop the final model to predict reproductive performance scale. Then the final algorithm is developed to estimate the goat annual reproductive performance index. To estimate the magnitude of abortion and associated factors (Chapter 4), a causal diagram was generated to identify causal relationships between the potential predictors and Zero-inflated negative binomial regression model was used to determine the associations. To determine the seroprevalence abortion causing pathogens (C. *burnetii*, *C. abortus*, *Brucella spp*., and *T. gondi*) and associated risk in sheep and

goat in three production systems of Ethiopia (Chapter 5), a multilevel mixed-effects logistic regression model was fitted to account for the clustering of animal within villages and households. The 2-PL Item Response Theory (IRT) model was fitted to determine the probability of a person appropriately respond to an item with a provided zoonotic disease KAP level.Based on developed algorithm to measure goat annual reproductive performance, the flocks were classified into good, moderately and poor performing. Good performing flocks has higher scores for reproductive output measures, lower scores for reproductive wastage and lower kidding interval. Many of the flocks were moderately affected by reproductive failures, consequently categorized as moderately performing flocks. Results showed that 142 (58.68%) goats and 53 (17.73%) sheep flocks reported abortions in the 12 months before the survey. The mean annual flock abortion percentages were 16.1% (±26.23) for does and 12.6% (±23.5) for ewes. The final zero-inflated negative binomial regression analysis result indicated that spending the night in traditional sheep house', 'providing supplementary feed for pregnant dams', 'presence of other livestock species and dog in the household' had a marked effect on the rate of abortion in sheep and goat flocks. In addition, exposure of the flock to *Brucella spp.* or anyone of four tested infectious agents significantly increased the risk of abortion in the flock. Overall, 65.41% of sheep and 92.22% goat flocks tested positive for one or more abortion-causing agents, namely, *C. burnetti*, *C. abortus*, *Brucella* spp., and *T. gondii;* mixed infection was found in 31.58% sheep and 63.33% goat flocks (Paper III). From the total tested animal, 231(16.48 %), 95 (6.78%), 124 (8.84%), and 137(11.42%) were found seropositive for *C. burnetii*, *Brucellas* spp., *C. abortus*, and *T. gondii*, respectively. Co-infections of abortion pathogens were observed where *C. abortus* (86.84%) showed the highest level followed by *Brucella* spp. (78.34 %) and *C. burnetii* (72.72%) (Paper IV). In the final chapter of this Ph.D. research (Paper V) The attitude subscale had the highest total mean score (37.3, ± 28.92 %) and the knowledge subscale had the lowest mean score (22.4, ± 33.6 %) among the three subscales. Pieces of work in this Ph.D. thesis highlighted the multifactorial nature of small ruminant abortion where animal management, agroecological and infectious disease factors play an important role in its occurrence. There is a need for an integrated approach that improves the nutritional state of pregnant dams through targeted supplementary feeding, abortion management through appropriate biosecurity practices, and vaccination programs for major infectious causes of abortion and herd health management through better veterinary services. Serological study of the four infectious abortion-causing agents demonstrated that all the four infectious causes of abortion

are widely distributed across three agro-ecologies and production systems might play an important role in sheep and goat abortion and impact the health of the public. Future studies which aim at identifying and characterization several possible abortion pathogens and their public health and socioeconomic impact should be done. The part of this Ph.D. thesis revealed overall low zoonotic disease knowledge, low attitude towards zoonotic disease risk, and common risk behaviors among smallholder farmers and pastoralists. There is a need to continue public education programs to raise the awareness of the communities towards the proper installation of personal protection measures and appropriate disposal of abortion materials to reduce the public health risk from zoonotic diseases.

Keywords: Abortion; Co-infection; Goat; Multi-pathogens; Risk factors, reproductive problems; Sheep; KAPs; IRT; Zoonotic diseases

Chapter 1

General introduction

1.1 Background

Sheep and goat in Ethiopia are managed under a traditional extensive system and it is subsistence by nature. Sheep and goats are largely produced in mixed crop-livestock, specialized pastoral, and agropastoral systems (Solomon Gizaw *et al*., 2013; Otte & Chilonda, 2002). Goats and sheep are highly valued livestock species kept by farmers and pastoralists mainly for milk and meat production, as well as income generation from live animal sales (Wodajo *et al*., 2020). However, low productivity per animal and flock limits the potential contribution of sheep and goats for rural households in the rural areas of Ethiopia (Feldt *et al*., 2016, Dereje *et al*., 2015).

Low productivity of the flock is linked to poor nutritional status due to poor pasture quantity and quality, and a high burden of disease, which together contributes to reproductive failure and poor growth rates (Mayberry *et al*., 2018). Reproductive performance is a key determinant of the efficiency of sheep and goat production (Delgadillo *et al*., 2015). The reproductive performance of small ruminants kept under different production systems in Ethiopia is generally low. Small ruminant reproductive rates average as low as 55 lambs and 56 kids born per 100 mature females/year in central highlands (Hirpa & Abebe, 2008). In comparison in optimized systems, this rate can be as high as 110 lambs/kids per 100 mature females/year (Ibrahim, 1998). Reproductive output is further reduced by the high rate of newborn losses which was reported to reach as much as 50% of all lambs and kids born/year (Petros *et al.*, 2014, Tsedeke, 2007).

Reproductive failures in sheep and goats are often multifactorial, in which variation in management factors such as health care, feeding and watering practices, nutrition management for pregnant animals, agroecological factors which affect feed and water availability and disease occurrence, production systems, extreme weather condition, hormonal dysfunction and seasonality can have significant effects (Ayalew *et al*., 2003; Gebremedhin *et al*., 2013; Mayberry *et al*., 2018).

Suboptimal reproductive performance can be caused by any of the following in small ruminants: Low ovulation rates, low conception rates, early fetal loss/reabsorption, late fetal death, abortion, stillbirth, dystocia, and prolapse, poor mothering ability, and male infertility. However, pregnancy wastage in the form of abortion, stillbirth, and perinatal lamb/kid mortality remains significant issues to the sheep and goat production in Ethiopia and are recognized as the single largest contributor to economic losses for small ruminant production sector worldwide (Díaz-Cao *et al*., 2018; Feldt *et al*., 2016; Palomares Reséndiz *et al*., 2020; Robertson *et al*., 2017).

In Ethiopia, it was reported that abortions, stillbirths, and neonatal losses were reported in 57.5%, 28.9%, and 47.9% of farmers' flocks in central Ethiopia, respectively (Gebremedhin *et al*., 2013). A study undertaken in various livestock production systems of Ethiopia reported a mean annual abortion/ stillbirth rate of 7–36.4% and lamb mortality rate range between 14.9–36% (Fentie *et al*., 2016). Moreover, about 20%, 10%, and 65%, the sheep flock experienced abortion, stillbirth, and neonatal losses annually in the highland of Ethiopia, respectively (Gebretensay *et al*., 2019).

Abortions in sheep and goat are of great concern to farmers because the fetus that would form replacement stock is lost and a prolonged period of uterine disease and infertility or sterility may follow leading to unproductive females being maintained for long periods (Ali *et al*., 2019). Therefore, abortion in small ruminant brings not only loss of replacement stock for the flock but also caused loss of milk for family members which is an important source of food and nutrition (Desta *et al*., 2020). Evidence from Kenya indicated that abortion could reduce milk production from the small ruminant flock by 85% (Lokamar *et al*., 2020). Moreover, the high prevalence of abortion in the flock resulted in the reduction of good quality animals in the flock which affects the access to food at the household level and livestock and livestock products export of the country in general (Van Ginkel *et al*., 2013; Alemayehu *et al*., 2015).

Both non-infectious and infectious agents can cause abortion in small ruminant. Non-infectious causes such as inadequate nutrition, pregnancy toxemia, stress, poor handling, vaccination, transport, dog worry, and concurrent disease have been implicated for abortion (Hindson & Winter, 2002).

Infectious causes of abortion play an important role in small ruminant abortion (Holler, 2012). The most diagnosed infectious causal agents of abortion are C*oxiella burnetii (C. burnetii), Chlamydia abortus (C. abortus),* and *Brucella* spp., *Leptospira* spp., *Campylobacter* spp., *Listeria* spp. and *Toxoplasma gondii* (Holler, 2012; Moeller, 2001). These pathogens are also zoonotic and thus can pose serious infection risks for farming communities (Borel *et al*., 2014; Ganter, 2015).

1.2. Problem statements

Infectious causes of small ruminant abortion are prevalent and widely spread in all livestock production systems in Ethiopia (Balako, 2013; de Vries & de Boer, 2010; Gebremedhin *et al*., 2013; Gebretensay *et al*., 2019; Teklue *et al*., 2013; Tulu *et al*., 2020). There are no national prevention and control strategies for those diseases in Ethiopia and largely neglected in most African countries because of a lack of information and awareness about the extent of the problem (WHO, 2009; Gebreyes *et al*., 2014). Many of the infectious agents which cause animal abortion have the potential to cause serious human illnesses. Infected animals shed large quantities of infectious agents during an abortion that pose considerable risk to humans in contact with birth products and the environment (Klous *et al*., 2016; Lejeune & Kersting, 2010). In addition, there were substantial knowledge gaps and high-risk behavioral practices towards zoonotic disease risk from livestock birth products among communities in Ethiopia which increases the risk of exposure to those zoonotic diseases(Legesse *et al*., 2018; Tolossa *et al*., 2014).

Increasing flock reproductive performance can be achieved through different interventions, including better management practice, nutrition, genetics, and healthcare adopted by the producers and extension agents (Mayberry *et al*., 2018). To ensure the cost-efficiency of interventions, the resulting reproductive performance change should be measured appropriately and objectively in a way that can provide a comprehensive picture of past reproductive performance, current changes, and future expectations. Moreover, it is important to estimate the occurrence of various reproductive disorders such as abortion and their causes and/or risk factors under different agroecologies and production systems to design more realistic and efficient control programmes in smallholder settings. A rapid and accurate diagnostic investigation of the cause of small ruminant abortion is critical to inform possible control options or research on management strategies to reduce reproductive losses. It is also vital in evaluating and understanding knowledge, attitudes, and practices (KAP) on zoonotic risks from livestock birth products among three rural communities in Ethiopia to develop evidence-based disease prevention campaigns and efficient veterinary public health interventions.

1.3. Objectives

1.3.1. General Objective

The general objective of the thesis was to investigate the magnitude, risk factors of small ruminant reproductive health problems, and public health risk of infectious cause of abortion in Ethiopia.

1.3.2. Specific Objectives

1. To systematically assess and provide a quantitative summary of the prevalence of selected infectious abortifacient agents in the aborted fetuses and small ruminants worldwide (Paper I).
2. To assesses common goat flock reproductive performance measures in dryland areas of Ethiopia and proposes a novel quantitative approach for defining the annual reproductive performance index (Paper-II).
3. To estimate the extent of abortion and identify the major causes, and associated risk factors in small ruminants in three agroecologies and production systems of Ethiopia (Paper III).
4. To estimate seroprevalences of multi-pathogen zoonotic abortion-causing agents in small ruminant and associated risk factors in three production systems in Ethiopia (Paper IV).
5. To assess knowledge, attitudes, and practices (KAP) on zoonotic risks from livestock birth products among three rural communities in Ethiopia (Paper V).

1.4. Thesis outline

This thesis is organized into seven chapters, including this general introduction (Chapter 1) and a general discussion (Chapter 7). Chapters 2 to 6 address each of the specific objectives of the study. Figure 1 provides a schematic overview of the structure of the thesis along with its information flow. To obtain insight on the relative importance of infectious agents in small ruminant abortion, appropriate samples, and diagnostic techniques, a systematic review and meta-analysis were carried out for three bacterial and one protozoal cause of abortion worldwide in chapter 2. Chapter 3 assesses commonly used measures for goat flock reproductive performance in dryland systems of Ethiopia and proposes a novel quantitative approach for defining annual reproductive performance by combining performance indicators into a goat-specific index. Chapter 4 tried to estimate the extent of abortion and identify the major causes, and associated risk factors in small ruminants in three agroecology and production systems of Ethiopia, and chapter 5 determine seroprevalences of multi-pathogen zoonotic abortion-causing agents, their co-infection, and associated risk factors in small ruminants in three production system in Ethiopia. Chapter 6 assess knowledge, attitudes, and practices (KAP) on zoonotic risks from livestock birth products among three rural communities in Ethiopia. Finally, the concluding chapter (7) provides a synthesis of the main findings of this thesis, outlines the policy implication of the main findings, point out the limitations of the study, highlights future research needs, and draws the main conclusions on the study results, and forward recommendations based on the main conclusions.

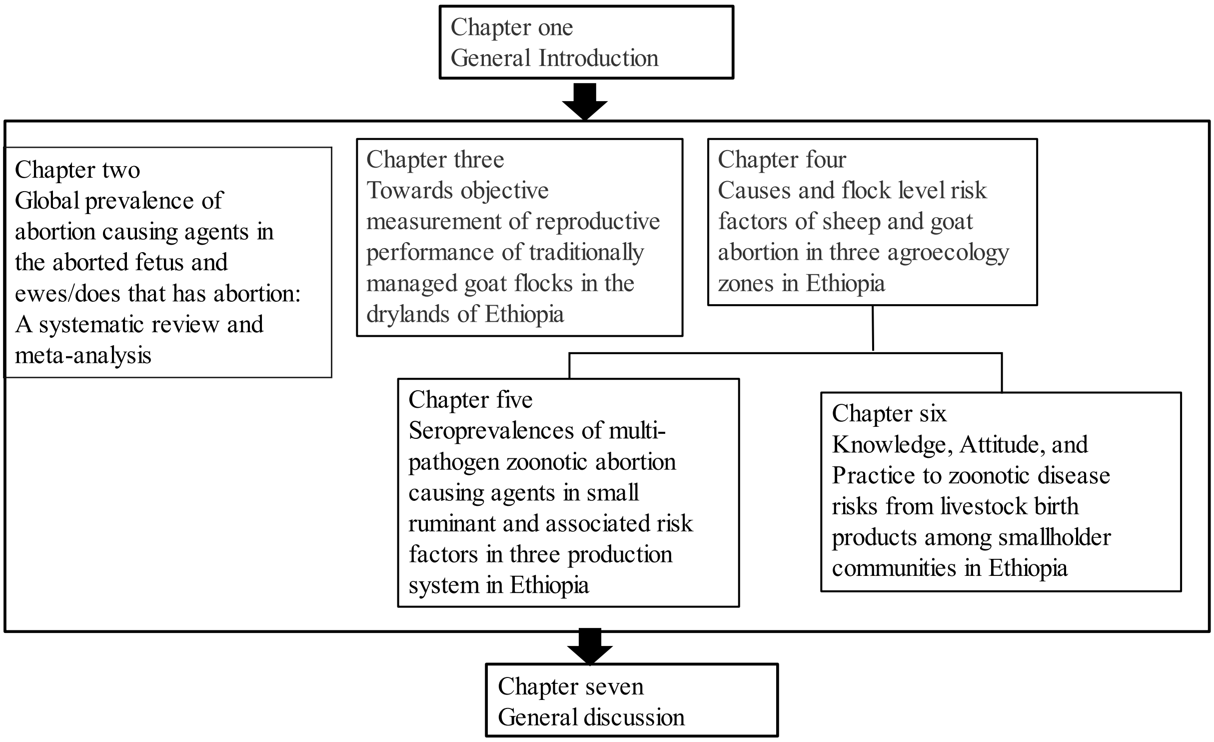

Figure 1.1. Schematic overview of the structure of the thesis showing the interrelationships between the specific components of the study.

Chapter 2
The prevalence of selected infectious abortifacient agents in the aborted fetuses and small ruminants: Systematic Review and Meta-Analyses

Gezahegn Alemayehu, Gezahegne Mamo, Gemechu Chala, Aynalem Mandefro, Samson Leta, Barbara Wieland

Under review by Animal Health Research Reviews Journal

Abstract

This systematic review and meta-analysis assessed the proportion of small ruminant abortion cases caused by four abortion-causing agents namely *Coxiella burnetii (C. burnetii)*, *Chlamydia abortus (C. abortus)*, and *Brucella* spp., and *Toxoplasma gondii* (*T.gondii*). A web-based literature search was performed in PubMed, Web of Science, and Google Scholar databases to access studies published between January 2000 and April 2021. Data on methods used and pathogens detected were extracted using a predefined template. A random-effects model was fitted to derive pooled estimates of the proportion of samples testing positive for different pathogens aggregated by small ruminant species. Separate analyses were conducted for each pathogen for the aborted fetus, placental, and maternal samples. A total of 78 articles which includes multi-pathogens (21), *C. abortus* (15), *C. burnetii* (12), *Brucella* spp. (7), and *T.gondii* (24) articles fulfill the inclusion criteria. From random effect meta-analyses results, highest pooled prevalence was estimated in maternal sera samples for *C. abortus* (ES=24.70%; 95 % CI: 16.81%-33.41%), *C. burnetii* (ES=24.75; 95% CI:18.83%-31.16%) and *T.gondii* (ES=43.37%; 95% CI: 27.39%-60.07%) and in fetal tissues for *Brucella* spp. (ES=42.25%; 95% CI: 16.55%-70.37%). Nevertheless, the lowest pooled prevalence was estimated in aborted fetal tissue for *C. abortus* (ES=19.67%; 95% CI: 12.93- 27.33%) and *T.gondii* (ES=13.68%; 95%CI; 9.59%-18.26%), aborted dam clinical samples(vaginal swab and milk) for *C. burnetii* (ES=12.21%, 95% CI: 6.08%-19.85%) and in aborted animal maternal sera for *Brucella* spp. (ES= 11.78%; 95% CI: 5.76%-19.40%)). Based on the results of the current study, infectious agents could be considered a potential risk factor for small ruminant abortion. It is recommended to carry out well-designed studies to evaluate the relative importance of infectious causes of abortion in small ruminants to assist in the designing more effective strategies for the prevention and control of zoonotic infectious abortion-causing agents in sheep and goat around the world as well as reduce significant economic losses to the small ruminant industry.

Keywords: Abortion; infectious causes; Goat; Meta-analysis; small ruminant; Sheep; zoonotic,

2.1 Introduction

Reproductive failure due to abortion remains a significant challenge for the productivity and welfare of flocks and is responsible for considerable economic losses in the small ruminant industry worldwide (Kardjadj *et al*., 2016; Lievaart-Peterson *et al*., 2012; Merdja *et al*., 2015). Although the relative roles of specific causes of abortion differ in each country or region, generally the most commonly diagnosed causal agents of abortion in sheep and goats are C*oxiella burnetii (C. burnetii), Chlamydia abortus (C. abortus),* and *Brucella* spp., *Leptospira* spp., *Campylobacter* spp., *Listeria* spp. and *Toxoplasma gondii* (Moeller, 2001; Chanton-Greutmann *et al*., 2002; Martins *et al*., 2012; van den Brom *et al*., 2012). These pathogens are also zoonotic and thus can pose serious infection risks for farming communities (Ganter, 2015, Pospischil *et al*., 2002).

To ensure that intervention strategies such as vaccines or antibiotics are successful, accurate diagnosis of abortive pathogens is critical. Accurate abortion diagnosis in small ruminants involves input from the producer, practitioner, and diagnostician. Appropriate collection and submission of samples for abortion diagnosis is critical for diagnostic success (Holler, 2012). Typical samples submitted are placental tissue, fetus, fetal tissue, blood, vaginal swabs, and serum and urine from the dam. These samples are then subjected to the available diagnostic tests for pathogens. There are various methods for the diagnosis of bacterial causes such as culture, histology, immunohistochemistry, and molecular methods (Borel *et al*., 2014). Though it has little value in abortion diagnosis, serological analysis of dam serum remains the preferred option in many diagnostic laboratories to test the presence of antibodies against various bacterial agents (Mearns, 2007; Holler, 2012; Menzies, 2011; Crilly and Gascoigne, 2016).

Many investigations end up with a low etiological diagnosis rate. This might be due to the reason that the majority of abortions are sporadic and that the submissions of samples from those cases are not economically feasible. Moreover, diagnostic laboratories primarily focus on the most likely etiologies and those with zoonotic potential because ruling out all the possible causes of abortion can prove costly.

An abortion risk higher than 5% was deemed unacceptable and worthy of aggressive diagnostic investigation (Menzies, 2011; Holler, 2012). A rapid and accurate diagnostic investigation is critical to inform possible control options or research on management strategies to reduce reproductive losses. Since *C. burnetii, C. abortus, Brucella* spp. and *T. gondii* are the most important causes for small ruminant abortions all over the world, this study aimed to systematically review the available literature and produce a quantitative summary of the proportion of abortion cases caused by those infectious agents worldwide.

2.2 Methodology

2.2.1 Protocol

A systematic review protocol that describes eligibility criteria, information sources, search strategy, study records, and data synthesis was developed based on the guideline of preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) statement (Moher *et al*., 2015) and reported accordingly (Moher *et al*., 2009).

2.2.2 Eligibility criteria

A study was deemed eligible for data extraction and meta-analysis if it was published in English in or after the year 2000, conducted in ewes or does suffering from naturally occurring abortion, reported the proportion of at least one abortion-causing agent under consideration from abortion cases and indicated data sources. In multi-pathogen studies, all pathogens under consideration in an eligible study were included in the meta-analysis.

2.2.3. Information sources

An extensive web-based literature search was performed through PubMed. Web of Science and Google Scholar databases. The names of infectious agents and abortion were the keywords used in electronic searches and combined with small ruminant species. Citations published in English in or after January 2000 were identified during the searches. The final search was done on May 24, 2021. Full-text articles were downloaded or obtained from the library of the International Livestock Research Institute (ILRI) and College of Veterinary Medicine and Agriculture, Addis Ababa University.

2.2.4 Search strategy

Electronic search strings were developed using the following keywords: (list of hazards) and (animal species) and (abortion). Hazards considered during the literature search were *C.*

abortus, C. burnetii, *Brucella* spp., and *T.gondii*. The terms small ruminants, sheep, goats, ewe, doe, ovine, and caprine were used to describe animal species. For example, to identify studies related to Chlamydial causes of abortion, *'Chlamydia abortus'** 'abortion'*' small ruminants' or 'sheep' or 'goats' or 'ewe' or 'doe' or 'ovine' or 'caprine' keywords were used.

2.2.5 Data management

Details of the studies identified were imported into EndNote version X7.8 software for duplicates identification and removal. Screening and selection of eligible studies based on title and abstract were then conducted in an EndNote and grouped into labeled subgroups for further evaluation and storage. Extracted data from the eligible studies were managed and stored in Microsoft Excel. Data coding and analysis were conducted using STATA software version 16 (Stata Corp, College Station, TX, USA).

2.2.6 Selection process

The downloaded titles and abstracts were screened by two independent reviewers. Articles selected by at least one reviewer were retained for further review. For cases where the two reviewers had disagreements, a consensus was sought through discussion. Articles that passed the preliminary screening were assessed against the predefined inclusion criteria by reviewing the full-text publications. Studies were excluded if the proportion of positive and negative samples at the animal level was not identifiable, the proportion of specific abortion-causing agents was not indicated, diagnostic test methods were not specified, country of the study was not specified, the language of the publication was not English, the reporting of the proportion was not consistent, the proportion was not from abortion cases only, the results were not clear, the full paper was not available if the proportion was not aggregated according to small ruminant species, samples type, and diagnostic method, or the study was not relevant (e.g.

experimental abortion). The PRISMA flow diagram (Moher *et al*., 2009) describes the number of articles retrieved, screened, and included or rejected as presented in Fig. 2.1

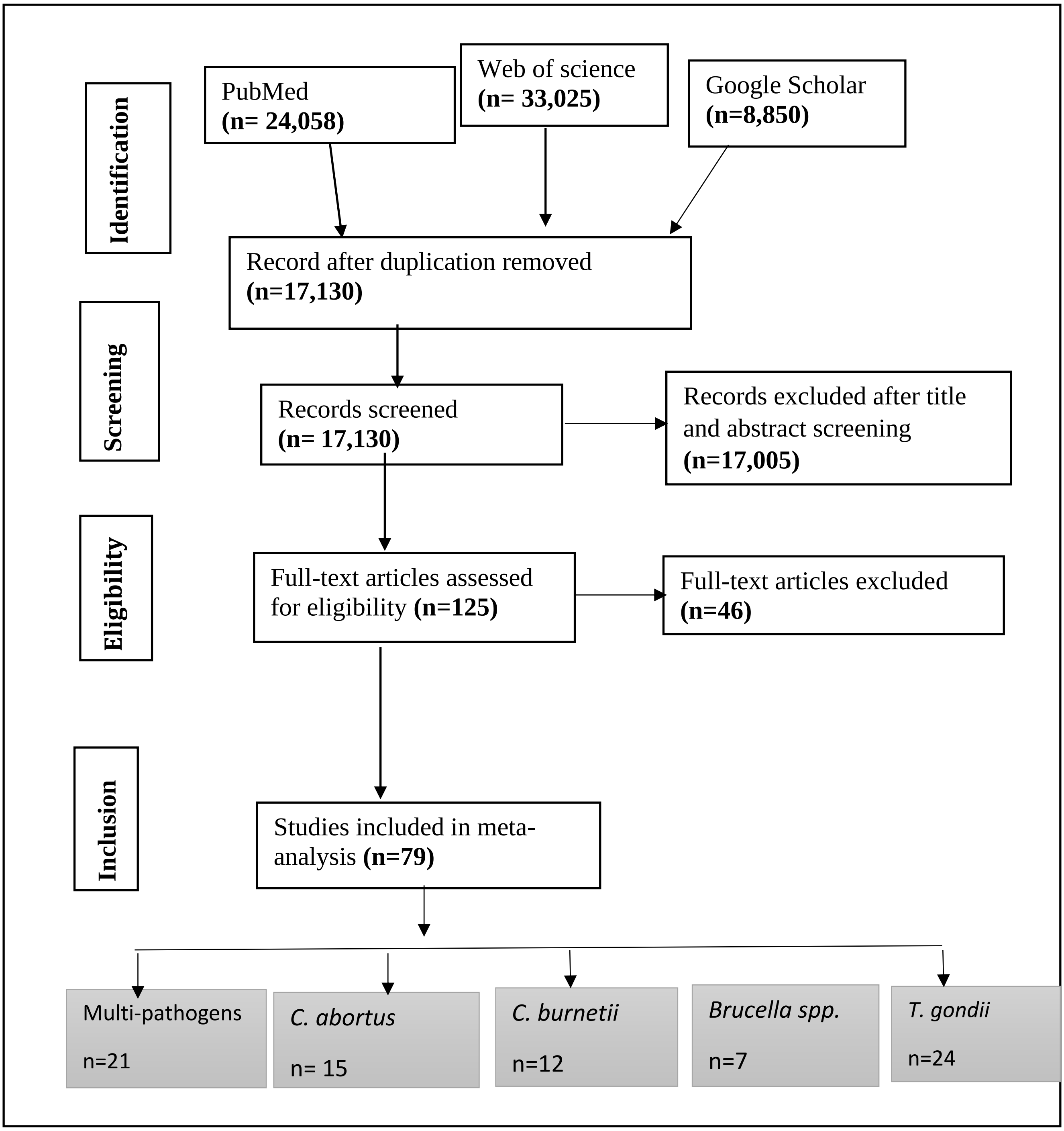

Figure 2.1 Flow diagram of the selection of eligible articles

2.2.7 Data collection process

Data extraction from all eligible studies was completed by two reviewers independently into a data extraction template prepared in Microsoft Excel. For multi-pathogen studies, data were extracted for all infectious agents included in this systematic review, even if tested negative. Once the extraction was completed, the data were cross-checked against the original article by the first author.

2.2.8 Data items

Data extracted from each study included: first author, year of publication, year of study, location (country), data source, pathogens, animal species, test methods, type of sample, number of abortion cases, number of positives, number of negatives, and proportion of positives.

2.2.8 Outcomes and prioritization

The outcomes of interest for this systematic review and Meta-analyses were the proportion of aborted fetus, placenta, and ewe/does test positive *C. abortus, C. burnetii*, *Brucella* spp., and *T.gondii* As some studies reported multiple samples from sheep and goat using various diagnostic methods from various abortion from multiple data sources (e.g. Outbreak-associated and surveillance and cross-sectional study design), it was possible for multiple estimates to be reported from the same study for the proportion of abortion cases caused by different infectious agents, small ruminant species and test method. Therefore, the term 'outcome' was used to describe the probability of cases of aborted fetus, placenta, and aborted ewes/does positive with the infectious agents under consideration.

2.2.9 Data Synthesis

A Der Simonian and Laird random-effects model was used for pooling proportions and presenting subgroup and overall pooled estimates with inverse-variance weights (DerSimonian and Laird, 1986).

The Freeman-Tukey double arcsine transformation was performed to compute the weighted pooled estimate and was back-transformed to the proportion estimate. The Freeman-Tukey double arcsine transformation was used to retain all the studies and guaranteed to have admissible confidence intervals for each study as well as for the pooled proportion (Miller, 1978). The robustness of a pooled estimate was tested by a single study omitted influence analysis. An individual data point was considered to not influence if the pooled estimate without it was within the 95 % confidence limits of the overall mean.

Inter-study heterogeneity was assessed with Cochrane's Q-test reported as P-value. The percentage of total variation between studies due to heterogeneity was evaluated by inverse variance index (I^2) measures. I^2 values of 25 %, 50 %, and 75 % were represented as low, moderate, and high degrees of heterogeneity among studies, respectively. I^2 value 0% indicates no observed heterogeneity among the studies (Higgins and Thompson, 2002).

Data were graphically displayed in forest plots for each abortion-causing bacterial agent and point estimates of proportion within squares of variable size (representative of the weights given to the studies based on the precision of the effect size (ES), with 95% confidence intervals (CI) were displayed.

2.3 Results

2.3.1 Literature search results and eligible studies

A total of 17,130 papers were identified through the electronic search for selected pathogens for this systematic review and meta-analyses after duplicate removal. Of these, 17,005 were excluded based on their titles and abstracts. Of the remaining 125 articles, 79 full-text papers met the eligibility criteria for inclusion in the meta-analysis. The reasons for the exclusion of 47 studies during the full paper evaluation were as follows: i) it was not possible to define the proportion of positives and negatives at animal level (5), ii) the proportions of specific abortion-causing agents was not indicated (2), iii) diagnostic test methods were not specified (1), iv) country of the study was not specified (1), v) language of the publication was not English (3), vi) inconsistent reporting of the proportion (2), vii) specified result was not from abortion cases only (5), viii) the number of tested and positive animals were not indicated (4), ix) results were not clear (4), x) full papers were not available (2), the result were not aggregated with animal species(5) and test methods(2) and sample type(4) and xi) study was not relevant (7).

2.3.2 Study characteristics

The eligible studies were conducted and published between 2001 and 2021. They covered 30 countries in five regions of the world. Risk-based surveillance, outbreak investigation, and cross-sectional study were used as data sources for the included papers. Detailed characteristics of the studies included are described under each pathogen and listed in Table 1-11.

2.3.3 Prevalence of *C. abortus* in the aborted fetus and aborted ewe/doe

Data from 8098 samples derived from 77 data points reflecting animal species and sample types were extracted from 33 eligible papers from 22 countries in 4 continents were used for this systematic review and Meta-analyses. The studies were conducted on aborted fetal tissues (n= 28), placenta (n=20), maternal sera (n= 22), vaginal swabs (n=6) and abomasum content(n=1) using molecular (PCR), histopathological and serological methods. Sample types used in the original study aggregated by small ruminant species are presented in Fig 2.2 Detailed characteristics of the studies included are given in Table 1 to 11)

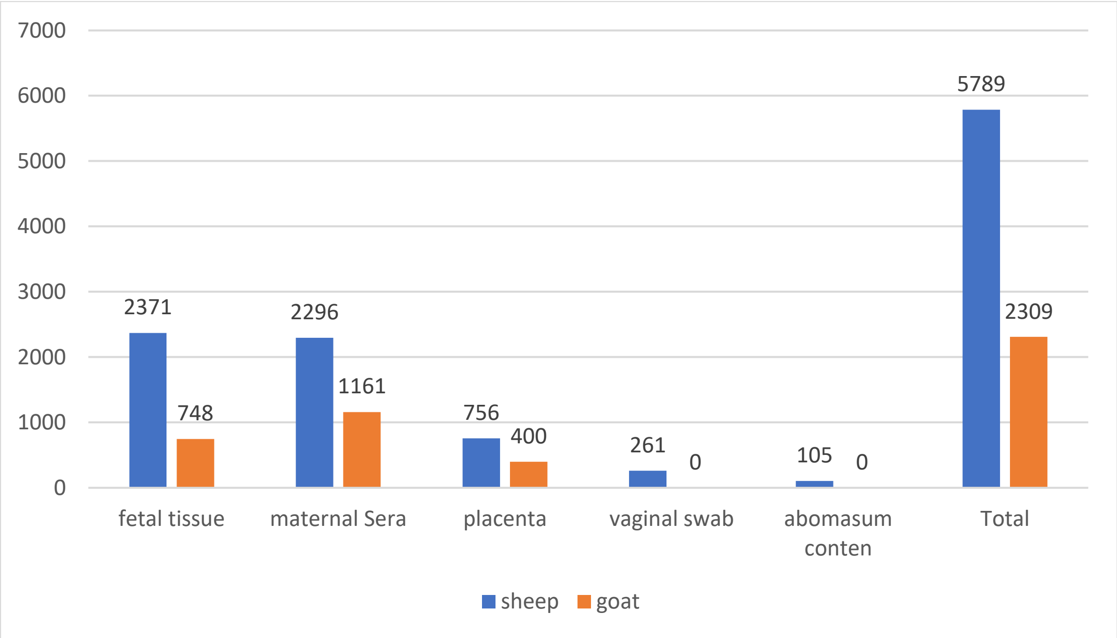

Figure 2.2 Sample types used to detect *C. abortus* from sheep and goat abortion cases

2.3.3.1 Prevalence C. abortus in aborted fetus

A total of 2785 samples of an aborted fetus and fetal abomasum content were tested in original studies for the presence of *C. abortus* using PCR (1589 samples), IHC (1187 samples), and IFA (9 samples). From the total samples, *C. abortus* was detected in 459 cases using upper mentioned diagnostic test techniques (Table 2.1). According to the obtained results, the pooled prevalence of *C. abortus* in the aborted fetuses of a small ruminant is found to be 19.67% (95% CI: 12.93- 27.33%) and it was estimated 20.29% (95 % CI: 12.04%-29.91%) for sheep and

18.70% (95 % CI:6.87% - 34.05%) for goat. There is significant heterogeneity in the prevalence estimates among different studies (Fig 2.3).

Table 2.1 Characteristics of the included studies for the prevalence of *C. abortus* in the aborted fetuses of sheep and goat.

Study	**Origin of the study**	**Animal species**	**Test method**	**No of sample**	**No positive**	**Apparent prevalence**
Kalender *et al*. (2013)	Turkey	Caprine	PCR	7	1	14.29
van den Brom *et al*. (2012)	Netherlands	Ovine	IHC	506	49	9.68
Heidari *et al*.2017	Iran	Ovine	PCR	183	15	8.20
Kalender *et al*. (2013)	Turkey	Ovine	PCR	64	4	6.25
Clemente *et al*. (2011)	Portugal	Caprine	PCR	66	32	48.48
Szeredi *et al*. (2006)	Hungary	Ovine	IHC	75	13	17.33
van den Brom *et al*. (2012)	Netherlands	Caprine	IHC	360	15	4.17
Hässig *et al*. (2003)	Switzerland	Ovine	PCR	20	3	15.00
Masala *et al*. (2007)	Italy	Ovine	PCR	292	7	2.40
Bagdonas *et al*. (2007)	Lithuania	Ovine	IFA	8	5	62.50
Clemente *et al*. (2011)	Portugal	Ovine	PCR	59	28	47.46
Hazlett *et al*. (2013)	Canada	Ovine	PCR	18	11	61.11
Hazlett *et al*. (2013)	Canada	Caprine	PCR	14	12	85.71
Hireche *et al*. (2015)	Algeria	Ovine	PCR	10	6	60.00
Masala *et al*. (2005)	Italy	Caprine	PCR	59	0	0.00
Heidari *et al*. (2017)	Iran	Caprine	PCR	117	18	15.38
Szeredi *et al*. (2006)	Hungary	Ovine	IHC	246	113	45.93
Hamedi *et al*. (2020)	Iran	Ovine	PCR	150	36	24.00
Hamedi *et al*. (2020)	Iran	Caprine	PCR	50	11	22.00
Aras *et al*. (2021)	Turkey	Ovine	PCR	105	7	6.67
Aras *et al*. (2021)	Turkey	Caprine	PCR	15	0	0.00
Simeonov and Chilingirova (2018)	Bulgaria	Caprine	PCR	24	6	25.00

Simeonov and Chilingirova (2018)	Bulgaria	Ovine	PCR	43	18	41.86
Barati *et al*. (2017)	Iran	Ovine	PCR	108	24	22.22
Arif *et al*. (2020)	Iraq	Ovine	PCR	30	1	3.33
Abadi *et al*. (2019)	Iran	Ovine	PCR	78	0	0.00
Schnydrig *et al*. (2017)	Switzerland	Ovine	PCR	41	17	41.46
Schnydrig *et al*. (2017)	Switzerland	Caprine	PCR	36	8	22.22

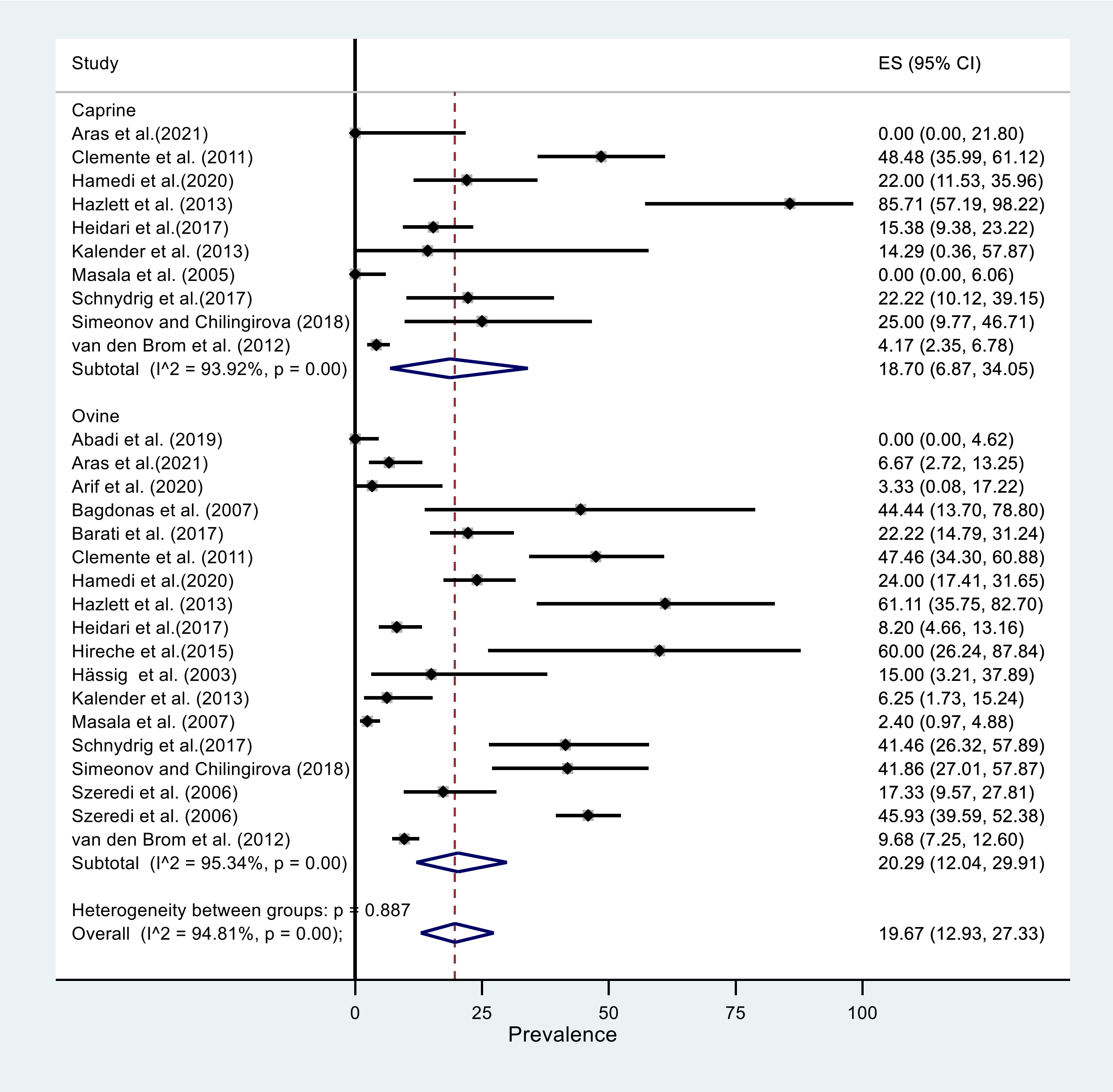

Figure 2.3 The pooled prevalence of *C. abortus* in the aborted fetuses and stillbirths of sheep and goats.

2.3.3.2 Prevalence of C. abortus in placental samples

From the total of 1156 placental samples investigated from aborted ewe and does, the presence of *C. abortus* was detected from 320 abortion cases using PCR (190/665), IHC (76/123), IFA (36/220), and HP (18/148) (Table 2.2). The overall pooled prevalence estimates of *C. abortus* in the placenta of an aborted small ruminant is found to be 37.57% (95 % CI:25.69%- 50.19%) and it is estimated at 41.95% (95 % CI: 24.77%- 60.11%) and 31.46% (95 % CI:15.97%- 49.07%) in the placenta of aborted sheep and goat, respectively (Fig 2.4). There is a high degree of heterogeneity in prevalence estimates among studies for both sheep and goat (Fig 2.5).

Table 2.2 Characteristics of the included studies for the prevalence of *C. abortus* in the placental tissues of sheep and goat

Study	**Origin of the study**	**Animal species**	**diagnostic test methods**	**Sample size**	**Number positive**	**Apparent prevalence**
Ababneh *et al*. (2014)	Jordan	Ovine	PCR	46	28	60.8
Masala *et al*. (2005)	Italy	Caprine	PCR	11	1	9
Navarro *et al*. (2015)	Spain	Caprine	IHC	23	10	43.5
Masala *et al*. (2007)	Italy	Ovine	PCR	76	5	6.5
Hazlett *et al*. (2013)	Canada	Ovine	PCR	176	37	21
Hireche *et al*.2015	Algeria	Ovine	PCR	8	4	50
Ababneh *et al*. (2014)	Jordan	Caprine	PCR	20	10	50
Moeller (2001)	California	Caprine	IFA	211	30	14
Bagdonas *et al*. (2007)	Lithuania	Ovine	IFA	9	6	66.7
Hazlett *et al*. (2013)	Canada	Caprine	PCR	99	49	49
Masala *et al*. (2005)	Italy	Ovine	PCR	137	7	5.1
Masala *et al*. (2007)	Italy	Caprine	PCR	8	1	12.5
Oporto *et al*. (2006)	Spain	Ovine	HP	148	18	12
Szeredi *et al*. (2006)	Hungary	Ovine	IHC	6	4	66.67
Navarro *et al*. (2015)	Spain	Ovine	IHC	35	22	62.8

Kreizinger *et al*. (2015)	Hungary	Caprine	PCR	5	2	40
Szeredi *et al*. (2006)	Hungary	Ovine	IHC	59	40	67.7
Navarro *et al*. (2015)	Spain	Ovine	PCR	35	21	60
Navarro *et al*. (2015)	Spain	Caprine	PCR	23	9	3.1
Kreizinger *et al*. (2015)	Hungary	Ovine	PCR	21	16	76.2

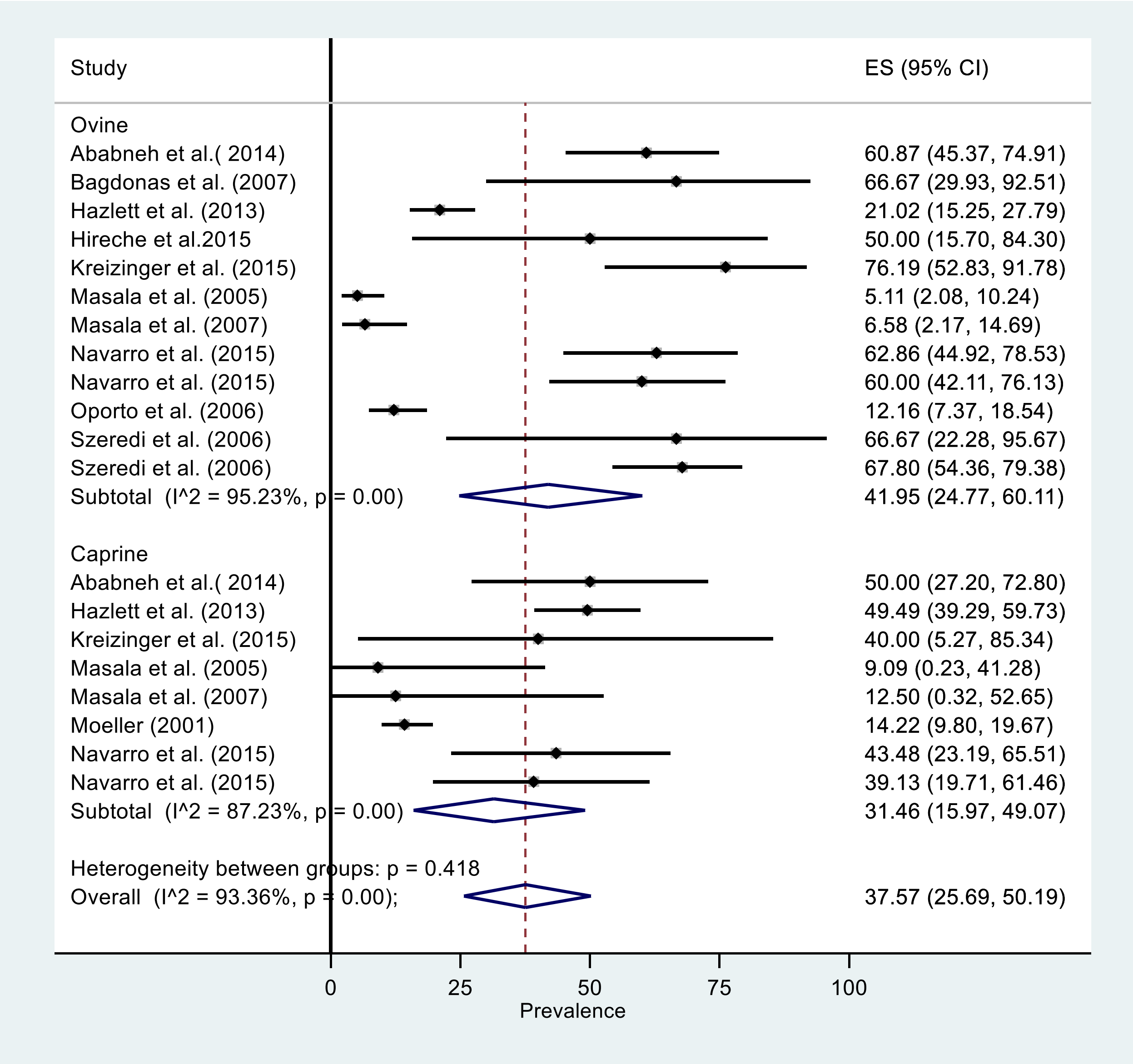

Figure 2.4 The pooled prevalence of *C. abortus* in the placental tissue of sheep and goat.

2.3.3.3 Prevalence C. abortus in maternal samples

A total of 3705 (3444 maternal sera and 261 vaginal swabs) samples were collected and tested to detect the exposure and presence of *C. abortus* from aborted dams. Anti-*C. abortus* antibodies were detected from 524 maternal sera using various serological methods. In addition, *C. abortus* was detected from vaginal swabs of 33 abortion cases using PCR (26/ 249) and IFA (7/ 12) (Table 2.3). The overall pooled prevalence of *C. abortus* was estimated at 24.59% (95 % CI: 6.71% - 47.37) from aborted ewe vaginal swabs (Fig.2.6). There is a wide variation in the prevalence estimates among different studies in vaginal swab samples (I^2 = 88.02% %, P = 0.00). Pooled seroprevalence of *C. abortus* is found to be 24.70% (95 % CI: 16.81%-33.41%) in aborted small ruminants where 29.09% (95 % CI: 17.99 %- 41.55%) and 18.94% (95 % CI: 9.11%-31.04%) was estimated in aborted ewes and doe sera, respectively (Fig 2.6). There is also a high degree of heterogeneity in prevalence estimates among studies for both ewes (I^2 = 96.89%, P = 0.00 and does (I^2 = 90.88%, P = 0.00).

Table 2.3 Characteristics of the included studies for the prevalence of *C. abortus* in aborted ewes and does.

Study	Origin of the study	Animal species	Test methods	Sample type	No tested	No positive	Apparent prevalence
Abd *et al*. (2011)	Saudi Arabia	Caprine	ELISA	Sera	706	40	5.66
Ababneh *et al*. (2014)	Jordan	Ovine	PCR	vaginal swab	15	7	46.70
Bisias *et al*. (2009)	Greece	Ovine	ELISA	Sera	289	43	14.90
Bisias *et al*. (2009)	Greece	Caprine	ELISA	Sera	174	37	21.20
Marsilio *et al*. (2005)	Italy	Ovine	PCR	vaginal swab	117	6	5.12
Abdelkadi *et al*. 2017	Algeria	Ovine	ELISA	Sera	180	55	31.00
Benkirane *et al*. (2015)	Morocco	Caprine	ELISA	Sera	106	16	15.10
Spilovska *et al*. (2009)	Slovakia	Ovine	ELISA	Sera	382	52	13.60
Abd *et al*. (2011)	Saudi Arabia	Ovine	ELISA	Sera	879	40	4.55
Špičić *et al*. (2015)	Bosnia and Herzegovina	Ovine	ELISA	Sera	14	4	28.60
Krkalić *et al*. (2015)	Bosnia and Herzegovina	Caprine	ELISA	Sera	12	11	91.70
Bagdonas *et al*. (2007)	Lithuania	Ovine	CFT	Sera	64	43	67.18
Benkirane *et al*. (2015)	Morocco	Ovine	ELISA	Sera	202	55	27.20
Szeredi *et al*. (2006)	Hungary	Ovine	CFT	Sera	38	15	39.40
Ababneh *et al*. (2014)	Jordan	Ovine	PCR	vaginal swab	15	6	40.00
Bagdonas *et al*. (2007)	Lithuania	Ovine	IFA	vaginal swab	12	7	58.30
Kalender *et al*. (2013)	Turkey	Ovine	PCR	vaginal swab	100	6	6.00
Špičić *et al*. (2015)	Croatia	Caprine	ELISA	Sera	69	8	11.50

Oh *et al*. (2017)	Korea	Caprine	ELISA	Sera	47	1	2.10
Špičić *et al*. (2015)	Croatia	Ovine	ELISA	Sera	93	19	20.00
Szeredi *et al*. (2006)	Hungary	Ovine	CFT	Sera	104	58	56.00
Simeonov and Chilingirova (2018)	Bulgaria	Caprine	ELISA	Sera	19	5	26.32
Simeonov and Chilingirova (2018)	Bulgaria	Ovine	ELISA	Sera	30	10	33.33
Díaz-Cao *et al*. (2018)	Spain	Ovine	PCR	vaginal swab	2	1	50.00
Schnydrig *et al*. (2018)	Switzerland	Ovine	ELISA	Sera	21	8	38.10
Schnydrig *et al*. (2020)	Switzerland	Caprine	ELISA	Sera	15	4	26.67

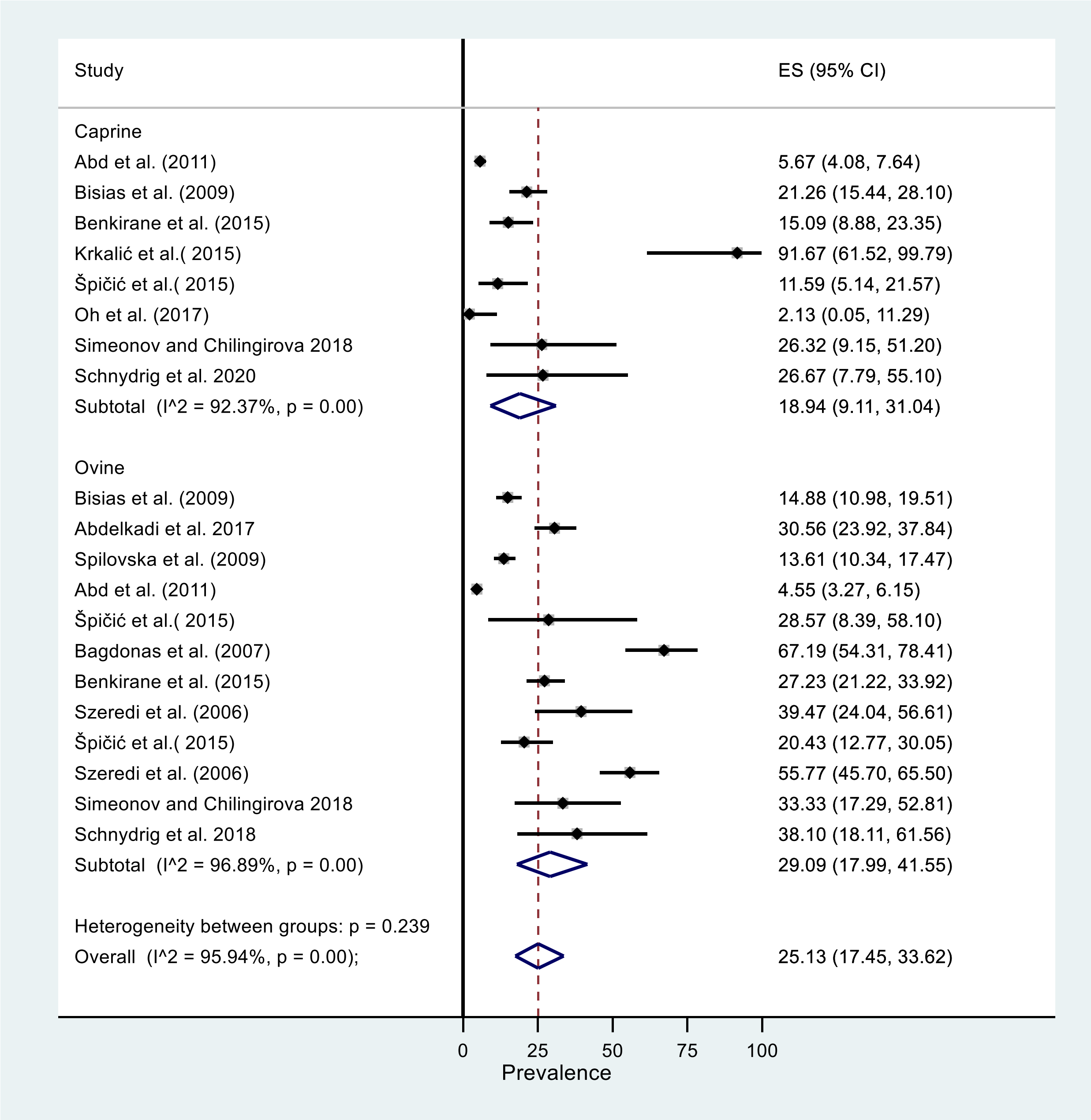

Figure 2.5 The pooled prevalence estimates of *C. abortus* in the vaginal swab of aborted ewes and does

2.3.4 The prevalence of *C. burnetii* in the aborted fetus and aborted ewes/does

Data was extracted from 88 reports collected from 19046 samples in 18 countries in 4 continents The studies were conducted on aborted fetal tissues (n= 22), placenta (n=36), maternal sera (n= 21), vaginal swabs (n=6), urine and fetal fluid (n=1) using PCR (n=53) ELISA (n=19), IHC (n=14) and IFA (2). The type and number of samples used in the original study aggregated by small ruminant species are presented in Fig 2.7. Detailed characteristics of the studies included are given in Table 2.4 to 2.6).

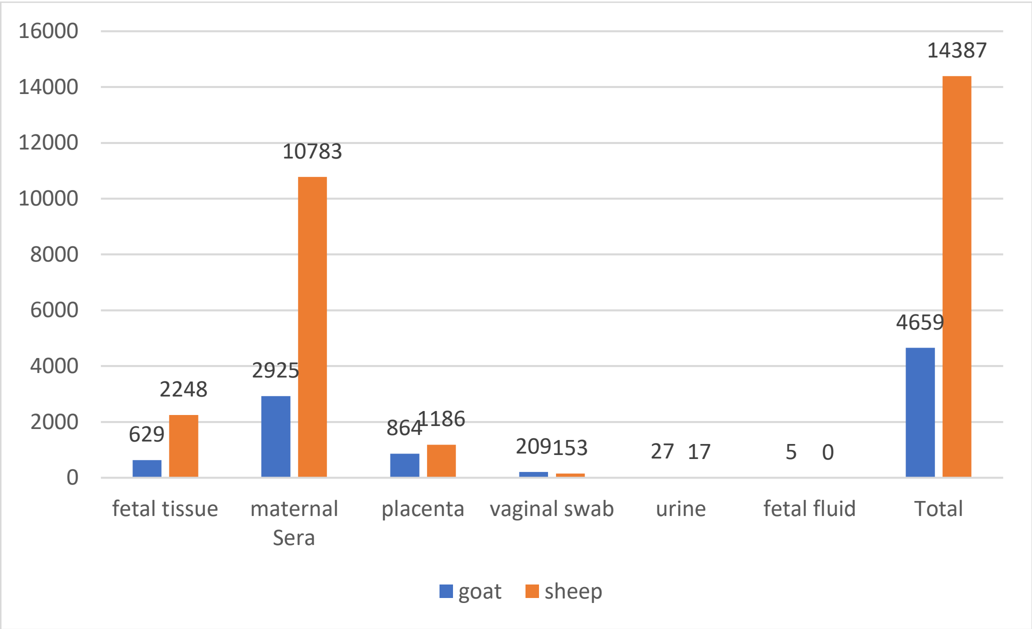

Figure 2.7 Sample types used to detect *C. burnetii* from sheep and goat abortion cases

2.3.4.1 Prevalence of C. burnetii in aborted fetus

To detect *C. burnetii* from aborted fetuses a total of 2882 fetal tissues were tested with PCR (1695 samples) and IHC (1187 samples). Overall, C. *burnetii* was detected from 290 cases using upper mentioned two diagnostic methods (Table 2.4). The pooled prevalence of *C. burnetii* was estimated at 15.77% (95% CI: 9.46%-23.12%) in small ruminant aborted fetus where 28.03% (95% CI: 9.27% -50.91% and 12.07% (95% CI: 6.47%-19.04%) (Fig 2.8) from goat and sheep, respectively. There is a high degree of heterogeneity in prevalence estimates among studies for both ewes (I^2 = 94.60%, P = 0.00 and doe (I^2 = 94.84%, P = 0.00).

Table 2.4 Characteristics of the included studies for the prevalence of *C. burnetii* in the aborted fetus of sheep and goat

Study	Origin of the study	Animal species	Test methods	No Sample	No positive	Apparent prevalence
Masala *et al*. (2004)	Italy	Caprine	PCR	50	3	6.00
Masala *et al*. (2007)	Italy	Ovine	PCR	292	32	10.90
Jones *et al*. (2013)	Britain	Caprine	PCR	7	4	57.10
Heidari *et al*. (2017)	Iran	Ovine	PCR	183	5	2.73
Jones *et al*. (2013)	Britain	Caprine	PCR	5	3	60.00
Abiri *et al*. (2016)	Iran	Caprine	PCR	2	0	0.00
Oporto *et al*. (2006)	Spain	Ovine	PCR	148	4	3.00
Szeredi *et al*. (2006)	Hungary	Ovine	IHC	246	5	2.00
van den Brom *et al*. (2012)	Netherlands	Caprine	IHC	360	31	8.60
Hazlett *et al*. (2013)	Canada	Ovine	PCR	42	28	67.00
Masala *et al*. (2004)	Italy	Ovine	PCR	372	40	10.00
Heidari *et al*. (2017)	Iran	Caprine	PCR	117	1	0.85
Szeredi *et al*. (2006)	Hungary	Ovine	IHC	75	1	1.00
van den Brom *et al*. (2012)	Netherlands	Ovine	IHC	506	9	1.80
Abiri *et al*. (2016)	Iran	Ovine	PCR	92	16	17.30
Hazlett *et al*. (2013)	Canada	Caprine	PCR	31	23	74.00
Kilicoglu *et al*. (2020)	Turkey	Ovine	PCR	79	8	10.13
Kilicoglu *et al*. 2020)	Turkey	Caprine	PCR	20	0	0.00
Mobarez *et al*. (2020)	Iran	Ovine	PCR	94	20	21.28
Mobarez *et al*. (2020)	Iran	Caprine	PCR	6	6	100.00
Roshan *et al*. (2018)	Iran	Ovine	PCR	78	13	16.67
Schnydrig *et al*. (2018)	Switzerland	Ovine	PCR	41	19	46.34
Schnydrig *et al*. (2018)	Switzerland	Caprine	PCR	36	19	52.78

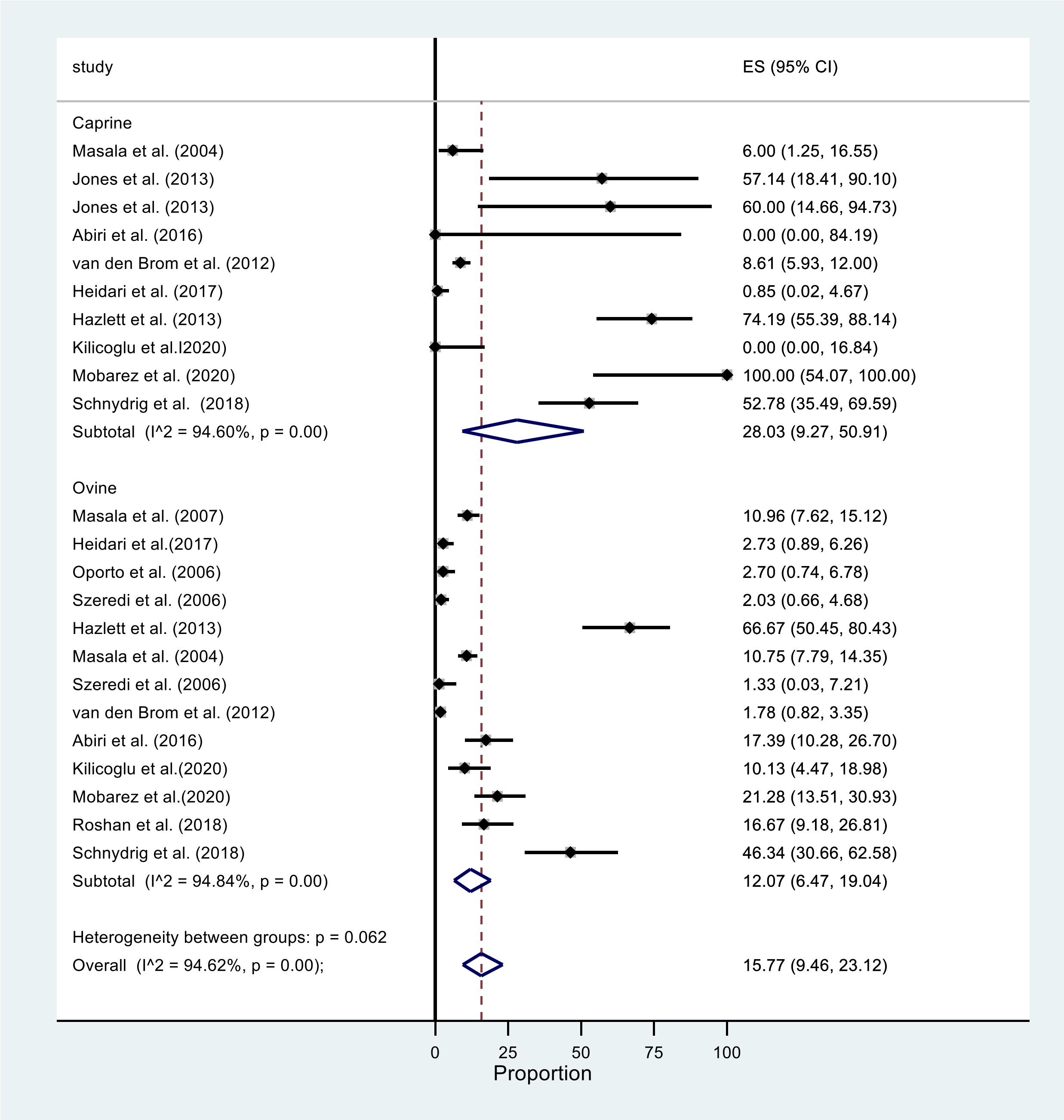

Figure 2.8 The pooled prevalence estimate for *C. burnetii* in the aborted fetus of sheep and goat

2.3.4.2 Prevalence rates C. burnetii in the placenta of aborted ewes/does

From the total of 1025 placental samples collected from aborted ewe and does, *C. burnetii* was detected from 272 abortion cases using PCR (243/730) and IHC (29/295) (Table 2.5). The overall pooled prevalence of *C. burnetii* is found to be 21.13% (95 % CI:25. 8.79%-36.48) in placental tissues of aborted small ruminant whereas it was estimated at 23.28% (4.83%- 48.19%) and 18.96% (95 % CI: 3.51%-41.79%) in aborted goat and sheep placenta, respectively. There is a high degree of heterogeneity in prevalence estimates among studies for both sheep (I^2 = 94.89%, P = 0.00 and goat (I^2 = 97.05%, P = 0.00).

Table 2.5 Characteristics of the included studies for the prevalence of *C. burnetii* in the placenta of aborted sheep and goat

Study	Origin of the study	Animal species	Test methods	No of sample	No of positive	Apparent prevalence
Moeller (2001)	California	Caprine	IHC	211	19	9.00
Kreizinger *et al*. (2015)	Hungary	Caprine	PCR	5	1	20.00
Abdel-Moein and Hamza (2017)	Egypt	Caprine	PCR	29	1	3.45
Navarro *et al*. (2015)	Spain	Ovine	PCR	35	4	11.43
Jones *et al*. (2013)	Britain	Caprine	PCR	15	14	93.33
Kreizinger *et al*. (2015)	Hungary	Caprine	IHC	5	0	0.00
Navarro *et al*. (2015)	Spain	Caprine	IHC	23	3	13.04
Hazlett *et al*. (2013)	Canada	Caprine	PCR	100	70	70.00
Oporto *et al*. (2006)	Spain	Ovine	PCR	148	4	2.70
Kreizinger *et al*. (2015)	Hungary	Ovine	IHC	21	3	14.29
Kreizinger *et al*. (2015)	Hungary	Ovine	PCR	21	10	47.62
Navarro *et al*. (2015)	Spain	Caprine	PCR	23	4	17.39
Masala *et al*. (2004)	Italy	Ovine	PCR	83	9	10.84
Navarro *et al*. (2015)	Spain	Ovine	IHC	35	4	11.43
Hazlett *et al*. (2013)	Canada	Ovine	PCR	174	116	66.67
Pritchard *et al*. (2014)	Britain	Caprine	PCR	9	1	11.11

Masala *et al*. (2007)	Italy	Ovine	PCR	76	7	9.21
Masala *et al*. (2004)	Italy	Caprine	PCR	12	2	16.67

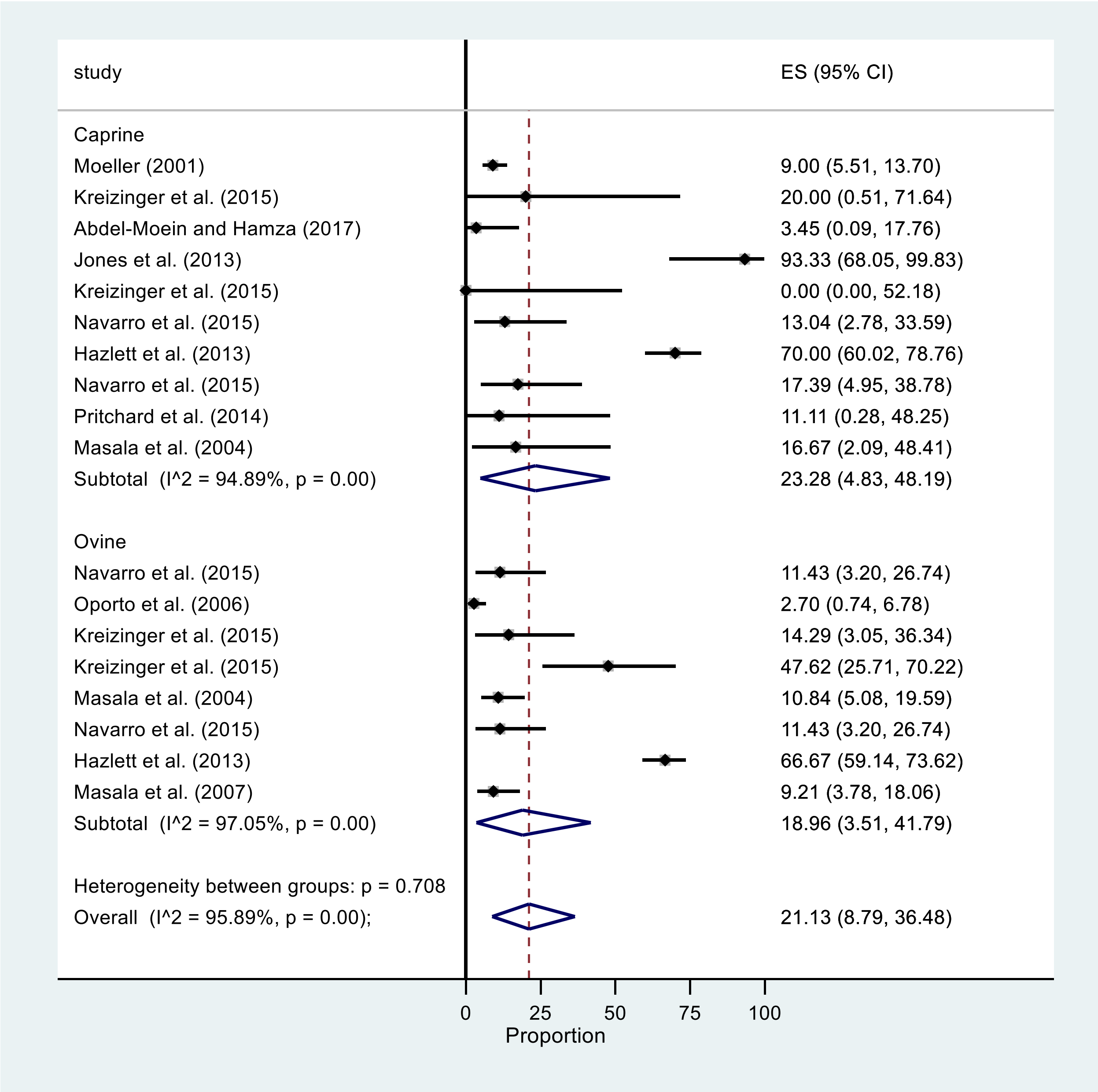

Figure 2.9 The pooled prevalence estimates for *C. burnetii* in the placenta of aborted ewes and does

2.3.4.3 Prevalence of C. burnetii in maternal samples

A total of 14417 maternal samples were collected and tested for *C. burnetii* using ELISA (13612), PCR (709), and IFA (96) diagnostic methods in original studies. The overall pooled seroprevalence estimate of ant-*C. burnetii* antibodies in maternal samples using ELISA and IFA was estimated at 24.75% (95% CI:18.83%-31.16%) for an aborted small ruminant. In aborted sheep and goats, the seroprevalence was estimated at 35.74% (95% CI: 19.28%-54.08%) and 16.92% (95% CI: 11.12 -23.61%), respectively. Other than maternal sera, 710 various maternal samples including feces (13), milk (34), urine (4), and vaginal swab (580) were tested from aborted dams. All these samples were tested using PCR. According to the obtained result, pooled estimate prevalence was found to be 12.21% (95% CI: 6.08%-19.85%). An almost similar result was obtained in sheep and goat samples with 12.54% (95% CI: 5.04% - 22.43%) and 12.25% (95% CI: 3.39%-24.80%) pooled prevalence estimate, respectively (the figure is not shown). There is a wide variation in the prevalence estimates among different studies for both sheep and goat (Fig.2.10).

Table 2.6 Characteristics of the included studies for the prevalence of *C. burnetii* in aborted ewes and does

Study	**Origin of the study**	**Animal species**	**diagnostic test methods**	**Sample type**	**No of sample**	**No positive**	**Apparent prevalence**
Vaidya *et al*. (2010)	India	Caprine	PCR	milk	36	1	2.78
Vaidya *et al*. (2010)	India	Ovine	PCR	VS	43	1	2.33
Bisias *et al*. (2009)	Greece	Ovine	ELISA	Sera	289	141	48.79
Asadi *et al*. (2013)	Iran	Ovine	ELISA	Sera	1100	215	19.55
Vaidya *et al*. (2010)	India	Ovine	ELISA	Sera	43	4	9.30
Bisias *et al*. (2009)	Greece	Caprine	ELISA	Sera	174	110	63.22
Vaidya *et al*. (2010)	India	Caprine	IFA	Sera	53	4	7.55
Benkirane *et al*. (2015)	Morocco	Caprine	ELISA	Sera	106	29	27.36
Masala *et al*. (2004)	Italy	Ovine	ELISA	Sera	7194	652	9.06
Asadi *et al*. (2013)	Iran	Caprine	ELISA	Sera	180	49	27.22
Vaidya *et al*. (2010)	India	Caprine	PCR	VS	53	1	1.89
Vaidya *et al*. (2010)	India	Ovine	PCR	Feces	43	2	4.65
Vaidya *et al*. (2010)	India	Ovine	PCR	milk	28	3	10.71
Rousset *et al*. (2007)	France	Caprine	ELISA	Sera	50	44	88.00
Oh *et al*. (2017)	Korea	Caprine	ELISA	Sera	47	36	76.60
Filioussis *et al*. (2017)	Greece	Ovine	ELISA	Sera	800	64	8.00
Benkirane *et al*. (2015)	Morocco	Ovine	ELISA	Sera	202	31	15.35

Abdel-Moein and Hamza (2017)	Egypt	Caprine	PCR	VS	29	1	3.45
Vaidya *et al*. (2010)	India	Caprine	PCR	Feces	53	0	0.00
Filioussis *et al*. (2017)	Greece	Ovine	ELISA	Sera	800	115	14.38
Masala *et al*. (2004)	Italy	Caprine	ELISA	Sera	2155	271	12.58
Vaidya *et al*. (2010)	India	Caprine	PCR	urine	27	3	11.11
Vaidya *et al*. (2010)	India	Ovine	PCR	urine	17	1	5.88
Vaidya *et al*. (2010)	India	Ovine	IFA	Sera	43	5	11.63
Oh *et al*. (2017)	Korea	Caprine	PCR	VS	47	20	42.55
Vaidya *et al*. (2010)	India	Caprine	ELISA	Sera	53	3	5.66
Abdelkadi *et al*. (2017)	Algeria	Ovine	ELISA	Sera	180	50	27.78
Oliveira *et al*. 2018	Brazil	Caprine	ELISA	Sera	27	15	55.56
Esmaeili *et al*. 2019	Iran	Caprine	PCR	milk	28	10	35.71
Esmaeili *et al*. 2020	Iran	Ovine	PCR	milk	56	20	35.71
HARDI *et al*. 2020	Iraq	Ovine	PCR	feces	48	6	12.50
HARDI *et al*. 2020	Iraq	Caprine	PCR	feces	12	5	41.67
Selim *et al*. 2018	Egypt	Caprine	PCR	VS	80	10	12.50
Selim *et al*. 2018	Egypt	Caprine	ELISA	Sera	80	13	16.25
Selim *et al*. 2018	Egypt	Ovine	PCR	VS	110	25	22.73
Selim *et al*. 2018	Egypt	Ovine	ELISA	Sera	110	37	33.64
Schnydrig *et al*. 2018	Switzerland	Ovine	ELISA	Sera	22	0	0.00

VS=Vaginal swab

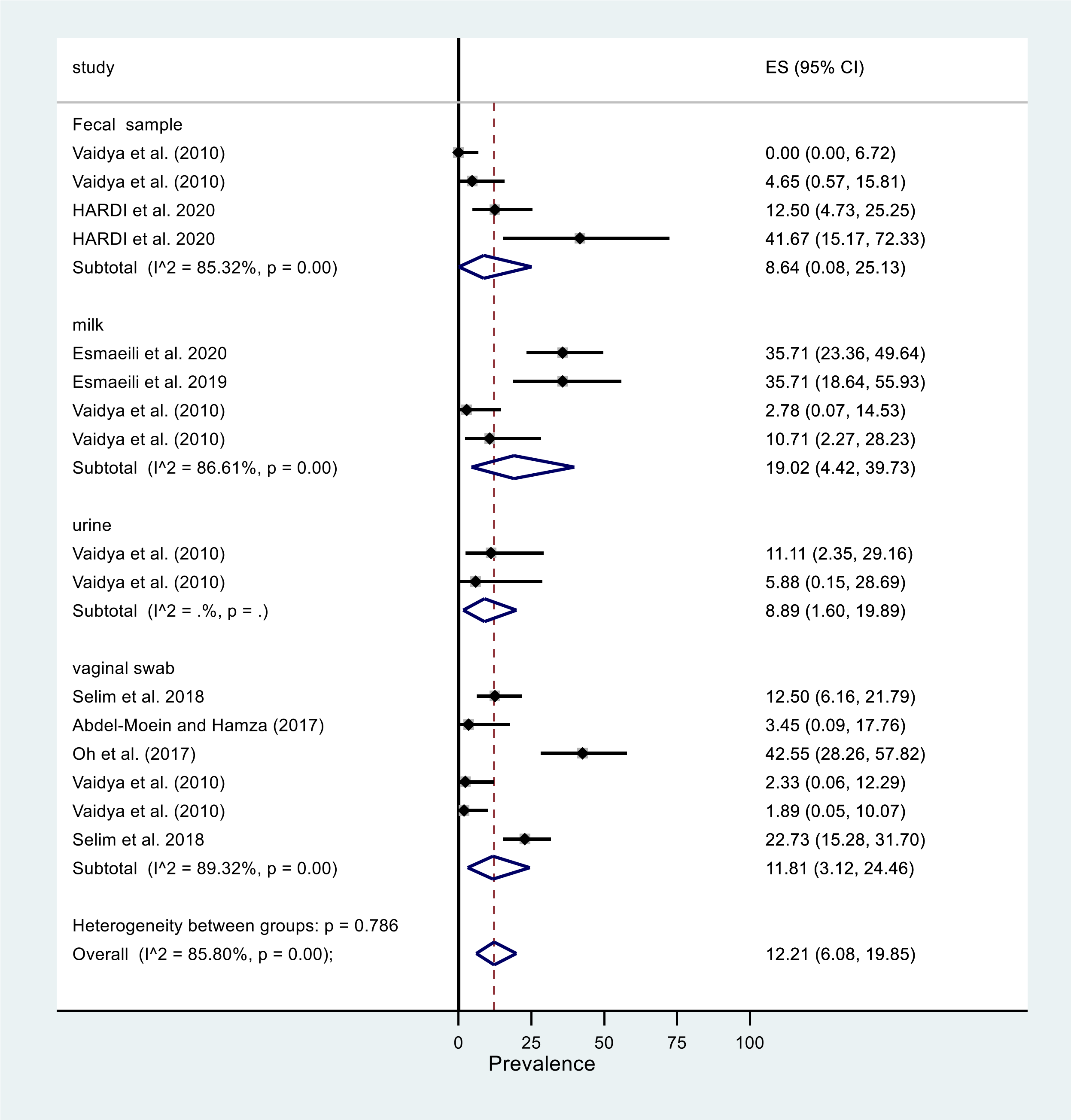

Figure 2.10 The pooled prevalence estimates of *C. burnetii* in the aborted sheep and goat

2.3.5 Prevalence of *Brucella spp.* in aborted fetus and sheep and goat

Data from 1986 samples extracted from 17 reports reflecting animal species and sample types were extracted from 12 eligible papers from 9 countries in 3 continents used for this systematic review and meta-analyses. The studies were conducted on aborted fetal tissues (n= 3), maternal sera (n= 17), and abomasum content (n=1) ELISA (n=7), RBPT(n=5), CFT(n=1), PCR (n=3) and IHC (n=1). Samples types used in the original study aggregated by small ruminant species are presented in Fig 2.11. Detailed characteristics of the studies included are given in Tables 2.7 to 2.8.

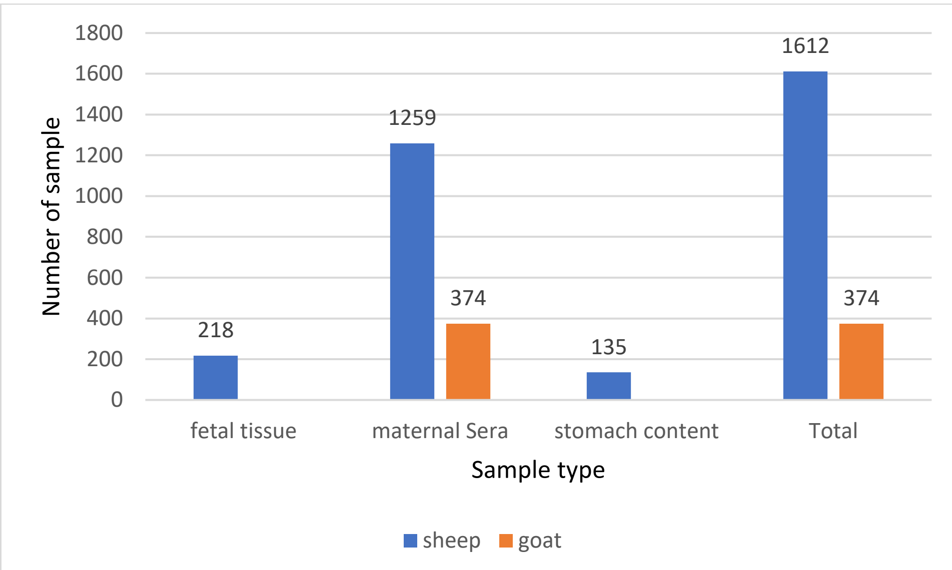

Figure 2.11 Sample types used to detect *Brucella* spp. from sheep and goat abortion cases

2.3.5.1 Prevalence of Brucella spp. *in the aborted fetus of sheep and goat*

A total of 353 aborted fetal samples which constitutes 218 aborted fetal tissue and 135 stomach content of aborted fetus were tested for *Brucella* spp. using PCR (243) and IHC (110) (Table 2.7). All the samples were collected from sheep abortion cases only. From total tested samples, 106 abortion cases were found positive for *Brucella* spp. with the overall pooled prevalence estimate of 42.25% (95% CI: 16.55%-70.37%) in sheep. The prevalence estimates among different studies showed very high heterogeneity (Fig 2.12)

Table 1.7 Characteristics of the included studies for the prevalence of *Brucella* spp. in the aborted fetus of ewes and does

Study	Origin of the study	Animal species	Test methods	No of sample	No of positive	Apparent prevalence
Ilhan and Yener (2008)	Turkey	Ovine	IHC	110	33	30.00
İlhan *et al*. (2007)	Turkey	Ovine	PCR	135	29	21.48
Roshan *et al*. (2018)	Iran	Ovine	PCR	78	15	19.23
Arif *et al*. 2020	Iraq	Ovine	PCR	30	29	96.67

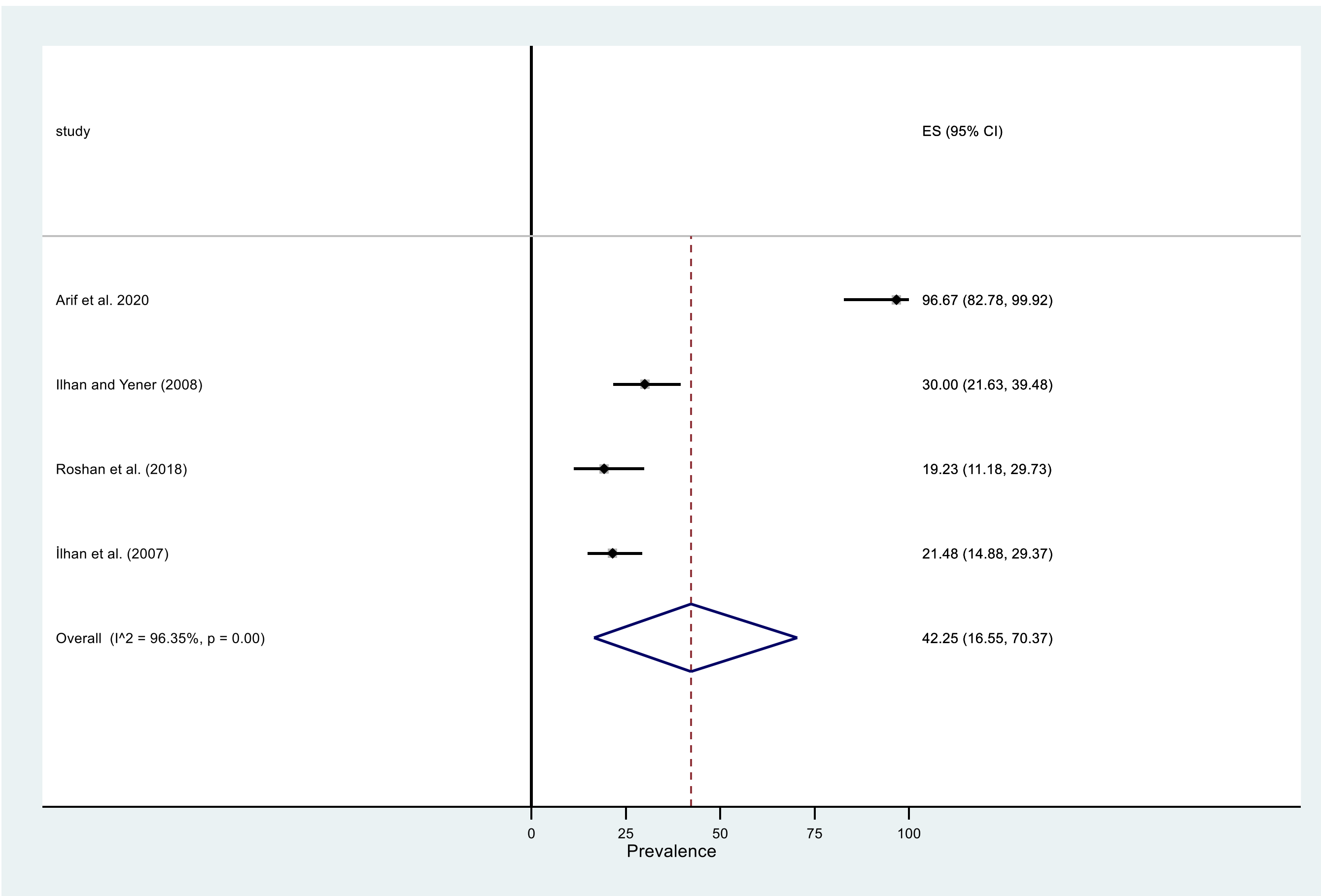

Figure 2.12 The pooled prevalence rates *Brucella* spp in the aborted fetus of sheep and goat

2.3.5.2 Prevalence of Brucella spp. in maternal samples

A total of 1632 sera of aborted dams were tested for the evidence of exposure for *Brucella* spp. using ELISA (661), CFT (400), and RBPT (571). From the total of tested aborted dams, evidence of brucellosis was detected in 308 cases. The overall pooled seroprevalence estimate of ant-*Brucella* spp. antibodies were estimated at 11.78 (95%CI: 5.76%-19.40%) regardless of animal species. However, the seroprevalence was estimated at 14.21% (95% CI: 6.23%-24.63) in aborted ewe and 8.35% (95% CI: 0.42%-22.35%) in doe. There is a wide variation in the prevalence estimates among different studies for both sheep and goat (Fig 2.8). Other than maternal sera 27 vaginal swabs and 31 milk from aborted animals were tested which were not included in the meta-analysis.

Table 2.8 Characteristics of the included studies for the prevalence of *Brucella* spp. in aborted ewe and doe

Study	**Origin of the study**	**Animal species**	**Sample type**	**Test methods**	**No sample**	**No positive**	**Apparent prevalence**
Benkirane *et al*. (2015)	Morocco	Caprine	RBPT	Maternal sera	106	14	13.21
Benkirane *et al*. (2015)	Morocco	Ovine	RBPT	Maternal sera	202	27	13.37
Bisias *et al*. (2009)	Greece	Ovine	ELISA	Maternal sera	289	44	15.22
Bisias *et al*. (2009)	Greece	Caprine	ELISA	Maternal sera	174	39	22.41
Celebi and Atabay (2009)	Turkey	Ovine	CFT	Maternal sera	400	135	33.75
Farag *et al*. (2021)	Egypt	Ovine	RBPT	Maternal sera	100	17	17.00
İlhan *et al*. (2007)	Turkey	Ovine	RBPT	Maternal sera	135	25	18.52
Ocholi *et al*. (2005)	Nigeria	Ovine	RBPT	Maternal sera	28	4	14.29
Oh *et al*. (2017)	Korea	Caprine	ELISA	Maternal sera	47	0	0.00
Szeredi *et al*. (2006)	Hungary	Caprine	ELISA	Maternal sera	38	0	0.00
Szeredi *et al*. (2006)	Hungary	Ovine	ELISA	Maternal sera	104	0	0.00
Wareth *et al*. (2015)	Egypt	Ovine	ELISA	Maternal sera	1	1	100.00
Wareth *et al*. (2015)	Egypt	Caprine	ELISA	Maternal sera	9	3	33.33
Tekle *et al*. (2019)	Ethiopia	Caprine	PCR	Vaginal Swab	27	3	11.11
Tekle *et al*. (2019)	Ethiopia	Caprine	PCR	milk	26	1	3.85
Ocholi *et al*. (2005)	Nigeria	Ovine	MRT	milk	5	5	100.00

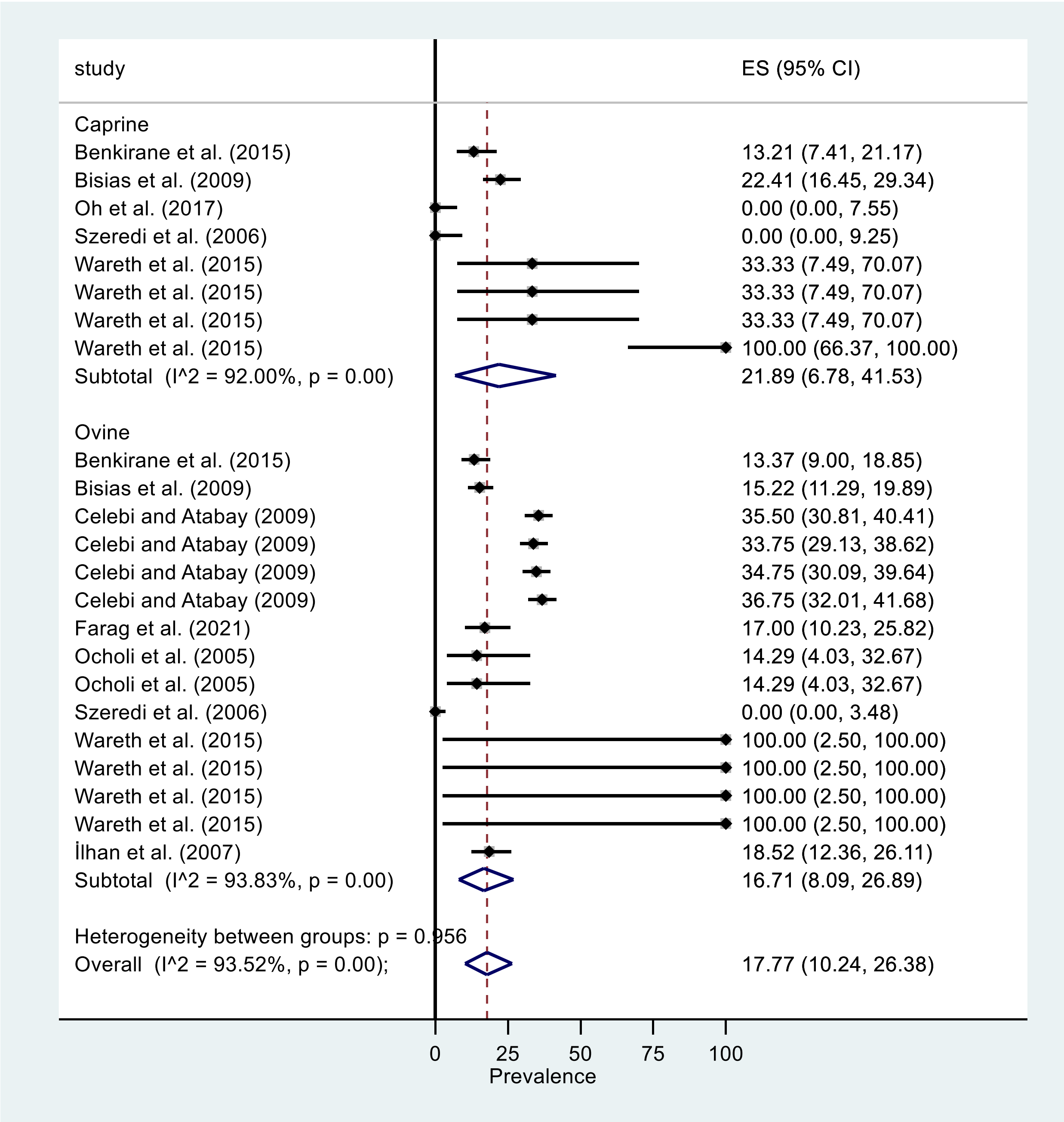

Figure 2.13 The pooled prevalence estimates for *Brucella* spp. in the aborted ewe and doe

2.3.6. Prevalence of *T. gondii* in aborted fetus and ewes/does

Data from 16982 samples extracted from 57 reports reflecting animal species and sample types were extracted from 38 eligible papers from 19 countries were used for this systematic review and meta-analyses. The studies were conducted on aborted fetal tissues (n= 30), maternal sera (n= 18), fetal fluid (n=2), placenta (n=6) and fetal sera (n=1) tested by ELISA (n=12), PCR (n=33), IFAT (n=5) and others (n=7). Sample types used in the original study aggregated by small ruminant species are presented in Fig 2.14. Detailed characteristics of the studies included are given in Table 2.9 to 2.11.

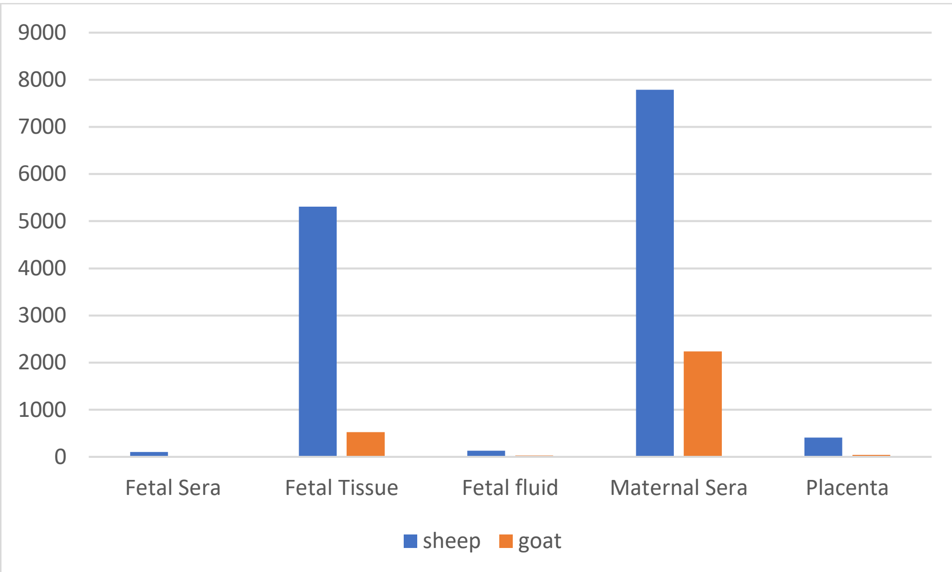

Figure 2.14 Sample types used to detect *T. gondii* from sheep and goat abortion cases

2.3.6.1 Prevalence of T. gondii in aborted fetus

A total of 6098 aborted fetal samples which constitutes 5834 aborted fetal tissues, 158 fetal fluid, and 106 fetal sera were tested for the presence of *T. gondii* using molecular, histological, and serological diagnostic methods. From the total, *T. gondii* were detected from 697 aborted fetal tissues, 30 fetal tissues, and 20 fetal fluids. From aborted fetal tissues, the overall pooled prevalence of *T. gondii* was estimated at 13.68% (95%CI; 9.59%-18.26%) in sheep and goat whereas it was estimated at 15.97% (95% CI: 11.05%-21.51%) in sheep and 6.41% (95% CI: 1.47%-13.38%) in goat. There is significant heterogeneity in the prevalence estimates among different studies for both sheep and goat (Figure 2.15).

Table 2.9 Characteristics of the included studies for the prevalence of *T. gondii* in the aborted fetuses of sheep and goat

Study	**Origin of the study**	**Animal species**	**Test methods**	**Sample type**	**No sample**	**No positive**	**Apparent prevalence**
Abu-Dalbouh *et al*. (2012)	Jordan	ovine	PCR	Fetal Tissue	133	43	32.33
Chessa *et al*. (2014)	Italy	ovine	PCR	Fetal Tissue	16	14	87.50
Danehchin *et al*. (2017)	Iran	ovine	PCR	Fetal Tissue	112	18	16.07
Filho *et al*. (2008)	Brazil	caprine	PCR	Fetal Tissue	8	2	25.00
Habibi *et al*. (2012)	Iran	ovine	PCR	Fetal Tissue	18	12	66.67
Masala *et al*. (2003)	Italy	caprine	PCR	Fetal Tissue	81	7	8.64
Masala *et al*. (2003)	Italy	ovine	PCR	Fetal Tissue	499	63	12.63
Masala *et al*. (2007)	Italy	caprine	PCR	Fetal Tissue	23	3	13.04
Masala *et al*. (2007)	Italy	caprine	PCR	Fetal Tissue	356	23	6.46
Masala *et al*. (2007)	Italy	ovine	PCR	Fetal Tissue	2421	271	11.19
Masala *et al*. (2007)	Italy	ovine	PCR	Fetal Tissue	292	53	18.15
Moreno *et al*. (2012)	Spain	ovine	PCR	Fetal Tissue	74	4	5.41
Moreno *et al*. (2012)	Spain	caprine	PCR	Fetal Tissue	26	1	3.85
Pereira-Bueno *et al*. (2004)	Spain	ovine	PCR	Fetal Tissue	173	12	6.94
Pereira-Bueno *et al*. (2004)	Spain	ovine	ELISA	Fetal Sera	106	30	28.30
Rassouli *et al*. (2011)	Iran	ovine	PCR	Fetal Tissue	200	27	13.50
Szeredi *et al*. (2006)	Hungary	ovine	HP	Fetal Tissue	246	2	0.81
Unzaga *et al*. (2014)	Argentina	caprine	IFAT	Fetal fluid	25	6	24.00

de Moraes *et al*. (2011)	Brazil	ovine	PCR	Fetal Tissue	35	5	14.29
van den Brom *et al*. (2012)	Netherlands	ovine	IHC	Fetal Tissue	506	42	8.30
Partoandazanpoor *et al*. (2019)	Iran	caprine	PCR	Fetal Tissue	10	1	10.00
Partoandazanpoor *et al*. (2019)	Iran	ovine	PCR	Fetal Tissue	111	9	8.11
Salehi *et al*. (2020)	Iran	ovine	PCR	Fetal Tissue	133	14	10.53
Gual *et al*. (2019)	Argentina	ovine	PCR	Fetal Tissue	2	2	100.00
Sah *et al*. (2019)	Bangladesh	ovine	PCR	Fetal Tissue	5	3	60.00
Sah *et al*. (2020)	Bangladesh	caprine	PCR	Fetal Tissue	5	1	20.00
Oliveira *et al*. (2018)	Brazil	caprine	PCR	Fetal Tissue	2	2	100.00
Salehi and Nezami *et al*. (2019)	Iran	ovine	ELISA	Fetal fluid	133	14	10.53
Arefkhah *et al*. (2020)	Iran	ovine	PCR	Fetal Tissue	100	2	2.00
Nourmohammadi etal. (2017)	Iran	ovine	PCR	Fetal Tissue	142	10	7.04
SahbaziI *et al*. (2018)	Iran	ovine	PCR	Fetal Tissue	75	48	64.00
Schnydrig *et al*. (2017)	Switzerland	ovine	PCR	Fetal Tissue	17	3	17.65
Schnydrig *et al*. (2017)	Switzerland	caprine	PCR	Fetal Tissue	13	0	0.00

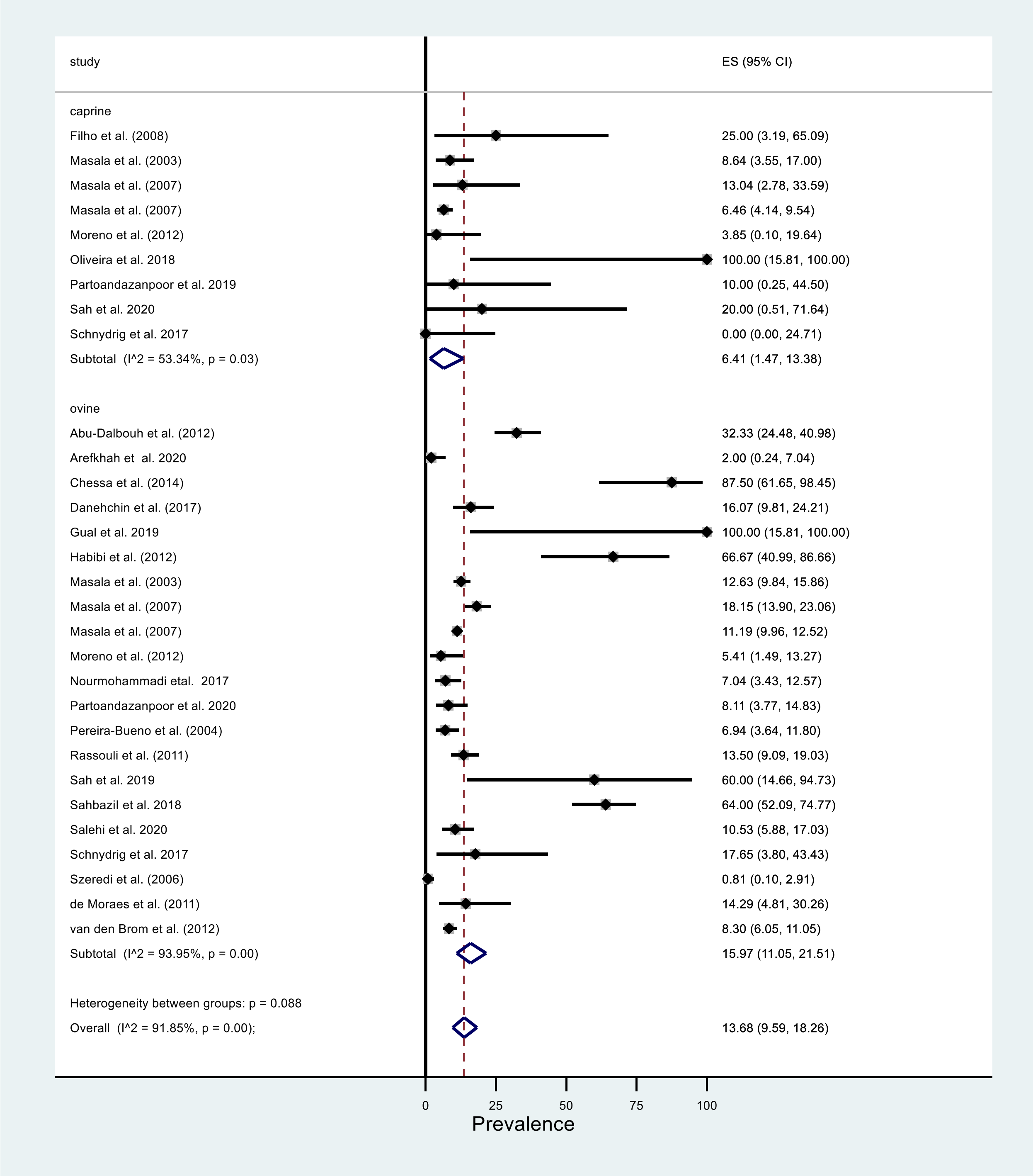

Figure 2.15 The pooled prevalence of *T. gondii* in the aborted fetuses of sheep and goat

2.3.6. 2 Prevalence of T. gondii in the placenta of aborted ewes/does

From the total of 430 placental samples collected from aborted ewe and does, *T. gondii* was detected from 70 abortion cases using PCR (65/371) and IHC (5/59) (Table 2.10). The overall pooled prevalence of *T. gondii* is found to be 17.39 % (95 % CI: 5.66%-

33.04%) in placental tissues of aborted small ruminant whereas it was estimated at 39.73% (95% CI: 18.33%-63.05%) and 12.73% (95%CI: 2.64%-28.32%) in aborted goat and sheep placenta, respectively. There is a high degree of heterogeneity in prevalence estimates among studies (Fig 6.16).

Table 2.10 Characteristics of the included studies for the prevalence of *T. gondii* in the placenta of aborted ewe and does.

Study	**Origin of the study**	**Animal species**	**Test methods**	**No of sample**	**No of positive**	**Apparent prevalence**
Masala *et al*. (2007)	Italy	caprine	PCR	8	2	25
Szeredi & Bacsadi (2002)	Hungary	ovine	IHC	59	5	8.4
Chessa *et al*. (2014)	Italy	ovine	PCR	142	5	3.5
Masala *et al*. (2003)	Italy	caprine	PCR	12	6	50
Masala *et al*. (2003)	Italy	ovine	PCR	133	42	35.5
Masala *et al*. (2007)	Italy	ovine	PCR	76	10	13.1

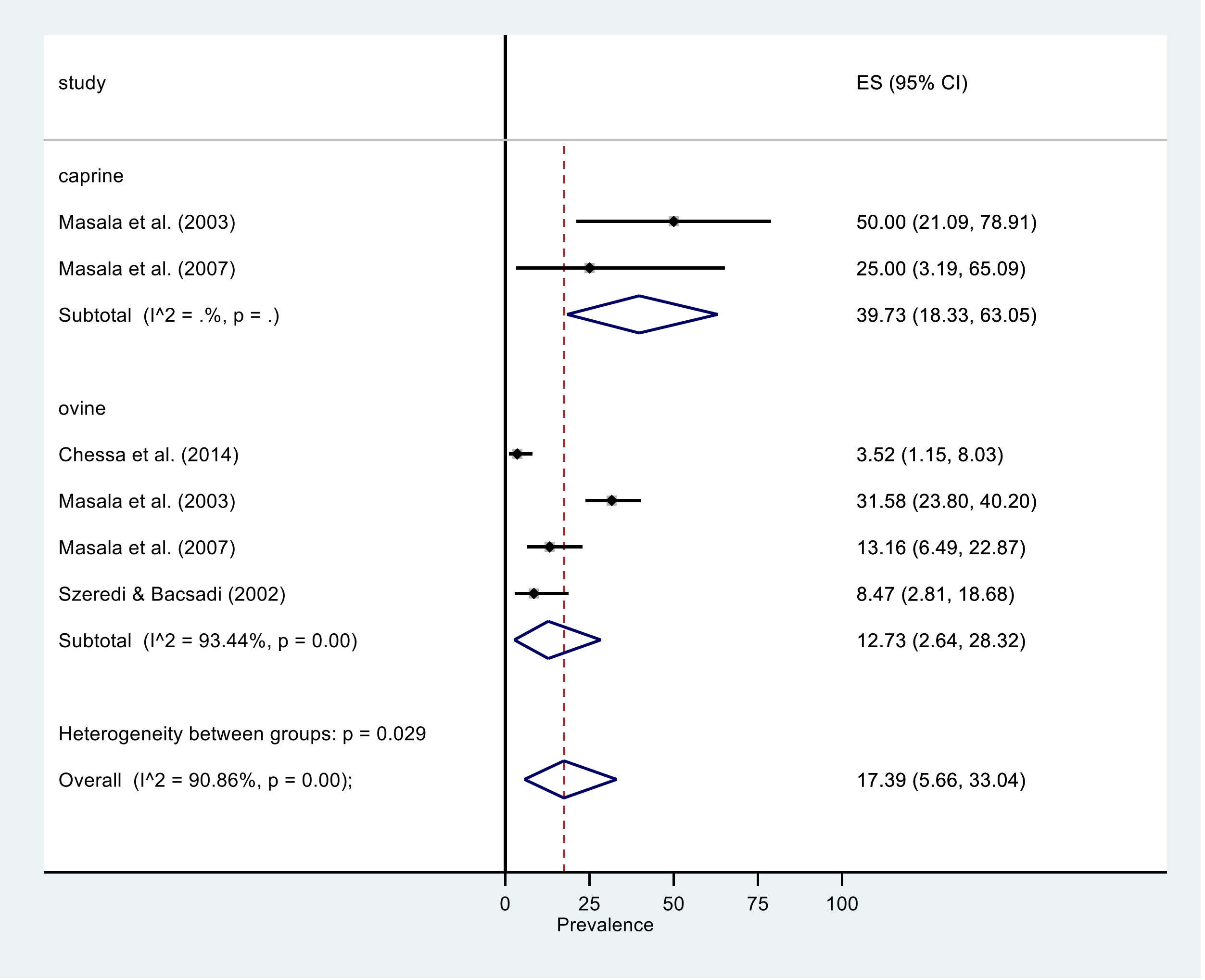

Figure 2.16 The pooled prevalence estimates for T. *gondii* in the aborted fetus of sheep and goat

2.3.6.3 Prevalence of T. gondii in maternal samples

A total of 5012 maternal sera were used to determine the seroprevalence of anti- *T. gondii* antibodies using various serological methods. Evidence of *T. gondii* infection was detected in 1139 aborted dams. From random effect meta-analyses results, the overall pooled prevalence estimate was 43.37% (95% CI: 27.39%-60.07%) in aborted ewes and does. The seroprevalence estimated by small ruminant species was 46.76% (95% CI: 26.04% -68.06%) for ewes and 36.77% (95% CI: 11.14% -67.15%) does. There is a high degree of heterogeneity in prevalence estimates among studies (Fig 2.16).

Table 2.11 Characteristics of the included studies for the prevalence of *T. gondii* in aborted ewe and does

Study	**Origin of the study**	**Animal species**	**Test method**	**No of sample**	**No positive**	**Apparent prevalence**
Abd *et al*. (2011)	Saudi Arabia	ovine	ELISA	879	78	8.87
Abd *et al*. (2011)	Saudi Arabia	caprine	ELISA	706	56	7.93
Benkirane *et al*. (2015)	Morocco	ovine	ELISA	202	42	20.8
Benkirane *et al*. (2015)	Morocco	caprine	ELISA	106	9	8.5
Bisias *et al*. (2009)	Greece	caprine	ELISA	174	52	29.9
Bisias *et al*. (2009)	Greece	ovine	ELISA	289	144	49.8
Borde *et al*. (2006)	Tobago	caprine	LAT	24	21	87.5
Edwards *et al*. (2013)	USA	ovine	MAT	39	37	94.8
Filho *et al*. (2008)	Brazil	caprine	IFAT	61	59	96.7
Hamidinejat *et al*. (2008)	Iran	ovine	MAT	100	80	85
Hamidinejat *et al*. (2008)	Iran	ovine	ELISA	100	85	85
Heidari *et al*. (2013)	Iran	ovine	ELISA	360	15	4.2
Hässig *et al*. (2003)	Switzerland	ovine	IFAT	1117	114	97.5
Oh *et al*. (2017)	Korea	caprine	ELISA	47	2	4.3
Rassouli *et al*. (2011)	Iran	ovine	IFAT	200	31	15.5
Spilovska *et al*. (2009)	Slovakia	ovine	ELISA	382	93	24.3
Gual *et al*. (2018)	Argentina	ovine	IFAT	15	10	66.7
Zangana *et al*. (2020)	Iraq	ovine	LAT	211	211	100

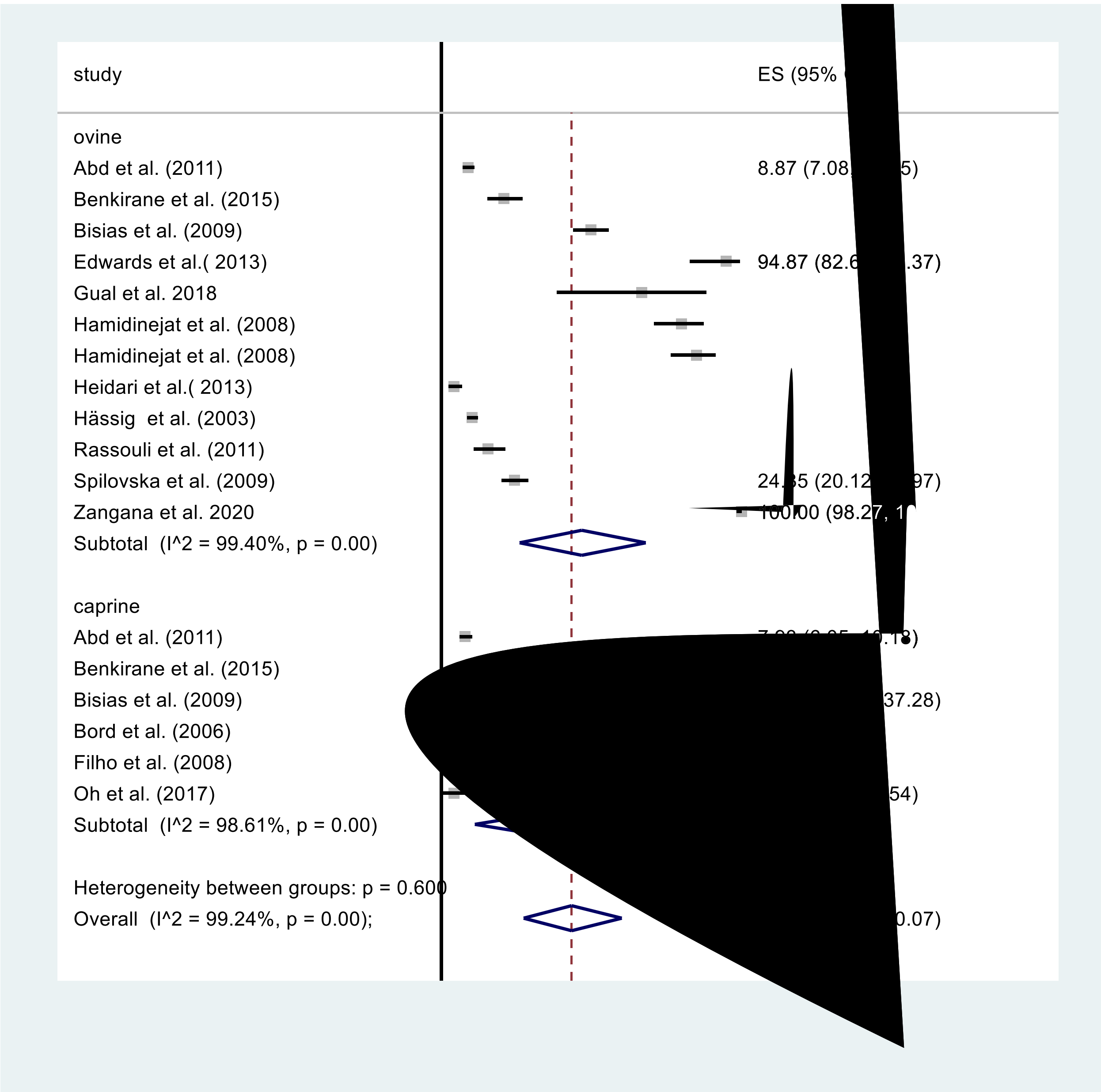

Figure 2.17 The pooled prevalence estimates for *T. gondii* in sera from aborted ewes and does

2.4. Discussion

This study aimed to systematically summarize the available evidence on the causes of small ruminant abortion and attempted to estimate the prevalence of *C. abortus, C. burnetii, Brucella* spp., and *T. gondii* of abortion cases globally. We used a systematic literature review and meta-analysis approach to estimate the prevalence of selected infectious abortifacient agents in aborted fetuses, placenta, and aborted populations of ewes/does. Looking at studies published over 200 years, we found 78 studies that could be included in meta-analyses for four abortion-causing agents. The majority of these are reported from European and Asian countries. Only very few articles were obtained from Africa and North and South America. This might indicate the perceived importance given to the condition in the regions and/or the diagnostic capacity in these regions.

Even though a lot of studies have been published on the seroprevalence of specific hazards in small ruminant populations all over the world, few studies describe the association of abortion with those hazards and provide confirmation on the agents involved. This might be related to the fact that many organisms can cause sporadic abortion (Buxton and Henderson, 1999); and submitting clinical material to laboratories from every abortion case is often not economically feasible. This minimizes the chance of investigating sporadic abortion and the chance of reaching a well-founded diagnosis (Hovers *et al*., 2014).

The result of this meta-analysis indicated that infectious agents play a comparable role in sheep and goat abortion. However, it is important to notice that very few multi-pathogen studies have been published. Since abortion is a complex and multifactorial phenomenon, it is impossible to draw any conclusion at the country level on the relative importance of single pathogen since causality is likely not fully confirmed. It is essential to consider underlying risk factors that fit the ecological context, production systems, and national abortion control measures which can differ between countries.

To investigate abortion, fetal materials, placenta, and aborted dam sera, and urine samples were used in the original studies. Single serum samples from the dam were the most frequently used sample for abortion investigations. Nevertheless, these are usually of little value in abortion diagnosis on an individual animal basis: since none of the currently available serological tests can differentiate vaccinated from naturally infected animals. However, in many cases, fetal and placental lesions may be absent, yet maternal titers may reflect current bacteremia or viremia and may help to diagnose infectious abortion. Paired serum samples on individual animals to detect changes in titers might be useful for demonstrating evidence of specific abortion agents (Borel *et al*., 2014, Holler, 2012).

A higher proportion of *C. abortus, C. burnetii,* and *T. gondii* positive was detected from placenta samples compared to fetal tissue samples. This indicated that placental membranes and cotyledons are the specimens of choice for diagnosing abortion outbreaks as these contain high levels of the pathogens (Borel *et al*., 2014). This result showed that infectious agents released into the environment through various routes can serve as a source of infection for animal populations and cause zoonotic risks for farming communities. Therefore, it is essential to reduce the spread of pathogens through appropriate disposal of placental and fetal material, cleansing and disinfection, and separation of pregnant animals from those who have just experienced abortion.

Effective control and prevention strategies require a definitive diagnosis of ovine and caprine abortion etiology. There are various methods for the diagnosis of pathogens such as culture, immunohistochemistry, histological, serological, and molecular methods. PCR was the most frequently used diagnostic test across the studies. PCR is one of the safest and modern advanced techniques used for accurate diagnosis of the causative agent which can reduce times on diagnoses and can be economical in comparison to conventional techniques (Holler, 2012; Borel *et al*., 2014). Recently, several PCR-based methods such as multiplex PCR, nested PCR, and real-time PCR have been developed for detection of causative agents in clinical specimens. New multiplex PCR formats are highly sensitive and allow rapid detection of multiple agents in a single test (Tramuta *et al*., 2011; Berri *et al*., 2009). However, its application is limited in developing countries where resources are limited due to its technical requirements and additional costs. The diagnosis of abortion etiology by serological responses through ELISA and other serological assays is useful in diagnosing non-endemic infections and/or infections against which animals are not vaccinated. However, robust and standardized commercial serological kits that separate actual exposure from vaccination are required for rapid and accurate diagnosis of the etiological agent of abortion in the field.

Abortion in sheep and goats has a significant impact on the productivity of flock in many countries worldwide which resulted in considerable economic losses to the agricultural industry (Givens and Marley, 2008; Hazlett *et al*., 2013). Moreover, several diseases that cause abortions in sheep and goats are zoonoses which confer health risks to people, especially for pregnant women. People get infected whilst assisting with the delivery of newborn animals or exposure to the contaminated environment from uterine fluids and discharge, post-abortion, or birth. Pregnant women may develop life-threatening infections, resulting in septic abortion and stillbirth (Pospischil *et al*., 2002; Ganter, 2015; George's, 2012). These call for better urgent tudies that investigate the contribution of each agent to abortion and the zoonotic impact resulted from these agents. Such data are urgently needed to inform management decisions at the country level, even more so in countries with low small ruminant reproductive performance.

The results of this review highlight massive knowledge gaps on the significance of abortive pathogens and invite for more powerful, well-designed studies to investigate possible infectious cases of abortion at the time and include important data such as the proportion of animals at risk that have aborted, age, stage of gestation at abortion, parity, vaccination history, and farming types. There is very limited data on the socio-economic impact caused by these agents—another important data gap.

This study demonstrates the role of infectious agents in small ruminant abortion. Estimated proportions from this meta-analysis are derived from existing studies on four infectious causes of abortion in small ruminants. However, estimates are subjected to limitations. Research on the role of those agents on abortion in small ruminants is scarce and the literature search is constrained by the diversity of terminologies used to refer to infectious abortion-causing agents. Furthermore, despite a comprehensive search, some studies likely conducted may have been missed due to the broadness of area coverage. Surveillance and routine diagnostic data are not published and widely accessible, and thus not included in the review even though such data would be very valuable. The pooled proportion was estimated based on data collected from different countries with different livestock systems, intervention measures, diverse sample types, and diagnostic techniques which resulted in a high degree of heterogeneity across the studies as expected. Therefore, the findings may not necessarily be conclusive of the actual role of infectious agents for abortion globally.

2.5 Conclusion

This comprehensive meta-analysis has collated data from different countries of the world and demonstrated the prevalence of major abortion-causing infectious agents. The result suggests that abortion in small ruminants is caused by multiple etiological agents that can be identified from abortion materials and maternal samples which need to be considered during abortion investigation. Overall, very few studies were found on this subject, especially for low- and middle-income countries, even though reproductive performance in these countries is lower. Accurate and timely diagnosis of abortion pathogens through the appropriate collection and submission of abortion samples along with a complete case history is essential to reduce the impact of abortion in small ruminant productivity. More research should be done looking at several possible pathogens at the same time and assess the socioeconomic impact caused by these pathogens.

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Chapter 3

Towards an objective measurement of reproductive performance of traditionally managed goat flocks in the drylands of Ethiopia

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Abstract

Reproductive performance is a key determinant for the efficiency of goat production. Regular monitoring of reproductive efficiency is essential to assess management and to avoid financial losses due to poor performance. To allow more objective measurement and comparisons over time, we propose a novel quantitative approach for defining annual reproductive performance by combining common performance indicators into a goat flock index. Commonly used reproductive performance measures were collected from 242 goat flocks in four districts in the dryland area between July 2018 to February 2019. Principal component analysis (PCA) was performed to identify biologically meaningful latent components that explain annual reproductive output (ARO) and annual reproductive wastage (ARW). Together with the remaining annual reproductive performance measures, the ARO and ARW components were included in a PCA to derive an algorithm for a goat annual reproductive performance index (G-ARPI). One component representing variation in kidding interval, $PC_{ARO}1$ and $PC_{ARW}1$ were extracted and normalized to a 10-scale value. The flocks were classified into good performing (15.63%) with index >8.5, moderately performing (48.21%) with index values ranging from 6.5-8.5, and poor performing (36.16%) with index <6.5. Good performing flocks have higher scores for reproductive output measures, lower scores for reproductive wastage, and lower kidding interval. The proposed G-ARPI can be used as an objective tool to compare reproductive performance between management systems, evaluate the costs of poor reproductive management, and will be useful for economic models that aim to identify the most cost-efficient intervention option and monitor the impact of interventions. We present here the index for goat production in dryland systems in Ethiopia, the approach can easily be adapted to other production systems elsewhere.

Keywords: Goat reproductive index; Kidding interval; Reproductive output; Reproductive wastage

3.1 Introduction

Goat production under extensive low-input systems plays an important role in ensuring food security and supporting rural livelihoods in arid and semi-arid areas where conditions for crop farming are limited (Muigai *et al*., 2017; Pulina *et al*., 2017). In these systems goats are an integral part of households, providing nutrition, employment, and easily accessible sources of income (Ørskov, 2011; Hassen and Tesfaye, 2014). Goats can survive in harsh environmental conditions and benefit from feed resource which is not used by other ruminants (Nardone *et al*., 2010; Gaughan *et al*., 2018). At the national level, they are an important source of foreign currency through live animal and meat export (FAOSTAT, 2019).

However, low productivity per animal and flock limit the potential contribution of goats for rural households in the arid areas of Ethiopia (Solomon *et al*., 2014; Feldt *et al*., 2016). Low productivity of the flock is linked to poor nutritional status due to poor pasture quantity and quality or lack of feed, and a high burden of disease, which together contribute to reproductive failure and poor growth rates (Mayberry *et al*., 2018).

Reproductive performance is a key determinant of the efficiency of goat production (Delgadillo and Martin, 2015). Accordingly, regular monitoring of reproductive efficiency is essential to assess management and often acts as an early indicator for health problems and thus, helps to avoid financial losses due to poor performance (FAO, 1993). Several parameters are commonly used to measure reproductive performance. The most common being fertility (the proportion of pregnant does exposed or mated to the buck), kidding percentage (number of kids born per doe exposed to the buck), prolificacy (the proportion of kids born alive), abortion rate (proportion of prematurely born kids), age at first kidding, kidding interval(interval between successive kidding), and weaning percentage (percentage of kids weaned per doe exposed to the buck). Kid losses are usually calculated as stillbirth rates and preweaning mortality rates as a proportion of kids weaned and kids born alive (Galina *et al*., 1995; Mellado *et al*., 2006; Song *et al*., 2006).

In Ethiopia, research undertaken on goat reproductive performance was largely based on a single trait, often used as part of a breed performance evaluation program on research stations (Belay *et al*., 2014; Solomon *et al*., 2014; Deribe *et al*., 2015). However, technological solutions developed through conventional station-based agricultural research have failed to achieve the expected results in the small-scale farming sector of the developing world (Stroud *et al*., 2000). The goals for reproductive performance vary tremendously between different goat production systems and need to take into account management systems and constraints at the flock level. The reproductive performance should be measured by obtaining an overall picture of the flock's reproductive performance preferably considering various individual components of reproductive activities and integrating them into an index. The minimum measures that should be included in an integrated index for annual flock performance are average age at first kidding, kidding interval, annual reproductive output, and annual reproductive wastage (Wilson, 1989; Ibrahim, 1998; Browning *et al*., 2011).

Increasing flock reproductive performance can be achieved through different interventions, including better management practice, nutrition, genetics, and healthcare adopted by the producers and extension agents (Mayberry *et al*., 2018). To ensure the cost-efficiency of interventions, the resulting reproductive performance change should be measured appropriately and objectively in a way that can provide a comprehensive picture of past reproductive performance, current changes, and future expectations.

This paper assesses commonly used measures for goat flock reproductive performance in goats in dryland systems in Ethiopia and proposes a novel quantitative approach for defining annual reproductive performance at the flock level by combining performance indicators into a goat-specific index.

3.2. Material and Methods

3.2.1 Study sites

This study was conducted in four locations (districts) in two regional states –Ziquala and Abergele in Amhara Region and Yabello and Elwaya in the Oromia region (Fig.3.1). Ziquala and Abergele represent the lowland mixed crop-livestock production system in the northern part of Ethiopia and Yabello and Elwaya represent the pastoral production system in the southern part of Ethiopia (Table 3.1). The sites are part of the CGIAR research program on Livestock (CRP Livestock) and had been selected based on agro-ecologies and production systems, the potential of the areas for goat production, accessibility and willingness of the community to participate in further studies, and the importance of sheep and goats to household livelihoods (Haile *et al*., 2019). Two "*Kebeles*" (= the smallest administrative unit in Ethiopia) were selected in each of the four districts. One of these *Kebeles* was an active CRP Livestock research site and one kebele had not seen any previous interventions. The CRP livestock intervention *Kebeles* had received animal health interventions such as vaccination for Pasteurellosis, Contagious caprine pleuropneumonia, Peste des petits ruminants, and goat pox, and were involved in community-based internal parasite control programs. They also had received animal health training on the following topics: 1) integrated herd health approach to reduce the impact of respiratory disease in small ruminants, 2) causes of reproductive health problems in small ruminants and possible control options, 3) community based strategies for internal parasite control in small ruminants. They were also a member of a community-based breeding program.

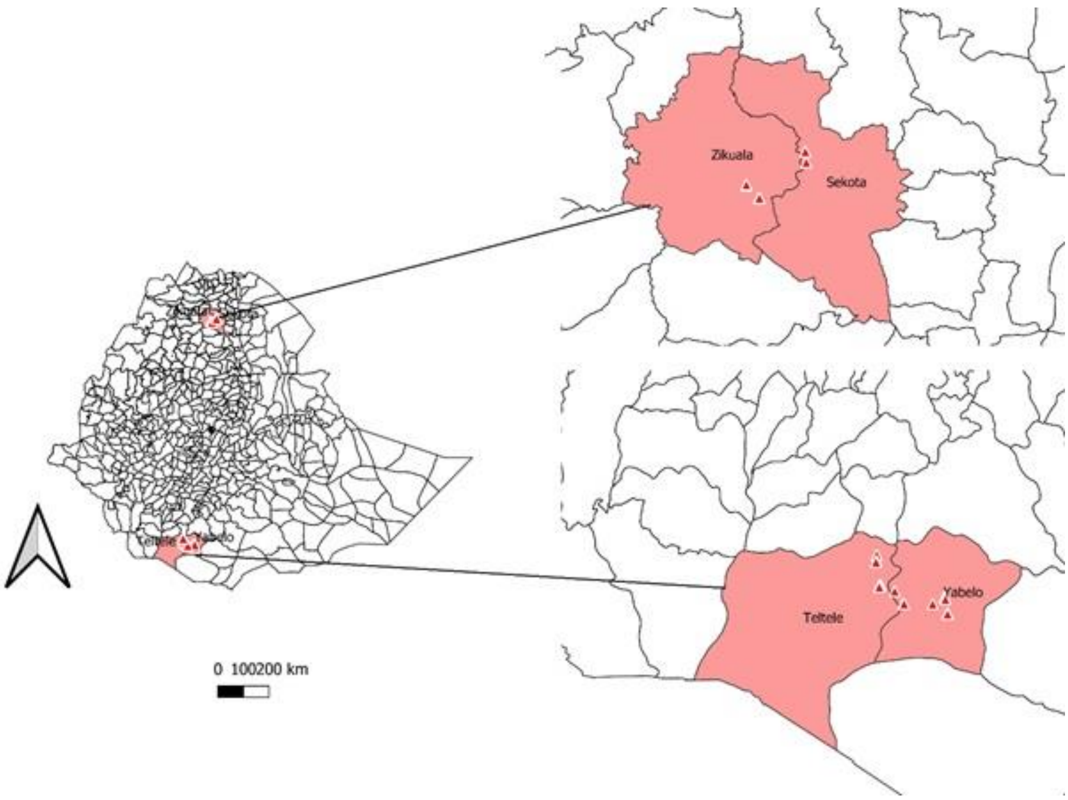


Figure 3.1 Map of Ethiopia showing the study districts

Table 3.1. Description of study areas in Ethiopia

No.	District	Production system	Village	Intervention status	Altitude (masl)	Rainfall (mm)	Average temperature (°C)
1.	Abergele	Lowland, mixed crop livestock	Sazba	CRP intervention	1348	647	24
			Belteharf	Control			
2.	Ziquala	Lowland, mixed crop-livestock	Bilaqu	CRP intervention	1486	732	22
			Tsitsika	Control			
3.	Yabello	Lowlands, pastoral	Derito	CRP intervention	1588	625	20
			Dida Yabello	Control			
4.	Elwaya	Lowlands, pastoral	Adegalchet	CRP intervention	1181	493	22
			Chari	Control			

3.1.2 Study households and flocks

In each *Kebele* 32 households were selected randomly, in total 242 households. Lists of households for each *Kebele* (smallholders' farmers/pastoralists in the study sites who own small ruminants) were obtained from enumerators of the CRP Livestock intervention *Kebeles* and from the key informants at control *kebele* one day before the survey. From these lists, households were selected randomly using the random function in Excel. Facilitators then contacted the household heads, asked for their willingness to participate, and planned the timing of the interview. Only households who own goats were considered in this study.

3.1.3 Data collection processes

Data were collected through a face-to-face structured interview using a questionnaire. Questions were designed based on a literature review and the experiences of researchers. To prevent ambiguity and obtain concise information, mainly closed questions were used. The questionnaire was pre-tested on a pilot group of 15 farmers who were not included in the study population and necessary adjustments were made after the pre-testing. The questions were coded using Epi Info™ 7.2.1.0 software and copied onto Galaxy Tab A (2016) for digital data collection. The recorded responses were transferred and stored on a personal laptop computer and subsequently exported to statistical software GeoDa (https://geodacenter.github.io/) where primary processing and quality checks were undertaken.

The interviews were conducted by four trained veterinarians and/or animal production experts from the National Agricultural research system, who spoke the local language of the respective study sites. They received training on the interview tool, interview approach, and digital recording of responses. The training ensured a common understanding of the meaning of each question and in what way to ask participants.

Interviews were conducted between July 2018 to February 2019 in a place where both interviewer and participant felt comfortable. The farmers/pastoralists were informed about the purpose of the study and the approximate time the interview will take, and their oral informed consent was sought before their participation in the survey.

3.1.4 Reproductive performance measures calculation

Reproductive performance measures included in the data collection were age at first kidding, kidding interval, number of pregnancy, number of kids born, number of kids survived to wean, number of abortion, number of defective birth (dystocia, retained placenta, weak kids, defective kids), number of kids died before weaning (three months), number of kid per doe lifetime. Each reproductive performance measure was estimated by farmers/pastoralists based on their information on reproductive events in the flocks during one year before the interview. The number of offspring produced per doe lifetime in each flock was estimated based on information on offspring produced from at least two culled/dead does in the last five years period. Respondents were also asked about their confidence in their estimation. If they were not confident about their estimates, the data entry was left empty and treated as missing. Each reproductive performance measure was estimated at flock level based on farmers/pastoralists' information and a separate data table was created in the database. The following reproduction traits were calculated in each flock as:

$$\text{Age at first kidding} = \frac{\sum age\ at\ first\ kidding\ in\ months}{\sum does\ kidded\ for\ first\ time\ in\ the\ flocks\ one\ year\ before\ the\ survey}$$

$$\text{Kidding interval} = \frac{\sum \text{months between successive kidding during one year prior to the survey}}{\sum does\ kidded\ \text{during one year prior to the survey}}$$

$$\text{Pregnancy rate} = \frac{\sum pregnant\ does\ during\ one\ year\ prior\ to\ survey}{\sum mature\ does\ in\ the\ flock\ exposed\ to\ buck\ during\ one\ year\ prior\ to\ survey} x100$$

$$\text{Annual kidding rate} = \frac{\sum \text{kids born}\ during\ one\ year\ prior\ to\ survey}{\sum mature\ does\ in\ the\ flock\ exposed\ to\ buck\ during\ one\ year\ prior\ to\ survey} x100$$

$$\text{Annual weaning rate} = \frac{\sum kids\ weaned\ \text{during one year prior to survey}}{\sum mature\ does\ in\ the\ flock\ exposed\ to\ buck\ \text{during one year prior to survey}} x100$$

$$\text{Abortion rate} = \frac{\sum kids\ lost\ before\ expected\ parturition\ \text{during one year prior to the survey}}{\sum \text{pregnant does in the flock during one year prior to the survey}} x100$$

$$\text{Mortality rate} = \frac{\sum kids\ died\ before\ 3\ months\ of\ age\ \text{during one year prior to the survey}}{\sum \text{all kids born alive in the flock during one year prior to the survey}} x100$$

$$\text{Birth defects rate} = \frac{\sum defective\ births\ \text{during one year prior to the survey}}{\sum \text{pregnant does in the flock during one year prior to the survey}} x100$$

3.1.5 Data analysis

Flock level reproductive performance was estimated by combining data on reproductive performance traits collected from 242 flocks in the drylands of Ethiopia. First, descriptive statistics, such as mean and SD, were used to summarize the data. As a first step, principal component analysis (PCA) was performed separately on data linked to annual reproductive outputs (pregnancy rate, kidding rate, and weaning rate) and annual reproductive wastage (abortion rate, kid mortality rate, and birth defective rate). Components with eigenvalues greater than 1 were identified and retained. As the second step, the retained components from step 1 were then combined with remaining indicators (kidding interval and age at first kidding) with PCA to derive an annual reproductive performance index of flocks. In all PCAs, resulting in components with eigenvalues greater than 1 were considered and only components loadings greater than 0.4 or below -0.4 were retained in the final model. This component is referred to as the 'goat annual reproductive performance index'(G-ARPI). Statistical analyses were performed in R (R Development Core Team 2010; R Foundation for Statistical Computing, Vienna, Austria) and GeoDa. Components scores were tested for normality and transformed into a normally distributed scale using appropriate transformation to have an easily understandable and communicable measure. The resulting values of the annual reproductive performance index were then used to define three ordered groups (poor, moderate, and good annual reproductive performance). In all analyses, confidence level (CI) was at 95% and $P \leq 0.05$ was considered significant.

3.3 Results

3.3.1 Flocks characteristics

Of the 242 goat flocks enrolled in the study, 114 (47.11%) were managed under a lowland mixed crop-livestock production system and 128 (52.89 %) under a pastoral production system. Long-eared Somali and Abergele were the predominant goat breeds kept by pastoralists in Borena and the lowlands of Waghimira respectively. Households kept goats for milk, meat, and immediate cash income. The median size of the flocks was 12.5 breeding does with many households (40.08 %) having between 10 and 20 does. Seventy-seven farms (31.82 %) had less than 10 does and 68 farms (28.1%) had more than 30 does with the largest having 100 does. Only 3 flocks (1.24%)

were goats only herds, while 12 farms (4.9%) managed their goat flock with at least one other livestock species and most of the farms (93.8%) managed their goat with two or more livestock species. Flocks were kept under traditional extensive management systems and therefore fully dependent on grazing lands, with overall limited inputs. The flocks were grazed freely on pastures during daytime and kept in open enclosure (74.38%) or house during the night (25.62%). All day-to-day herding decisions were made by the owner and breeding was uncontrolled. Fertile bucks were allowed to remain continuously with a group of females throughout the year.

3.3.2 Reproductive performance measures

The means and standard deviations for the 9 reproductive performance measures analyzed in this study, aggregated by the production system, are summarized in Table 3.2. The overall mean age at first kidding was 16.09 (±3.83) months. Pastoral flocks kidded at an earlier mean age (15.5±3.4 months) than mixed crop-livestock flocks (16.8±4.2), P-value =0.012. Overall mean months between successive kidding was 8.3±1.9 months, with pastoral flocks having lower kidding intervals than mixed crop-livestock flocks (P=0.005).

The average annual pregnancy rate reported in the present study for the dryland flocks was 83.9 % (±35.55). The average annual kidding and weaning rates were 71.54 % (±42.7) and 62.13% (±36.38), respectively. Mean offspring produced per lifetime of the doe was estimated at 10.22 kids (±3.47). Regarding reproductive failures, mean abortion, birth defects, and kid mortality rates per flock were 21.73% (±26.92), 11.06% (±20.84), and 18.17% (25.54), respectively.

Table 3.2. Summary of goat reproductive performance parameters in dryland areas of Ethiopia

Reproductive parameters	Flock	Overall mean(±SD)	Production system		P-value
			Crop livestock	Pastoral	
Age at first kidding, months	232	16.09(±3.83)	16.79(±4.18)	15.52(±3.44)	0.012
Pregnancy rate, %	241	83.9(±35.55)	83.16(±43.80)	84.57 (±26.19)	0.759
Kidding interval, month	231	8.31(±1.968)	8.71(±2.33)	7.98 (1.54)	0.005
Kidding rate, %	241	71.54(±42.77)	71.39(±42.78)	74.1 (±29.17)	0.584

Weaning rate, %	241	62.13(±36.38)	56.0 (±40.03)	67.57 (±31.95)	0.000
Abortion rate, %	236	21.73(±26.92)	19.36(24.81)	23.89(28.635)	0.197
Birth defects rate, %	240	11.06(±20.84)	12.89(±22.43)	9.39(19.23)	0.195
Kid mortality rate, %	239	18.17(25.54)	25.16(29.31)	11.8(19.59)	0.000
Kids per doe lifetime	231	10.22(±3.47)	9.84(±4.30)	10.52(±2.60)	0.140

3.3.3 Goat annual reproductive performance index (G-ARPI)

The annual reproductive performance index (G-ARPI) was predicted by combining reproductive performance traits.

First, two principal component analyses were performed to identify biologically meaningful latent components that explain annual reproductive output (ARO) and annual reproductive wastage (ARW) based on farmers' information in relation to reproductive performance measures. One component with eigenvalues greater than 1, representing 77.78% and 44.88 % of the common variance PC_{ARO} and PC_{ARW} were identified and retained (Table 3.3).

Table 3. 3. Loadings of annual reproductive output and wastage on the first two principal components with eigenvalue.

RP measures	PC1	PC2
Annual reproductive output (ARO) measures		
Pregnancy rate	0.53	0.80
Kidding rate	0.62	-0.14
Weaning rate	0.58	-0.58
Eigenvalue	2.33	0.53
% of explained variability	77.78	17. 74
Annual reproductive wastage (ARW) measures		
Abortion rate	0.64	0.17
Birth defects rate	0.49	0.83
Kid mortality rate	0.59	-0.52
Eigenvalue	1.346	0.89
% of explained variability	44. 88	29.97

In the next step, a PCA was performed combining ARO and ARW with the remaining annual reproductive performance measures. Significant loadings were identified for kidding interval, $PC_{ARO}1$, and $PC_{ARW}1$ (Table 3. 4).

Table 3.4. Loadings of components from the principal component analysis based on data of 224 dryland flock.

RP measures components	**PCA all variable**	**PCA with only relevant variable**
	Component score	**G-ARPI**
Kidding interval	**-0.53**	**-0.53**
Age at first kidding	-0.30	
Reproductive wastage ($PC_{ARW}1$)	**-0.58**	**-0.63**
Reproductive output ($PC_{ARO}1$)	**0.54**	**0.57**

Final PCA was conducted on components that had significant loading in the 2nd step and one component with an eigenvalue of 1.24 accounted for 41.5% of the total variance which was used to define the G-ARPI. G-ARPI had positive loadings from ARO and negative loadings for the kidding interval (KI) and ARW. Thus, it reflected the negative relationship between reproductive wastage and annual reproductive output. Fig 3. 2 shows 95% concentration *ellipses* for each production system and shows that variability among pastoralist herds is smaller than among mixed systems. Fig 3.3 shows the distribution of the reproductive performance measures and observed flocks along PC1 and PC2.

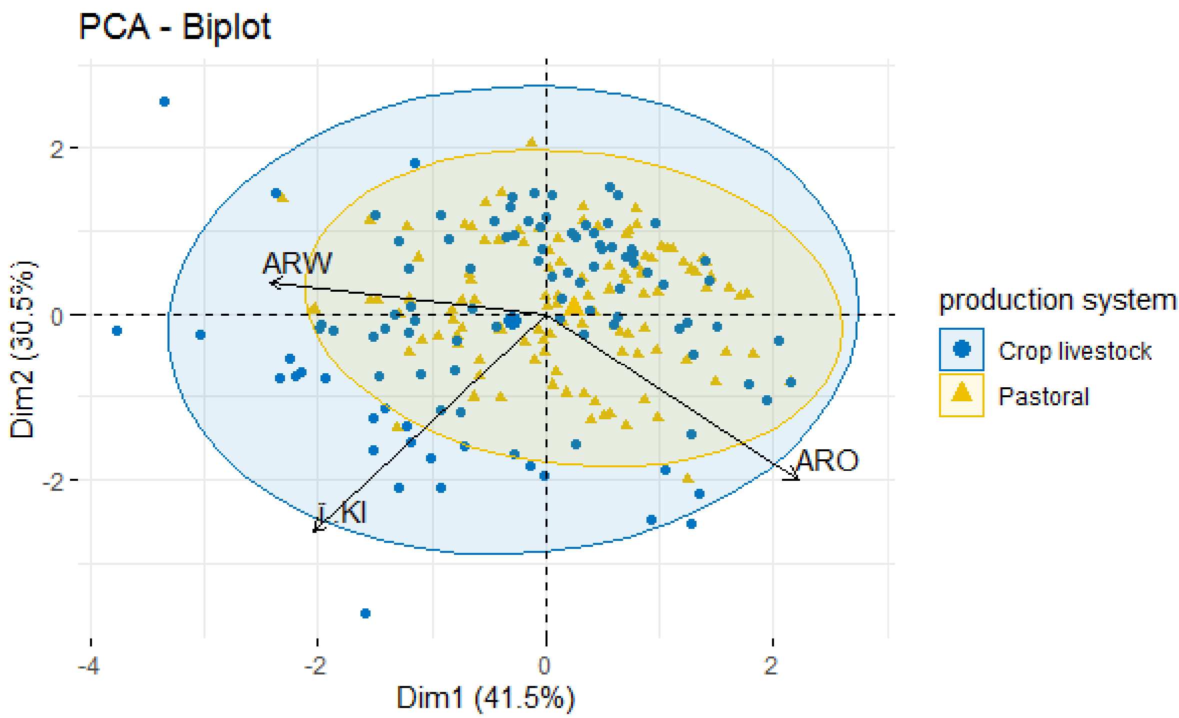


Figure 3.2 Goat flocks (dots) along with the first two PCs with 95% concentration ellipses

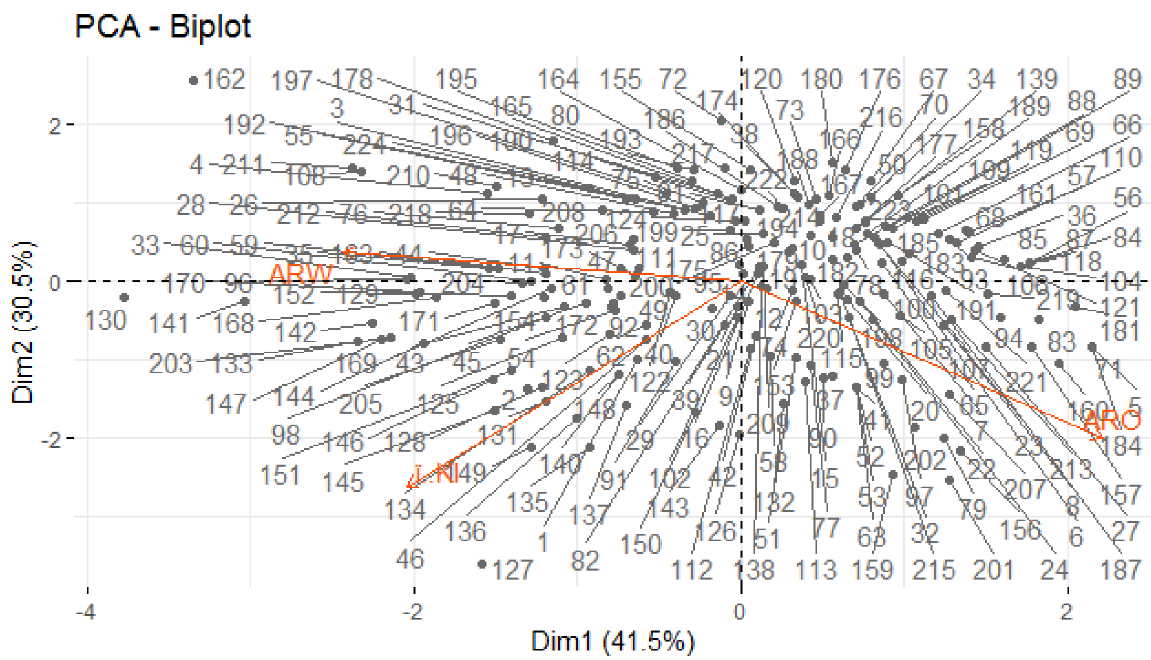
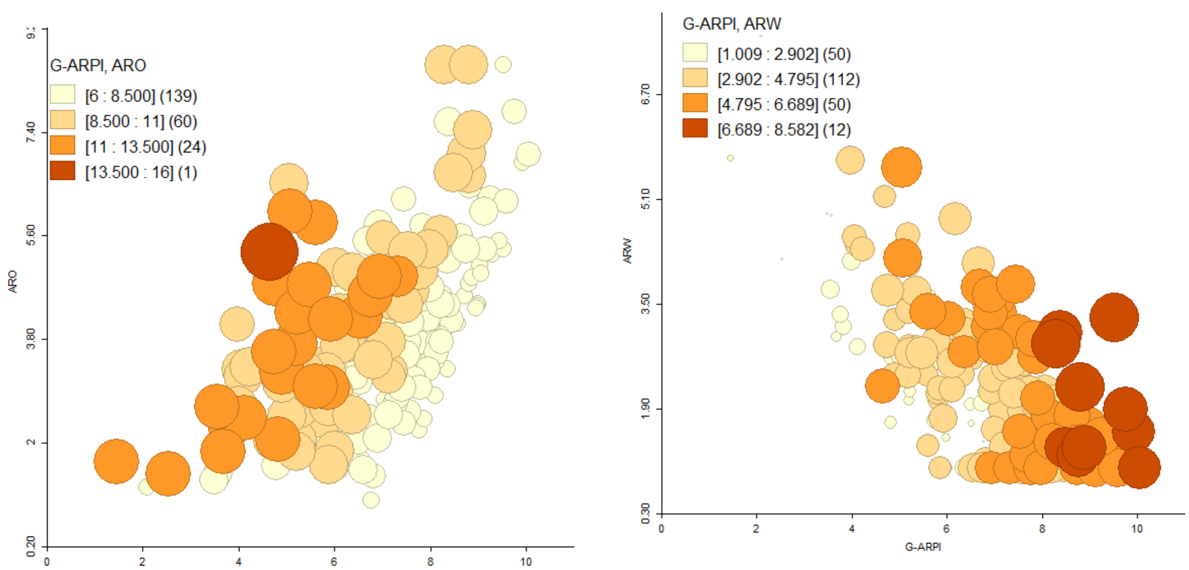


Figure 3.3 Goat flocks (dots) and reproductive performance measures (light red) along with the first two PCs

The calculated G-ARPI values were then transformed to a 10 scale initially by setting the minimum component score value as 1 by adding 4.78 and multiply by 1.4 to expand the distribution up to 10.

Eighteen observations had to be excluded from analysis due to one or more missing measures. Hence, G-ARPI was estimated for 224 flocks, resulting in a mean G-ARPI score of 6.93. To define performance categories, cut-offs were identified upon visual examination of the bubble charts (Fig 3.4). Mean values for underlying reproductive performance measures are shown in Table 3.5. The scatter plot matrix was used to visualize the relationship between underlying standardized reproductive performance measures and G-ARPI (Fig 3.5).



a) **b)**

Figure 3. 4. Bubble chart representing the relationship between G-ARPI, ARW, and ARO (a) G-ARPI, ARO, and KI (b) for 224 flocks in the dryland of Ethiopia.

Table 3.5. Mean value of underlying reproductive performance measures in dryland goat flocks of Ethiopia

RP categories	ARP index	N	PR	KR	WR	KI	AR	KMR	BDR
Poor	<6.5	81	71.38	49.04	36.93	9.72	39.33	29.56	18.8
Moderate	6.5-8.5	108	84.62	74.72	63.04	7.62	12.90	13.12	9.11
Good	>8.5	35	108.20	114.47	107.56	6.88	6.17	7.32	1.58

RP: reproductive performance; N: number of flocks; PR: pregnancy rate; KR: kidding rate; RW: weaning rate; KI: kidding interval; AR: abortion rate; KMR: kid mortality rate; BDR: Birth defect rate

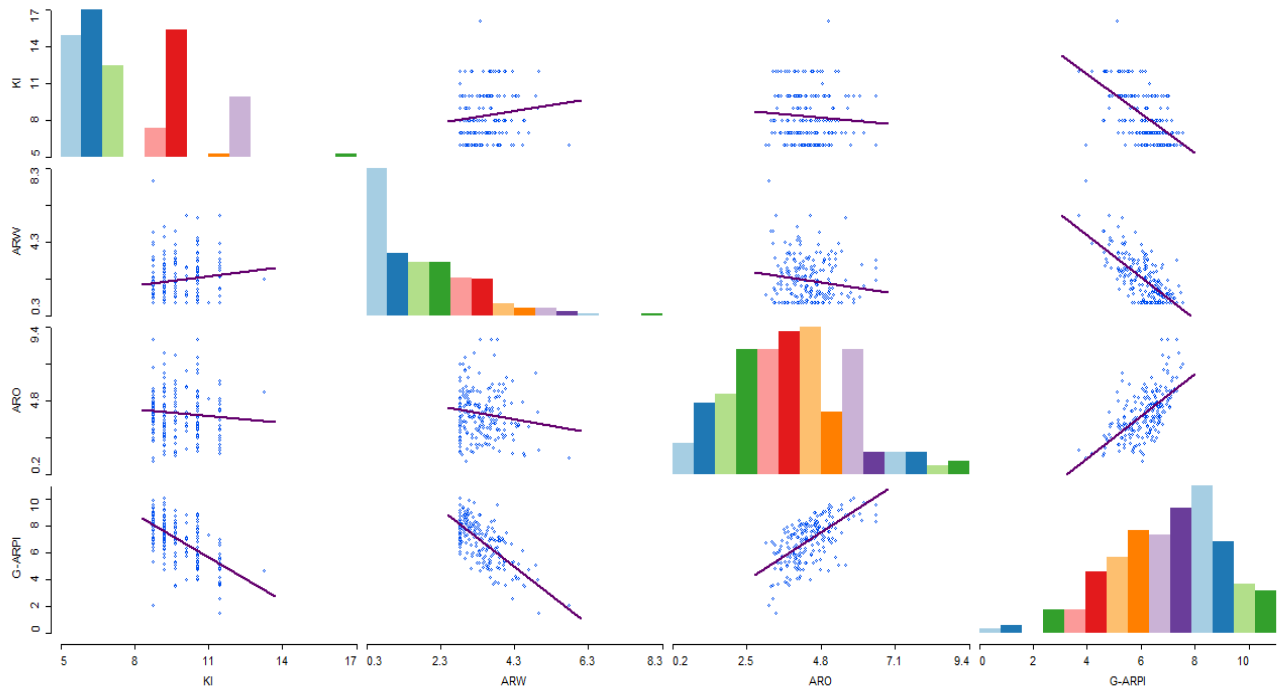


Figure 3.5. Scatter plot matrix showing the relationship between underlying reproductive performance measures and annual reproductive performance index for 224 flocks in the dryland of Ethiopia.

Flocks with a G-ARPI above 8.5 (15.63% of the flocks) were considered as good performing flocks, with higher scores for reproductive output measures and lower scores for reproductive wastage, while flocks with an index lower than 6.5 (36.16% of the flocks) were considered as poorly performing flocks. Many of the flocks (48.21%) had a score between 6.5 and 8.5, categorized as moderately performing flocks (Fig 3. 6). From the 104 flocks studied in mixed crop-livestock lowland production systems, only 13(12.50%) were classified as well-performing flocks, whereas 44 (42.31%) and 47 (45.19%) flocks were categorized as moderate and poor performers, respectively. In the pastoral production system, from a total of 120 flocks, 22 (18.33%) of flocks were categorized as well-performing flocks, whereas 64 (53.33%) and 34 (28.33%) flocks were categorized as moderate and poor performers, respectively (Fig 3. 6). The result of this study showed that a relatively higher number of flocks (18.26 %) from intervention sites categorized as good performers as compared to control sites flocks (12.84%). But the difference is not statistically significant (p>0.05).

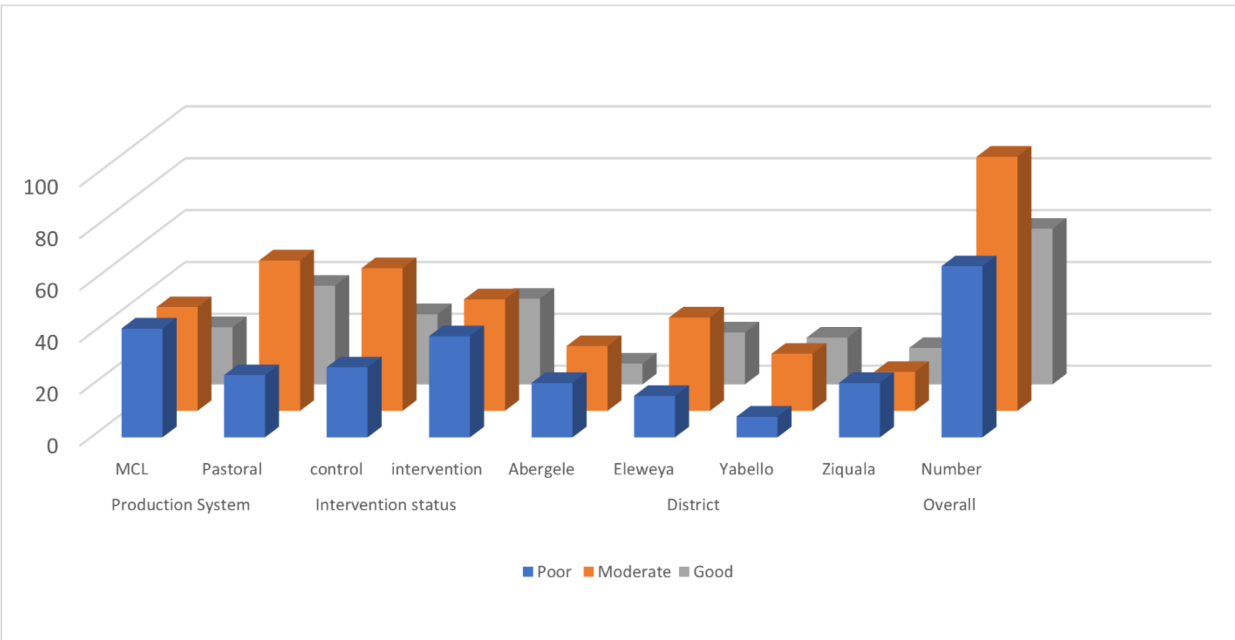


Figure 3. 6. Distribution of reproductive performance categories of goat flocks in drylands of Ethiopia by the production system, intervention status, and district

3.4 Discussion

Measures of reproduction in goats commonly used include fertility, age at first kidding, kidding interval, kidding rate, weaning rate, and kid mortality rate. However, none of these measurements on their own provides conclusive information on overall flock reproductive performance or manages to capture in an objective way level of performance. For livestock producers, development partners, and government initiatives that want to benchmark and compare the performance of flocks, approaches that quantify reproductive performance by combining various aspects of the reproduction process is useful as it allows better monitoring over time. In this study, we present a novel quantitative tool that allows farmers/pastoralists, veterinarians, and researchers to determine reproductive performance at the flock level. The reproductive performance of the flocks was estimated based on the annual reproductive output, kidding interval, and annual reproductive wastage. Information used to derive the index was obtained from smallholders' farmers/pastoralists and hence the resulting index can only be valid for the systems included. The difficulty for researchers to obtain a complete understanding of the flocks' reproductive performance during a single farm visit made the use of interview-based progeny history approaches appropriate. The outcomes of progeny history depend on the memory, perceptivity, and reliability of the interviewees. To improve the reliability of data and to reduce recall bias we used the one-year memory of farmers/pastoralists and enquired confidence of answers. The collected information yielded reliable estimates of reproduction data among the studied flocks and are comparable to reproductive measures reported by various researches conducted elsewhere in Africa (Wilson, 1989; Otte and Chilonda, 2002; Hary *et al.*, 2003; Dereje *et al.*, 2015). More reliable and precise data could be collected in a longitudinal approach that has shorter in-between visit intervals or approaches that allow ongoing data collection over time, such as for example information communication technology (ICT) assisted approaches.

Principal component analysis (PCA) is a powerful multivariable technique to identify latent components that are present in a biological process (Wang and Du, 2000). It has been used in animal breeding studies to condense the information contained in breeding values and reproductive traits predicted for all available traits into fewer uncorrelated (i.e. orthogonal) latent variables (Boligon *et al.*, 2016). It has been also used to generate variables representing biologically meaningful aspects of variation among qualitative and quantitative morbidity variables related to animal diseases, such as for example to quantify post-weaning multi-systemic wasting syndrome severity at farm level in England (Alarcon *et al.*, 2011). In this study, PCA was used both as reproductive measures dimensional reduction technique and to develop the final model to predict reproductive performance scale.

The presence and high negative loading of reproductive wastage on G-ARPI indicate its important contribution to poor reproductive performance, which needs to be addressed appropriately in any intervention plan to increase reproductive performance. This study indicated that the kidding interval appears to be an important descriptor of reproductive performance. The number of young produced per breeding female is of major economic importance (Wilson, 1989). One of the most important ways of increasing young produced per breeding female is through reduction of the kidding interval and, if done with optimal input, this may help in meeting the growing demand of the export trade (Abebe, 2008).

We normalized G-ARPI to a scale of 10 to facilitate communication and for its use in future projects and to allow comparison over time. Although the establishment of cut-offs values in this scale is subjective, clear cut-offs could easily be determined and seemed reasonable. While benchmark figures for reproductive performance in goat flocks in Ethiopia are difficult to find, the values of the underlining measures feeding into the index cut-offs are well aligned with optimum values reported for arid and semi-arid areas of Africa (Wilson, 1989; Otte and Chilonda, 2002; Hary *et al*., 2003; Solomon *et al*., 2014; Dereje *et al*., 2015).

Flocks presenting a score lower than 6.5 G-ARP indexes were considered as a poor performer while flocks with a score higher than 8.5 were considered as good performing flocks. Many of the flocks were moderately affected by reproductive failures, consequently categorized as moderately performing flocks. The categorization allowed the identification of extreme flocks, good performer or poor performer flocks. The presence of a larger number of moderately performed flocks gives opportunities for development partners and smallholders to upgrade those flocks with a moderate improvement of management practices, feed supplementation, and health care. Household modeling in Ethiopia by Mayberry *et al*. (2018) showed that reproduction, growth, and survival rates could be increased through better nutrition and healthcare. Providing improved nutrition by supplementation with low-cost, farm-generated feed resources potentially increase the reproductive performance of West African Dwarf goats (Amole *et al*., 2017). The distribution reflects the severity of reproductive wastage in the Ethiopian dryland goat population. To overcome these challenges, it important to implement integrated intervention packages that improve feed efficiency, reproduction, parasites, and disease control. The presented algorithm can be a useful and objective tool to compare reproductive performance between breeds, management systems, agro-ecological zones, and interventions, especially in projects targeting dryland production systems. Future activities should focus to validate and possibly to re-calibrate the index and its cut-offs and to adapt the approach for other production systems in different agro-ecologies.

3.5 Conclusions

The goat reproductive performance index presented here can serve as a basis for analysing reproductive performance and to identify husbandry and environmental factors affecting reproductive performance, study risk factors for reproductive wastage and it will also be used in measuring and monitoring reproductive performance to make flock management adjustments. Ultimately, the index is an ideal management tool to help to evaluate the costs of poor reproductive management, which are often hidden, and help to the development of economic models that aim to identify the most cost-efficient intervention option to increase reproductive performance. Besides, the G-ARPI can then also be used to monitor the impact of interventions implemented in these goat production systems.

3.6 Acknowledgments

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Chapter 4

Causes and flock level risk factors of sheep and goat abortion in three agroecology zones in Ethiopia

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Abstract

A cross-sectional survey was conducted to estimate the prevalence of small ruminant abortion and identify its major causes and potential risk factors in goat and sheep flocks in three agroecology and production systems of Ethiopia. Information on pregnancy outcomes and management risk factors were collected for 299 goat and 242 sheep flocks. Blood samples were collected from 133 sheep and 90 goat flocks and tested for *Coxiella burnetii*, *Brucella* spp, *Chlamydia abortus*, and *Toxoplasma gondii*. A causal diagram outlined relationships between potential predictor variables and abortion in the flock. The effect of management and exposure to infectious causes on the number of abortions in the flock across agroecology was tested using zero-inflated negative binomial regression. Results showed that 142 (58.68%) goats and 53 (17.73%) sheep flocks reported abortions in the 12 months before the survey. The mean annual flock abortion percentages were 16.1% (±26.23) for does and 12.6% (±23.5) for ewes. Farmers perceived infectious diseases, extreme weather conditions, feed shortage, physical traumas, and plant poisoning as the most important causes of abortion. A higher proportion of abortion was recorded during the short rainy season (March to May) and the start of the short dry and cold season (June to August) in the lowland mixed crop-livestock and pastoral agroecology and production system, respectively. Overall, 65.41% of sheep and 92.22% of goat flocks tested positive for one or more abortion-causing agents, namely, *C. burnetti*, *C. abortus, Brucella* spp., and *T. gondii;* mixed infection was found in 31.58% sheep and 63.33% goat flocks. Spending the night in a traditional house and providing supplementary feed for pregnant dams were important management factors that significantly ($p \leq 0.05$) decreased the risk of abortion by 2.63 and 4.55 times, respectively. However, the presence of other livestock species and dogs in the household and exposure of the flock to *Brucella* spp. or any one of the four tested infectious agents significantly ($p \leq 0.05$) increased the risk of abortion in sheep and goat flocks. In general, abortion is a challenge for small ruminant production in the study area especially in lowland agroecology, and calls for improvement in husbandry practices, health care, and biosecurity practices.

Keywords: Abortion; Infectious causes; Small ruminant; Reproductive failures; Risk-factors; Ethiopia.

4.1 Introduction

Abortion is a significant problem in pregnant ewes/ does and causes major financial losses for smallholder livestock producers in Ethiopia. It is a limiting production factor, as it decreases the potential number of replacement stocks for the flock and milk production and increases the number of unproductive females maintained for long periods in the flock (Ali *et al*., 2019; Lokamar *et al*., 2020). Thus, abortion in sheep and goats significantly impacts the food security and livelihoods of rural smallholder Ethiopian farmers, as sheep and goats are an integral part of households by providing nutrition, employment, and sources of income (Ørskov, 2011; Hassen and Tesfaye, 2014; Wodajo *et al*., 2020).

Causes of abortion can be broadly categorized into infectious and non-infectious causes. The commonly diagnosed infectious causes of abortion in sheep and goats are C*oxiella burnetii* (*C. burnetii*), *Chlamydia abortus* (*C. abortus*), *Brucella* spp., *Leptospira* spp., *Campylobacter fetus*, *Listeria* spp. and *Toxoplasma gondii* (*T.gondii*) (Menzies, 2011; Holler, 2012). These pathogens are also zoonotic and thus can pose serious infection risks for farming communities (LeJeune and Kersting, 2010; Ganter, 2015). Zoonotic causes of animal abortion are prevalent and widely spread in all livestock production systems in Ethiopia (Gumi *et al*., 2013; De Vries *et al*., 2014; Gebremedhin and Tadesse, 2015; Tadesse, 2016; Tulu *et al*., 2018; Gebretensay *et al*., 2019; Thomas *et al*., 2019; Tesfaye *et al*., 2020). Furthermore, substantial knowledge gaps and high-risk behavioral practices towards zoonotic disease risk from livestock birth products among communities in Ethiopia increase the risk of exposure to zoonotic diseases.

Low fertility has been reported in sheep and goats in Ethiopia (Solomon *et al*., 2014; Rekik *et al*., 2015; Goshme *et al*., 2020). Low productivity per animal and flock offtake affects the overall contribution of sheep and goats to households in the rural areas of Ethiopia. Since the production efficiency of a flock is directly related to the number of kids and lambs produced, controlling important abortion causes increases the profitability and access to animal source food for rural poor households. Abortion in sheep and goats is often multifactorial in nature. Variation in management factors such as health care, feeding and watering practices, nutrition management for pregnant animals have an impact on the survival of the fetus. Moreover, production systems, seasonality, and agroecological factors also significantly affect the occurrence of abortion.

Furthermore, abortion management strategies through appropriate biosecurity, confirmation of the cause of abortion, prevention of common disease conditions using appropriate vaccination schedules, and internal and external parasite control programs through regular anthelmintic and acaricidal treatments, respectively, are important to ensure the health of pregnant animals, as well as foetal survival, henceforth to reduce abortion.

Although multiple infectious causes and putative factors for abortion are identified in livestock, studies focusing on small ruminant abortions' major causes and their associated risk factors are limited in Ethiopia. Some studies (Gebremedhin *et al*., 2013; Gebretensay *et al*., 2019) tried to address various factors under extensive production systems in specific agroecology. However, it is important to estimate the frequency of occurrence and various factors under different agroecologies and production systems to design more realistic and efficient control programmes in smallholder settings. The objectives of this study were thus to estimate the prevalence of abortion and identify the major causes and associated risk factors in small ruminants in the three agroecologies and production systems of Ethiopia.

4.2 Materials and methods

4.2.1 Study areas

The study was conducted in eleven sites in five districts in three regional states in Ethiopia, namely, Amhara, Oromia, and Southern Nation, Nationality and People (SNNP) (Fig 5.1). Six of the sites were part of the CGIAR research program on Livestock (CRP Livestock) and had been selected based on agroecology and production systems, their potential for sheep and/or goat production, accessibility and willingness of the community to participate in further studies, and the importance of sheep and goats to household livelihoods (Haile *et al*., 2019). In addition, the CRP Livestock sites were complemented by control sites from the same districts and which, in contrast to the CRP Livestock sites, had not seen any livestock interventions in recent years. The CRP Livestock interventions included improved breeding programs, better access to feed and vaccination for key diseases, and control of parasites. The agroecology and production system characteristics of the study sites are summarized in Table 5.1. Livestock production in Ethiopia is broadly classified into pastoral, agro-pastoral, and mixed crop-livestock (MCL), peri-urban, and urban production systems (Gizaw *et al*., 2010).

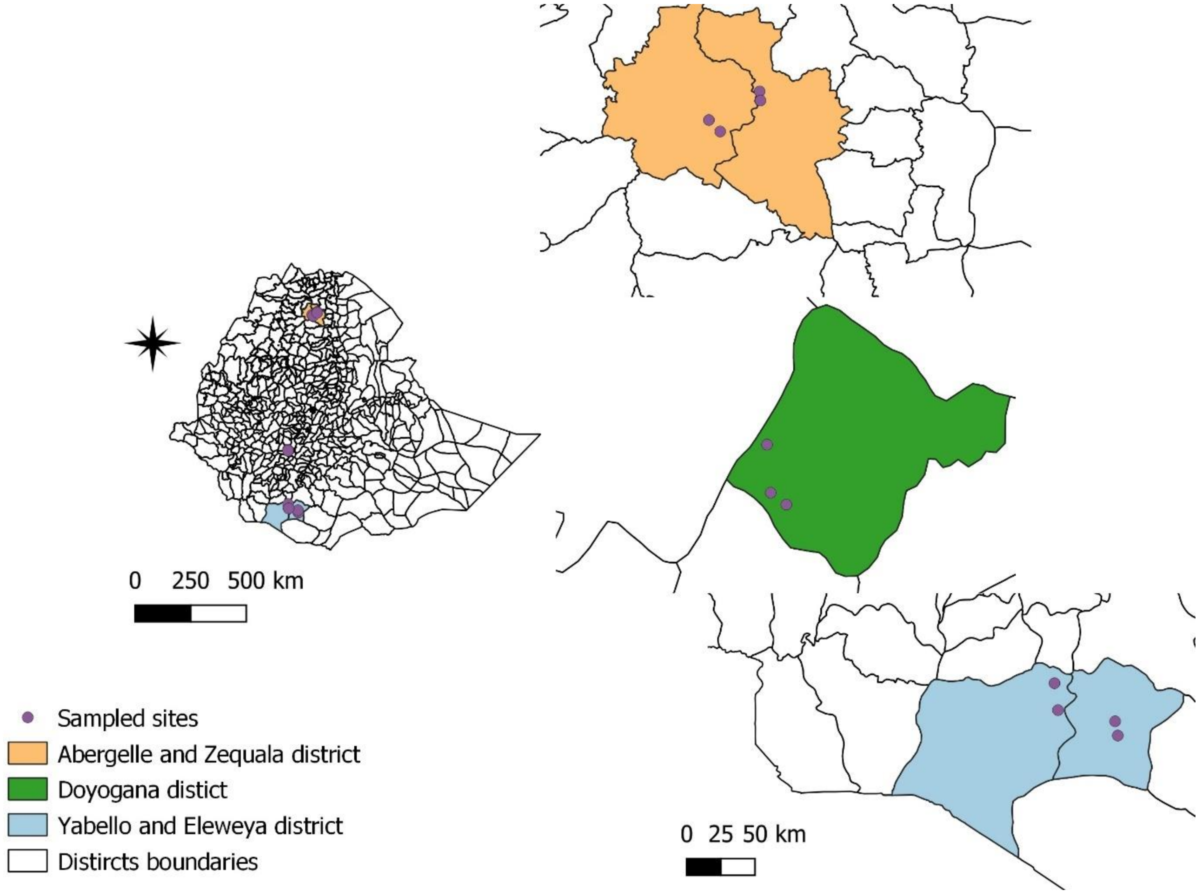

Figure 5.1 Map of study locations

The highland agroecology is typical for areas 2,200 meters above sea level (masl) and higher in which a mixed crop-livestock production system drives the predominant economic activities. Livestock husbandry and rain-fed cropping are closely interlinked to gain complementary benefits from an optimum mixture of crop and livestock and spreading income and risks over both crop and livestock production. Natural pastures, crop residues, and crop stubbles are used as major livestock feed. Doyogana district of the SNNP region represents this agroecology and production system.

The lowland agroecology with mixed crop-livestock production system denotes an elevation below 1,500 masl and is dominated by livestock production but practiced in proximity to and perhaps functional association with cropping farming. The productivity of crop farming is low in the area due to the shortage of rain. The livestock production activities are dominated by goats. The major feed resources for livestock in this production system are communal natural pastures, crop residues, crop stubbles, hay, browse plants, and weeds. Abergelle and Zequala districts in the Wagihimira zone of the Amhara region represented the lowland agroecology and mixed crop-livestock production system.

The lowland pastoral production system is typically characterized by sparsely populated pastoral rangelands, where the subsistence of pastoralists is mainly based on livestock and livestock products. Livestock husbandry in this system is dominated by goats, cattle, sheep, and camels. Pastoralists in this production system take advantage of the characteristic instability of rangeland environments through strategic mobility and fencing of communal land. Yabello and Eleweya districts in the Borena zone of Oromia region represent the lowland pastoral production system.

Table 5.1 Description of study areas in Ethiopia

No.	District	Production system	Village	Altitude (masl)	Rainfall (mm)	Average temperature (^oC)
1.	Abergele	Lowland, MCL	Sazba* Belteharf	1348	647	24
2.	Ziquala	Lowland, MCL	Bilaqu* Tsitsika	1486	732	22
3.	Yabello	Lowlands, pastoral	Derito* Dida Yabello	1588	625	20
4.	Elwaya	Lowlands, pastoral	Adegalchet* Chari	1181	493	22
5.	Doyogena	Highland, MCL	Ancha Sadicho* Hawara Arara* Gomora Gawada	2616	1,275	15

*received CRP livestock interventions.

4.2.2 Study design and sampling strategies

A cross-sectional survey was conducted between July 2018 to February 2019 to collect data on the incidence of small ruminant abortion and identify its major causes and potential risk factors in goat and sheep flocks. This was complemented with a retrospective one-year data set of pregnancy outcomes of small ruminants. After obtaining lists of households for each selected village from local field researchers, households who owned at least one sheep or goat were selected randomly using the random function in Microsoft Excel. Household heads were contacted by facilitators and asked for their willingness to participate and to plan the timing of the interview. Only one person per household, whether male or female, was interviewed to have 50% female respondents if possible. Assuming a standard error (SE) of 2.8%, the required sample size for respondents of the household questionnaire interviews was calculated using the formula ($n = 0.25/(SE^2$) given by Arsham (2005) with a 95 % confidence level. Accordingly, a total of 318 respondents were required for house-to-house interviews, resulting in 64 households per district.

The blood samples used to determine the serostatus of antibodies against abortive pathogens in the flock were collected from flocks of randomly selected households who participated in the interview. Due to financial and logistical limitations, diseases were prioritized according to their likely burden on small ruminant and human populations in Ethiopia and the availability of diagnostic kits in Ethiopia. Accordingly, six abortion-causing agents (*C. burnetii*, *C. abortus*, *Brucella* spp., *Leptospira* spp., *T. gondii,* and *Neospora caninum)* were selected to be included in this study based on existing literature and expert opinion. Unfortunately, we were not able to source the diagnostic kits for *Leptospira* spp. and *Neospora caninum*. Therefore, we only included *C. burnetii*, *C. abortus, Brucella* spp., and *T. gondi* for which we included the analysis result in this paper. The sample size was calculated using web-based Epidemiological Calculators (AusVet, 2017) with the following predetermined parameters: 50% of the expected individual prevalence of each pathogen, a confidence level (CL) of 95 %, and a desired level of precision (d) of 5%. Since animals within the same households tend to have more similar outcomes, the total sample size was adjusted for clustering at the household level using the formula described by Dohoo *et al*. (2009):

$$n' = n(1 + p(m - 1))$$ where

n' is the new sample size,

n is the original sample size estimate,

p is the intra-cluster correlation coefficient

m is the number of ewes /does sampled per flock.

The intra-cluster correlation coefficient *(P)* for the majority of infectious diseases is usually between 0.05-0.2 (Otte and Gumm, 1997). Accordingly, a value of 0.2 was taken for the initial sample size calculation. We planned to sample an average of 8 animals (5 goats and 3 sheep) from each household. Hence, a minimum of 922small ruminants was required for the study. However, the sample size was increased to 1,226 to allow for poor quality samples, drop-outs and to increase precision. Accordingly, a total of 154 households were targeted for a blood sample collection from their animals.

4.2.3 Data collection processes and tools

The data were collected through a face-to-face structured questionnaire interview. The questionnaire interviews were conducted by four trained veterinarians and/or animal production experts from the National Agricultural Research System (NARS) who spoke the local language of the respective study site. The participants were informed that the aim of the survey was to get information on small ruminant abortions. The questions were designed based on a literature review and the experiences of the researchers and pretested with 15 farmers who were not included in the study population. After necessary adjustments, questions were coded using Epi Info™ 7.2.1.0 software and copied on mobile tablet devices for digital data collection.

Questions addressed pregnancy outcomes of goats and sheep over one year before the interview, the season of the year, abortion strikes in the flock, perceived causes of small ruminant abortion, and flock management practices. Data on management-related potential factors included household demographics data (sex, age and educational level of household head, location), livestock keeping type (mixed crop-livestock pastoral), flock type and structure (small ruminant species, flock type, flock size, and presence of other livestock in the households), feeding and husbandry (grazing land, housing type, confinement level, source of water, distance travel to grazing pasture), management of pregnant dam approaching delivery (supplementary feeding, housing), breeding management (breeding buck/ram ownership, buck/ ram stay in the flock), biosecurity practices (routine manure cleaning, birth products disposal practice, action on frequently aborting dam, dog and cat access to the flock) and CRP livestock intervention status.

During the interview, respondents were asked about their confidence in the estimates provided in retrospective information. If they were not confident about their estimates, the data entry for that question was left empty and treated as missing. Moreover, information collected through the questionnaire interviews about the number of pregnant animals delivered and aborted was matched with data collected longitudinally on recorded sheets by recruited enumerators in the households belonging to intervention sites. In cases where information did not match, the flock owners and data enumerators were approached to validate the discrepancy and the data were corrected wherever possible.

4.2.4 Serum sample collection and processing

About 6-8ml blood was collected from the jugular vein into 10ml sterile plain vacutainer tubes. Individual animal biodata was gathered during sample collection. The tubes were then labeled with a unique identification number and kept protected from direct sunlight. The samples were placed in the slant position until the blood was clotted, and sera were separated. The sera were separated from clotted blood after centrifugation at 1500 g for 10 min at Yabello Pastoral and Dryland Agriculture Research Center, Sekota Dryland Agriculture Research Center, and Wolayita Sodo Regional Veterinary Laboratory. The serum was transferred into a sterile cryovial tube bearing the identification number and transported to the National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia, for laboratory analysis. The samples were transported to the laboratory at +4 using a portable fridge, plugged into a car, and then stored at -20°C until analyzed.

4.2.5 Laboratory analyses

Commercial enzyme-linked immunosorbent assays (ELISA) were used to detect antibodies against *C. burnetii*, *Brucella* spp., *C. abortus*, and *T. gondii* at NAHDIC. For *C. burnetti* the Antibody Test Kit, (IDEXX® Switzerland AG, CH-3097 Liebefeld-Bern Switzerland), for Chlamydia, the *C. abortus* Antibody Test Kit (IDEXX® Switzerland AG, 3097 Liebefeld-Bern Switzerland), for

Brucella spp, Svanovir TM Brucella-Ab c-ELISA test kits (Svanova Biotech, Uppsala, Sweden) and for Toxoplasma the *T.gondii* Antibody Test Kit, (IDEXX® Switzerland AG, 3097 Liebefeld-Bern Switzerland) were used. The test procedure, computation of the sample to positive ratios and final interpretation of the results were performed following the protocols provided by the respective kit manufacturers. Briefly, sera samples, negative and positive controls were diluted at 1:400 using wash solution. One hundred μL of pre-diluted negative and positive control and samples were added into a microtiter plate and incubated for one hour at 37°C. The plate was then washed 3 times and 100μL of the conjugate was added to each well. The plate was incubated at +37°C for 1 hour after the microplate was sealed using plate covers to avoid any evaporation. The plates were again washed 3 times. Finally, 100μL of the substrate was added to each well and incubated at 18–26°C for 15min. Then 100μL of stop solution was added to each well and the result was read at a wavelength of 450nm. The OD of the positive control ($PC\bar{x}$) and the OD of the samples (sample A_{450}) are corrected by subtracting the OD of the negative control ($NC\bar{x}$). Sample to the positive ratio (S/P %) was computed as $\frac{100 \times sample\ A450 - NC\bar{x}}{PC\bar{x} - NC\bar{x}}$. An animal was considered to be infected when the serum presented an S/P% ≥30 for *Brucellas* spp., ≥40 for *C. burnetii,* and *C. abortus,* and ≥ 50% for *T. gondii.*

4.2.6 Data management and analyses

The recorded responses from questionnaire interviews were transferred and stored on a personal laptop computer and subsequently exported to Microsoft Excel where data cleaning and integration were undertaken. Laboratory results were entered into Microsoft Excel version 15 and linked to the respective household data. The data were transformed to create flock-level tables in the database. The variables created from the laboratory data were crossmatched and combined with the questionnaire data. The data cleaning and statistical analyses were conducted in STATA 15.1 (Stata SE/15.1, Stata Corp., College Station, TX, USA).

The unit of analysis was the flock. A flock was defined in this study as sheep and goats owned by a household. The outcome of interest was the number of abortion cases in a sheep and goat flock. Annual abortion percentages were calculated as lamb/kids lost before the expected date of parturition divided by pregnant ewes/dose in the flock for one year multiplied by hundred.

Descriptive statistics such as mean, standard deviation (SD) were used to describe the number and percentage of abortions. Cross tabulation and frequency tables were used to describe proportions.

Unconditional association between potential risk factors and outcomes of interest was tested in univariable models. The maximum likelihood method was used to estimate parameters describing the relationship between predictor variables and outcomes of interest. Incidence rate ratio (IRR) was used to measure the effect of various predictors on the outcomes of interest. The Poisson distribution has been considered in the context of regression analysis for describing count data where the sample means and sample variances are almost equal. The data was considered overdispersal if the sample variance was significantly greater than the sample mean (Coxe *et al*., 2009). Since the count of abortions in this study showed overdispersion, the negative binomial regression has been found to fit our data well. Moreover, Zero-Inflated models have been used for modeling the count data set, which showed a large proportion of zeros. It was assumed that the excess zeros in our dataset were from two sources, either there was no pregnancy or no abortion in the flock. The model goodness of fit was examined by likelihood using the Akaike Information Criteria (AIC) and the Bayesian Information Criteria (BIC). The likelihood ratio test was used to compare between negative binomial and zero-inflated negative binomial regression models. Models with smaller values of AIC and BIC were considered to fit the data. A random-effects regression model was used to account for the clustering of flocks within villages. Variables showing an unconditional association with the outcome were selected for multivariable analysis, using $p < 0.15$ as the criterion for inclusion.

A causal diagram was generated in the browser-based environment DAGitty® (Textor, 2015) to identify causal relationships between potential predictors and abortion in the flock (Fig.5.2). This diagram was used to identify plausible predictors of abortion in the flock and intervening variables (the variable lies along the path between the exposure and the outcome of interest) and potential confounders (variables antecedent to the exposure variable). Multivariable models were built with the number of abortions and the main factor (s) of interest related to management factors and exposure to abortion-causing agents. Variables in the causal diagram, which could be potential confounders, were retained in the model if the parameter estimates of any explanatory variable changed more than 20%. Variables between the exposure variables and outcome variables were excluded from the model as they were intervening variables. Plausible biological interactions between variables were evaluated and included if significant. The likelihood ratio test was used to evaluate the significance of variables. P≤0.05 was considered significant.

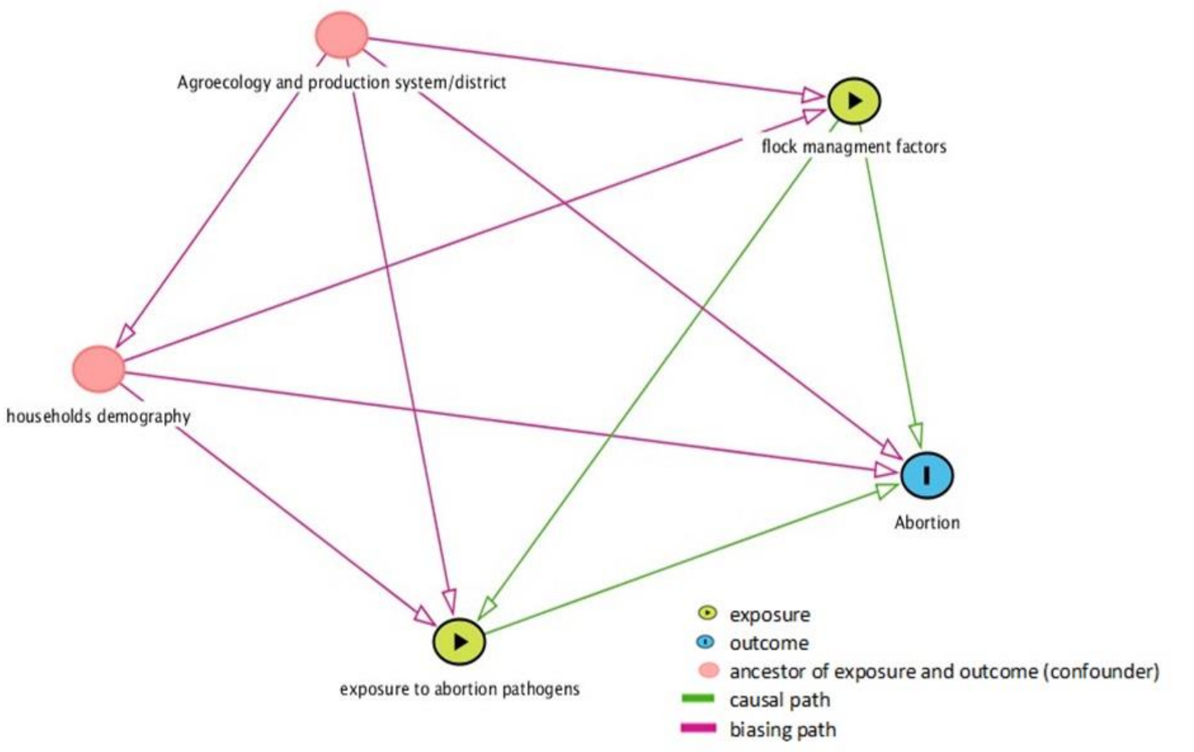

Figure 5.2 Causal diagram generated in DAGitty (Textor, 2011) postulating the relationships between the potential predictors and small ruminant abortion sampled in three-agroecology and production system of Ethiopia.

4.3 Results

Information on pregnancy outcomes and management risk factors were collected from a total of 299 goat and 242 sheep flocks. Blood samples were collected from a total of 1226 animals from 223 (133 sheep and 90 goat) flocks and were tested for *C. burnetii*, *Brucella* spp., and *C. abortus* and 994 samples from 192 (103 sheep and 85 goat) flocks were tested for *T. gondii*. Fig 5.3 shows the study subject enrolment flow.

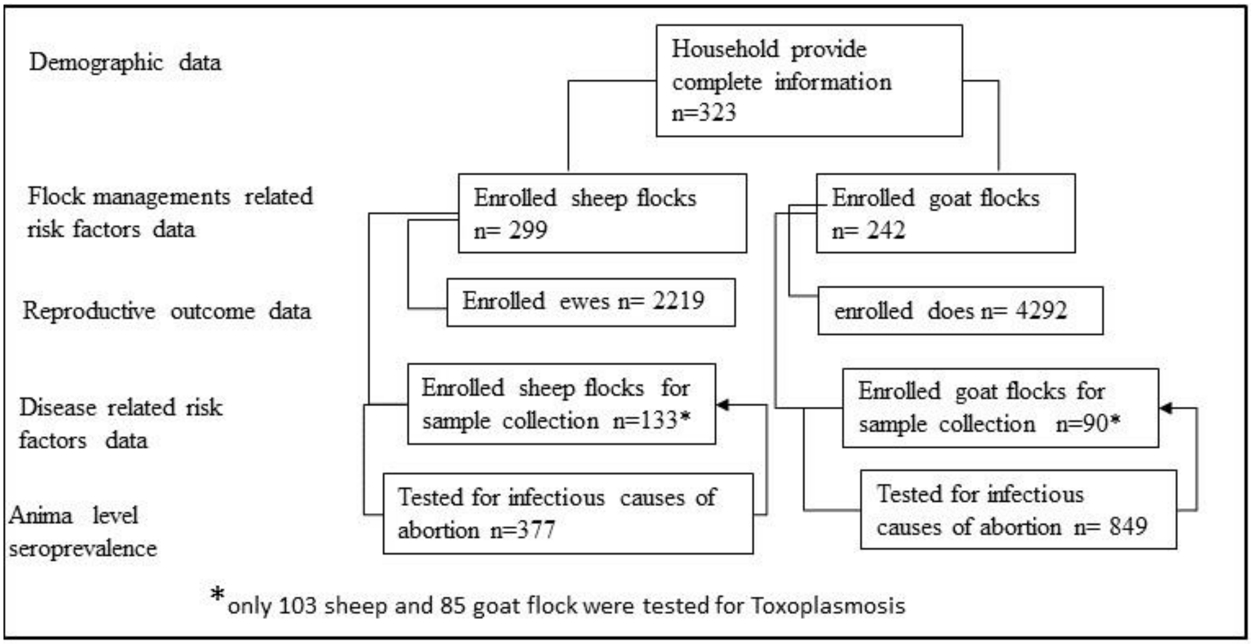

Figure 5.3 Study subject enrolment flow diagram

4.3.1 Flocks enrolled in the study

From the total 242 goat flocks enrolled in the study, 114 (47.11%), and 128 (52.89%) were managed under a lowland MCL and pastoral agroecology, respectively. Whereas from the total 299 sheep flocks, 107 (35.79%), 126 (42.14%), and 66 (22.07%) were lowland MCL, pastoral, and highland MCL agroecology and production systems, respectively.

Long-eared Somali and Abergele were the predominant goat breeds kept by pastoralists in the lowland Borena zone and the lowland of Waghimira, respectively. Adilo, Sekota, and Blackhead Somali are the predominant sheep breeds kept in the Doyogena district, Waghimira, and Borena zones, respectively. The mean (± s.d.) flock size of goats and sheep was 30.19 (±25.36, median = 13) and 13.6 (±13.4, median = 5) animals, respectively. The majority of the flocks were mixed flocks with both sheep and goats (81.33%), while 14.23% and 4.44% were sheep-only and goat-only flocks, respectively. Flocks were kept under traditional extensive management systems and therefore fully dependent on grazing land, with overall limited input. The majority of the flocks (89.28%) were grazed freely on pastures during the daytime while few flocks were tethered (0.74%). About 41.59% of small ruminant flocks spent the night in an open enclosure, while 58.41% of the flocks spent the night in the traditional shoat house. All day-to-day herding decisions were made by the owner and breeding was uncontrolled. Ewes/ does in 84.84% of the flocks were mainly mounted by rams/bucks from the same flock, while 15.16% of the flocks' utilized rams/bucks from other flocks. Fertile bucks/rams remained continuously with a group of females throughout the year.

4.3.2 Estimation of sheep and goat abortion

Of the 242 goat and 299 sheep flocks observed, 142 (58.68%) of goat and 53 (17.73%) sheep flocks reported abortions in the 12 months before the study. The occurrence of at least one abortion in the flock was significantly higher ($P \leq 0.000$) in goat flocks (142/242, 58.68 %) than sheep flocks (53 of 299, 17.73%). Overall, very few numbers of flocks from highland mixed crop-livestock production systems (2 of 66, 3%) reported abortions, which was significantly ($P \leq 0.00$) lower than other production systems. However, comparable proportions of flocks were affected in the lowland mixed crop-livestock production system (40.27%) and the lowland pastoral production system (40.94%) (Fig 5. 4).

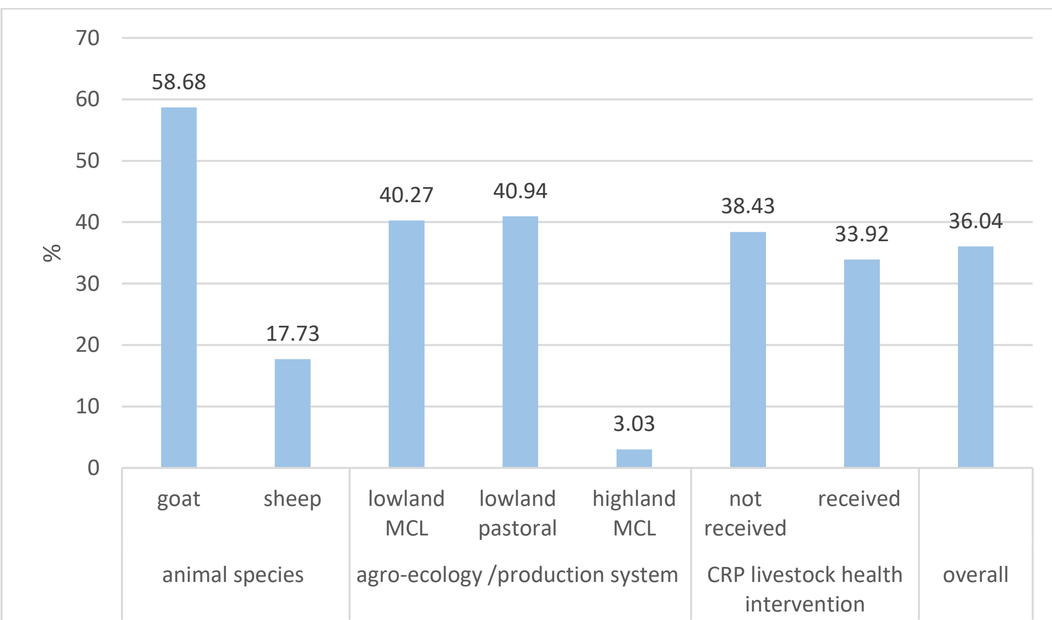

Figure 5.4 Proportion of flock with abortion in sheep and goat in three agroecology and production system of Ethiopia

A total of 860 does and 153 ewes abortion cases were recorded from 4,995 pregnancies of 3,380 does and 1,615 ewes monitored over one year, respectively. On average, 3.55 does and 0.5 ewes aborted per flock. The annual number of abortions per flock ranged from 0 to 60 (median =1) and 0 to 12 (median =0) for the sheep flocks. The mean annual flock abortion percentages were 16.1% (±26.23) for does and 12.6% (±23.5) for ewes. Annual flock abortion percentage was higher (P <0.05) among small ruminant flocks in lowland mixed crop-livestock production system than highland mixed crop, livestock, and lowland pastoral production systems. Table 5.2 shows the annual abortion percentage in three agroecologies and production systems aggregated by small ruminant species.

Table 5.2 Mean annual sheep and goat abortions percentage in three agroecologies and production systems of Ethiopia

Ago-ecology and production	Species	No. of flock examined	No. at risk	No. aborted (mean)	abortion percentage (%) (±SD)
Overall		541	4995	1013 (1.9)	14.15 (24.78)
Lowland MCL	Goat	114	1682	380 (3.33)	22.23 (27.71)
	Sheep	102	643	72 (0.67)	21.20 (26.19)
Lowland Pastoral	Goat	128	1698	480 (3.75)	10.52 (23.56) **
	Sheep	123	828	79 (0.63)	12.01 (24.25)8*
Highland MCL	Sheep	65	144	2 (0.03)	0.00 (0.00) **
Total	Goat	242	3380	860 (3.55)	16.11 (26.23)
	Sheep	290	1615	153 (0.51)	12.55(23.45)

*significant at P≤0.01, ** P≤0.001, SD = standard deviation

4.3.3 Perceived causes of small ruminant abortion

Abortion was called "Koyisayech" by the Agew community in the lowland of Waghimira zone, "Salesa" by Borena pastoralists of the Oromo community in lowland Borena, and "Kara" by the Kembata community in the highland of Doyogana. Goat was considered as the most affected livestock species by abortion by 99.2% and 98.4% of respondents in lowland MCL and pastoral production system, respectively. Of the 1011 abortion cases recorded in the lowland MCL and pastoral agroecology and production system, animal owners recognized only the causes of 509 (50.34%) abortion cases during the individual interview. From the recognized causes, extreme weather conditions (30.21%), disease (26.89%), and feed shortage (25.68%) were the first, second, and third most important causes of abortion in lowland MCL agroecology and production system, respectively. Nevertheless, in lowland pastoral agroecology and production system, disease (56.74%) was considered the major cause, followed by feed shortage (17.42%) and plant poisoning (14.61%) (Table 5.3). The two abortion cases in the highland MCL agroecology and production system were caused by physical trauma as perceived by the farmers.

Table 5.3. Perceived causes of sheep and goat abortion in lowland MCL and pastoral agroecology and production system in Ethiopia

Cause of abortion	**The relative contribution of causes, proportion, n (%)**		**P-value**
	Lowland MCL (n=331)	**Lowland pastoral (n=178)**	
Infectious diseases	89 (26.89)	101 (56.74)	0.000
Extreme weather condition	100 (30.21)	15 (8.43)	0.034
Feed shortage	85 (25.68)	31 (17.42)	0.000
Physical traumas	53 (16.01)	5 (2.81)	0.000
Plant poisoning	4 (1.21)	26 (14.61)	0.000

MCL= Mixed crop-livestock

The monthly incidence of abortion in sheep and goat flocks in the dryland ecosystem is presented in Fig 5.5. A higher proportion of abortion was found in the lowland MCL agroecology and production system during the short rainy season-*Belg* (March to May). The abortion numbers peaked in May, the hottest month of the year. However, in lowland pastoral agroecology and production system, higher proportions of abortions were found to occur at the start of the short dry and cold season -*Adoolessa* (June to August) followed by the long dry season-*Bona Hagayya* (December -February).

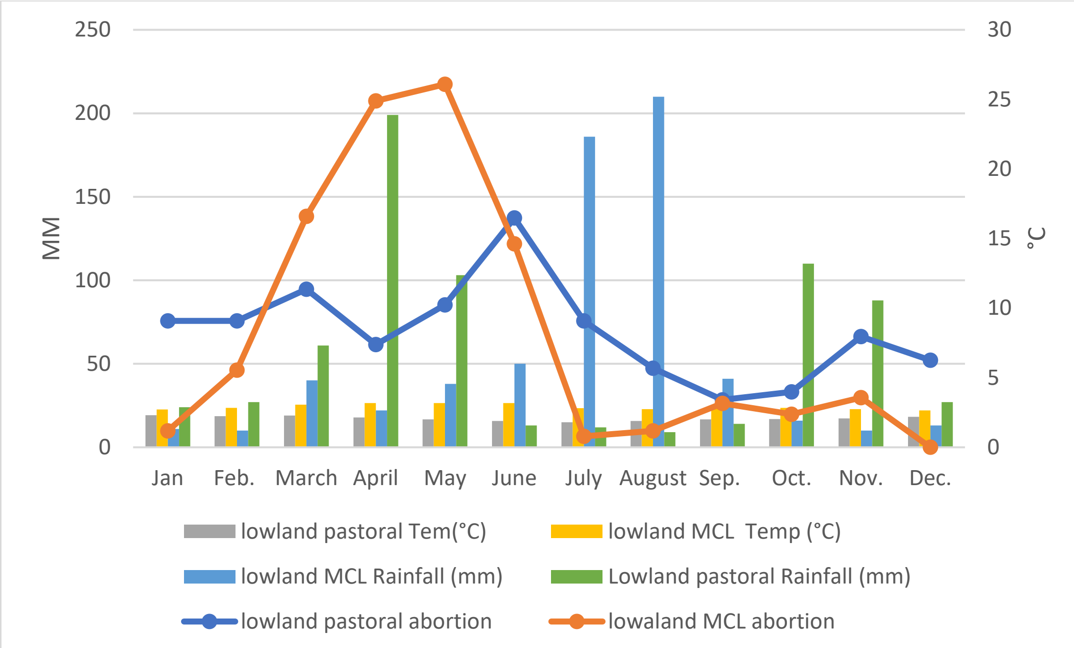

Figure 5.5 Monthly levels of sheep and goat abortion in comparison with mean monthly temperature and rainfall distribution for lowland MCL and pastoral agroecology and production system

4.3.4 Flock level seroprevalence of major infectious causes of abortion

Overall, 76.23% of sheep and goat flocks tested positive for one or more abortion-causing agents, namely, *C. burnetti*, *C. abortus*, *Brucella* spp., and *T. gondii;* mixed infection was found in 44.39% of the 223 flocks tested. 96 (43.05%), 73 (32.74%), and 57 (25.56%) flocks tested positive to *C. burnetti*, *C. abortus,* and *Brucella* spp, respectively. *Toxoplasma gondii* infection was detected in 91(47.4%) of the 192 flocks tested. Details, including statistically significant differences across the small ruminant species flocks and agroecology and production system, are presented in Table 5.4.

Table 5.4. Flock level seroprevalence of major infectious causes abortion in small ruminant in three agroecology and production systems of Ethiopia

		Prevalence estimates (%) (calculated at p = 0.05, CI = 0.95)					
Category	variable	*C. burnetii* (n= 223)	*C. abortus* (n= 223)	Brucella Spp. (n= 223)	*T.gondii* (n= 192)	at least one infection (n=223)	Mixed infection (n=223)
Flock type	Goat	73.3 (64.0, 82.6)	42.2 (31.8, 52.6)	24.4 (15.3, 33.4)	48.2 (37.4, 59.1)	92.2(86.6, 97.9)	63.3 (53.2, 73.45)
	Sheep	22.6 (15.4, 29.8) **	26.3 (18.7, 33.9) *	26.3 (18.7, 33.9)	46.7(37.1, 56.3)	65.4 (57.2, 73.6) **	31.6 (23.6, 39.6) **
Production system	Lowland MCL	62.9 (50.5, 75.3)	37.1 (24.7, 49.5)	6.45 (0.2, 12.7)	31.6 (19.1, 44.0) **	70.9 (59.3, 82.6)	46.8(34.0, 59.5)
	Lowland pastoral	57.6 (47.7, 67.5) **	26.3 (17.4, 35.1)	24.2 (15.7, 32.8) **	57.6 (47.6, 67.5)	79.8 (71.7, 87.8)	50.5(40.5, 60.5)
	Highland MCL	0	38.7 (26.2, 51.1)	46.7 (33.9,59.5)	44.4 (27.3, 61.5)	75.8 (64.8, 86.8)	32.3(20.3, 44.2)
Overall		43.1 (36.5, 49.6)	32.7 (26.5, 38.9)	25.5 (19.7, 31.3)	47.4 (40.2, 54.5)	76.2 (70.6, 81.9)	44.4 (37.8, 50.9)

*Significant at p≤0.05, ** p≤0.001

4.2.5 Factors affecting the number of abortions in sheep and goat flocks

The effect of management and exposure to infectious causes on the number of abortions in the flock across agroecologies was tested using univariate zero-inflated negative binomial regression. The casual diagrams (Fig 5.2) helped to describe the postulated links between sheep and goat abortion and potential predictors. The diagram illustrates that sheep and goat management practices as a group (flock type and structure, feeding and watering, housing, pregnant dam management, breeding management, biosecurity practices, and herd health intervention) and exposure to abortion pathogens are direct exposure variables associated with abortion. The demographic factors of household head, district, and agroecology and production system were assumed to be potentially confounding variables, associated with both exposure and outcome variables, but not a consequence of exposure to it. However, the district was removed from the model due to the multicollinearity effect of the production system. Unconditional association between potential predictors and the occurrence of abortion in the flock is presented in Table 5.5.

Table 5. List of all predictors relating to small ruminant management practice, household demography descriptions, exposure to abortion pathogens, and unconditional association with the history of abortion and number of abortions.

Viable	Category	No. flock observed	No. flock affected	Odds Ratio	95% CI	P value	IRR	95% CI	P value
Agro- ecology and production system	Lowland MCL	221	89 (40.27)	1			1		
	Lowland Pastoral	254	104 (40.94)	1.03	0.71, 1.48	0.88	1.08	0.75, 1.56	0.66
	Highland MCL	66	2 (3.03)	0.05	0.01, 0.19	0.00	0.05	0.01, 0.29	0.00
District	Abergele	112	49 (43.75)	1			1		
	Doyogena	66	2 (3.03)	0.04	0.01, 0.17	0.00	0.05	0.01, 0.30	0.00
	Eleweya	152	70 (46.05)	1.10	0.67, 1.79	0.71	1.27	0.80, 2.04	0.32
	Yabello	102	34 (33.33)	0.64	0.37, 1.12	0.12	0.79	0.46, 1.36	0.40
	Ziquala	109	40 (36.7)	0.75	0.43, 1.28	0.29	1.01	0.59, 1.74	0.96
Sex of household head	female	61	18 (29.51)	1			1		
	male	480	177 (36.88)	1.40	0.78, 2.49	0.26	1.90	0.96, 3.78	0.07
Age of household head in years	<30	185	66 (35.68)	1			1		
	30-60	324	119 (36.73)	1.05	0.72, 1.52	0.81	0.80	0.54, 1.17	0.25
	>60	32	10 (31.25)	0.82	0.37, 1.83	0.63	0.67	0.29, 1.52	0.34
Education level household head	none	402	164 (40.8)	1			1		
	primary	98	22 (22.45)	0.42	0.25, 0.70	0.00	1.00	0.57, 1.74	1.00
	secondary and above	41	9 (21.95)	0.41	0.19, 0.88	0.02	0.84	0.34, 2.11	0.72
Flock type	goat	242	142 (58.68)	1			1		
	sheep	299	53 (17.73)	0.15	0.10, 0.22	0.00	0.25	0.17, 0.36	0.00
Small ruminant flock mix	goat only	24	9 (37.5)	1			1		
	sheep only	77	4 (5.19)	0.09	0.02, 0.34	0.00	0.33	0.08, 1.28	0.11
	mixed	440	182 (41.36)	1.18	0.50, 2.74	0.71	1.51	0.65, 3.50	0.34

Presence of other livestock	Small ruminant only	17	2 (11.76)	1			1		
	≤2 other livestock species	134	40 (29.85)	3.19	0.70, 14.61	0.14	2.17	0.34, 13.7	0.41
	>2 other livestock species	390	153 (39.23)	4.84	1.09, 21.47	0.04	3.81	0.62, 23.3	0.15
Flock size	<15	351	80 (22.79)	1			1		
	15,30	110	60 (54.55)	4.07	2.59, 6.38	0.00	1.93	1.25, 2.99	0.00
	>30	80	55 (68.75)	7.45	4.37, 12.72	0.00	5.40	3.39, 8.62	0.00
Grazing land	communal and private	113	41 (36.28)	1			1		
	communal only	374	147 (39.30)	1.14	0.74, 1.76	0.56	1.99	1.29, 3.06	0.00
	privately owned only	54	7 (12.96)	0.26	0.11, 0.63	0.00	4.64	1.64, 13.16	0.00
Distance to grazing land	<2km	215	58 (26.98)	1			1		
	2- 4km	219	92 (42.01)	1.96	1.31, 2.94	0.00	1.36	0.90, 2.06	0.15
	>4	107	45 (42.06)	1.96	1.21, 3.20	0.01	2.14	1.30, 3.52	0.00
Source of water	river/spring	255	89 (34.9)	1			1		
	tab water	19	0 (0)				0(0)		
	Stagnant	264	105 (39.77)	1.23	0.86, 1.76	0.25	1.13	1.63, 0.5	0.78
Feeding system	free grazing	483	194 (40.17)	1			1		
	tethered	4	0 (0)				0(0)		
	both	54	1 (1.85)	0.03	0.00, 0.20	0.00	0.03	0.00, 0.30	0.00
Supplementary feed for pregnant	no	447	178 (39.82)	1			1		
	yes	94	17 (18.09)	0.33	0.19, 0.58	0.00	0.33	0.18, 0.60	0.00
A separate house for pregnant	no	399	157 (39.35)	1			1		
	yes	142	38 (26.76)	0.56	0.37, 0.86	0.01	0.84	0.53, 1.32	0.45
Breeding male ownership	no	82	26 (31.71)	1			1		
	yes	459	169 (36.82)	1.26	0.76, 2.07	0.38	1.40	0.79, 2.46	0.25
	>2	62	7 (11.29)						

Ram/buck stay in the flock	2-4	199	80 (40.2)	5.28	2.29, 12.19	0.00	0.44	0.17, 1.09	0.08
	>4	280	108 (38.57)	4.93	2.17, 11.23	0.00	0.39	0.16, 0.97	0.04
CRP livestock health intervention	not received	255	98 (38.43)	1			1		
	received	286	97 (33.92)	0.82	0.58, 1.17	0.28	1.27	0.89, 1.83	0.19
Housing /shelter at night	none shelter paddock	225	102 (45.33)	1			1		
	traditional house	316	93 (29.43)	0.50	0.35, 0.72	0.00	0.48	0.33, 0.68	0.00
Routine manure cleaning	no	166	74 (44.58)	1			1		
	yes	375	121(32.27)	0.59	0.01, 0.41	0.86	0.63	0.43, 0.92	0.02
Birth products disposal practice	bury/burn	44	2 (4.55)	1			1		
	feed to dog	379	157 (41.42)	14.85	0.00, 3.54	62.26	17.22	3.13, 94.80	0.00
	disposed to environment	118	36 (30.51)	9.22	0.00, 2.12	40.16	22.83	4.02, 129.7	0.00
Action on frequently aborting dam	keep in the flock	132	62 (46.97)	1			1		
	sell	267	101 (37.83)	0.69	0.08, 0.45	1.05	0.64	0.43, 0.95	0.03
	slaughter	36	21 (39.00)	1.58	0.23, 0.75	3.33	1.15	0.58, 2.28	0.69
Dog access to the flock	not at all	152	39 (25.66)	1			1		
	yes, neighbor dog	139	54 (38.85)	1.84	0.02, 1.12	3.03	2.11	1.21, 3.67	0.01
	yes, own dog	239	99 (41.42)	2.05	0.00, 1.31	3.20	2.49	1.53, 4.06	0.00
Cat access to the flock	not at all	211	90 (42.65)	1			1		
	yes, neighbor cat	108	38 (35.19)	0.73	0.20, 0.45	1.18	1.00	0.61, 1.64	1.00
	yes, own cat	200	62 (31)	0.60	0.02, 0.40	0.91	1.31	0.87, 1.96	0.20
C. burnetii infection	negative	127	28 (22.05)	1			1		
	positive	96	45 (46.88)	1.61	0.78, 3.32	0.198	1.05	0.57, 1.95	0.840
Brucella Spp. infection	negative	166	110 (66.27)	1					
	positive	57	40 (70.18)	1.30	0.57, 2.96	0.531	1.86	0.9, 3.83	0.094
C. abortus infection	negative	150	44 (29.33)	1			1		
	positive	73	29 (39.73)	1.97	0.96, 4.04	0.066	1.04	0.55, 1.94	0.877
T. gondii infection	negative	166	56 (33.73)	1			1		
	positive	57	17 (29.82)	0.51	0.25, 1.04	0.063	1.22	0.66, 2.27	0.644

At least one infection	negative	53	12 (22.64)	1					
	positive	170	61 (35.88)	2.03	0.92, 4.51	0.081	2.23	1.03, 4.81	0.000
Mixed infection	negative	124	33 (26.61)	1			1		
	positive	99	40 (59.6)	1.49	0.78, 2.85	0.227	1.27	0.70, 2.3	0.591

IRR= Incidence rate ratio, CI=Confidence interval

Nineteen variables with P ≤ 0.15 were included in the multivariable analysis, which retained seven variables in the final regression model. The result of the final zero-inflated negative binomial regression analysis is presented in Table 5.6. Agroecology and production system were controlled as potential confounders in the model. Spending the night in traditional sheep houses' and 'providing supplementary feed for pregnant dams' were important management factors that significantly (p≤0.05) decreased the risk of abortion by 2.63 and 4.55 times, respectively. The presence of other livestock species and dogs in the household' had a marked effect on the risk of abortion in sheep and goat flocks. Moreover, exposure of the flock to *Brucella* spp. or any one of the four tested infectious agents significantly (p≤0.05) increased the risk of abortion in the flock by 1.66 and 1.68 times compared to nonexposed flocks, respectively.

Table 5.6. The final model of multivariable Zero-inflated negative binomial regression analysis for the effect of management and exposure to abortion pathogen on abortion in the flock.

Variable	**Category**	**IRR**	**95% CI**	**P-value**
Agroecology and production system	Lowland MCL	1		
	Lowland Pastoral	0.53	0.34, 0.81	0.003
	Highland MCL	0.18	0.03, 1.06	0.058
Presence of other livestock	small ruminant only	1		
	≤2 other livestock species	2.69	1.33, 5.42	0.006
	>2 other livestock species	2.22	1.15, 4.30	0.018
Housing /shelter at night	none shelter paddock	1		
	traditional house	0.38	0.25, 0.58	0.000
Dog access to the flock	not at all	1		
	yes, neighbor dog	1.77	0.85, 3.67	0.127
	yes, own dog	2.45	1.38, 4.37	0.002
Supplementary feed for pregnant	no	1		
	yes	0.22	0.07, 0.70	0.01
Brucella spp. infection	negative	1		
	positive	1.73	1.27, 2.36	0.001
At least one infection	negative	1		
	positive	1.85	1.38, 2.47	0.000

IRR=Incidence rate ratio, CI= Confidence interval

4.4 Discussions

This study provided important insights on the occurrence, causes, and potential risk factors of abortion in small ruminants in smallholder systems in Ethiopia. The present study revealed that abortion is an important problem of small ruminant production in lowland mixed crop-livestock, and pastoral agroecology and production system. This study found an overall annual abortion percentage of 16.1% in doe and 12.5% in the ewe, which is in the range of previous reports from the mixed crop-livestock and pastoral production systems in Ethiopia, 9.3-40.9% for doe and 7.5-36.4 % for ewes (Fentie, 2016). Compared to the international figures, a higher abortion percentage of 43.7% for does and 35.6% for ewes was reported from Egypt (Ahmed *et al*. 2008). On contrary, a lower abortion rate has been reported from Jordan, 10.6% in does and 2.0% in ewes (Megersa and Obeidat, 2020); from Nigerian, 10.8% in does (Yakubu *et al*., 2014) and from Mexico, 3.5% in does (Mellado *et al*., 2006). Similarly, the higher abortion percentage in lowland flocks than in highland flocks agrees with the report of Gebremedhin *et al*. (2013) and Fentie (2016) in Ethiopia. Mixed crop-livestock and pastoral production systems are practiced in dryland agro-ecosystems where multiple stressors such as the cumulative effects of poor nutrition, excessive heat, and the need to walk long distances to source feed and water compromise the production and reproduction performance of small ruminants (Sejian *et al*., 2012). The results from the household survey also indicated feed shortage as the second most important abortion cause in lowland pastoral and the third in lowland MCL agroecology and production system as perceived by livestock keepers. Lack of adequate year-round feed resources because of erratic rainfall could be the most important factor contributing to high reproductive failures such as abortion in arid and semiarid areas (Makkar *et al*., 2018). Poor availability of quality feed in the drylands leads to low levels of energy during pregnancy, which markedly affects fetal survival, thus abortions and stillbirths are major causes of economic loss for the small ruminants managed in dryland areas under extensive management conditions (Gunn *et al*., 1995; Salem and Smith, 2008). Lower levels of glucose in the maternal blood due to nutritional stress trigger the hyperactivity of the adrenal glands of the fetus, which then releases the estrogenic precursors that leads to the expulsion of the live fetus and, hence, abortion occurs (Gaughan *et al*., 2018). The monthly distribution of abortion incidence documented in this study corresponds to the shortage of rain, which in turn affects the availability of feed in the study areas.

Furthermore, extreme ambient temperatures in lowland agroecology might contribute to the higher abortion rate in sheep and goats (Silanikove, 2000; Coloma-García *et al*., 2020). Heat stress in this agro-ecosystem leads to hyperthermia and may indirectly affect feed intake (Al-Dawood, 2017). Thermal stress during pregnancy is responsible for the abnormal development of the fetus due to impaired normal placental vascular development and less chance of survival as a result of compromised passive immunity (Mellado and Meza-Herrera, 2002; Marai *et al*., 2007). The respondents from the lowland MCL agroecology and production system also highlighted the extreme weather conditions in May as the most important cause of sheep and goat abortion in the area.

Our findings revealed that abortion is widely prevalent in goat flocks compared to sheep flocks. The present study is consistent with previous studies in Ethiopia (Gebremedhin *et al*., 2013; Fentie, 2016; Megersa and Obeidat, 2020) and elsewhere (Ahmed *et al*., 2008). Almost all interviewed sheep and goat owners also confirmed the higher susceptibility of does to abortion than ewes. The reason for this might be genetic factors, physiological or higher susceptibility to risk factors present. In addition, the higher infection rate of three of four tested abortion-causing agents in goat might be attributed to this higher number of abortions in doe than ewes. Moreover, the reproductive behavior of does is that they can tolerate moderate weight loss due to feed shortage at mating and still get pregnant. However, the fetuses might be maintained or expelled depending upon feed availability (Goonewardene *et al*., 1997; Mellado *et al*., 2004).

The risk of abortion was significantly higher ($p \leq 0.05$) in the small ruminant flocks which spent the night in non-shelter paddocks compared to the flock in the traditional shelters. In low-input extensive production systems, small ruminant flocks confined in non-shelter paddocks expose the animal to bad weather conditions, hence compromising the health and welfare status of the animal. Furthermore, in the majority of cases, households who have larger flock size confine their animals in non-shelter paddocks with insufficient area for resting of the animal, increased moisture, manure accumulation, and an overall decreased hygiene status, which increase contact with pathogenic agents.

This study found that the presence of more than two livestock species in the household significantly increases the abortion risk in the flock. Since the majority of abortion-causing agents are shared among livestock species (Givens and Marley, 2008), keeping more animal species at the household level may increase animal density and chance of contact between animals, thus facilitating cross-transmission between livestock species which increase the chance of acquiring infection. Another possible explanation for this is that livestock species such as cattle are considered the most important livestock species in lowland MCL and pastoral agroecosystem (Wodajo *et al*., 2020) and thus receive preferential treatment if resources are scarce resulting in a low standard of management and inadequate feeding of sheep and goats.

Our findings revealed that the presence of dogs in the household could increase the risk of abortion significantly. A study in Algeria by Ghalmi *et al*. (2009) also found the presence of dogs significantly associated with the occurrence of abortion in cattle. One possible explanation for this is that dogs might play a role in transmitting abortion-causing agents to the sheep and goat populations which in turn increases the risk of abortion in the flocks (Shaapan, 2016; Givens and Marley, 2008). Moreover, the dogs might be mechanically spread infectious agents while feeding on the infected placenta and aborted fetuses. Application of biosecurity precautions including burying or incinerating placentas and aborted fetuses helps to prevent the spread of the infectious organism and can reduce reproductive losses in sheep and goats (Menzies, 2011). The presence of dogs in the household might increase the chance of chasing ewe/does by dogs. The stress of worrying by dogs can cause the pregnant dams to miscarry their lambs/kids (Davies, 2019).

This study also found that providing supplementary feed for dams in the last stage of pregnancy could significantly decrease the risk of abortion in the flock. Supplementary feeding will greatly depend on the availability and quality of forage. Where an animal is in an environment where feed resources are scarce, grazing needs to be supplemented with some level of concentrate feeding because the forage is not being balanced in terms of energy, protein, minerals, and vitamins (Blache *et al*., 2008). During the final stage of pregnancy, the fetus(es) can develop rapidly to acquire up to 75–80% of their future birth body weight (Fthenakis *et al*., 2012). Hence, supplementing the dam with available feed resources in addition to grazing is important to fulfill the energy requirements of pregnant dams, and hence to increase pre-and post-natal survival of lambs and kids and birthweight and production for life.

Infectious causes of abortion play an important role in small ruminant abortion (Holler, 2012). Those pathogens are released into the environment through the aborted fetus, placenta, uterine fluids, and vaginal discharge of infected dams. These can serve as a source of infection for animal populations and cause zoonotic risks for farming communities (Givens and Marley, 2008; Menziess, 2011; Berri *et al*., 2002). Serological analysis of serum might be useful for demonstrating evidence of exposure and estimating their role in sheep and goat abortion. The result of the present serological investigation indicated that all four infectious causes of abortion are widely distributed across three agro-ecologies and production systems might play an important role in sheep and goat abortion. This study found a higher infection rate of *T. gondii* than other investigated abortion-causing agents. This might be related to the availability of favorable conditions for the maintenance and spread of this agent across the agroecology and production systems. The presence of both definitive hosts of *T. gondii* (cat) and intermediate hosts (rodents) in the area may influence the likelihood of contamination of feed, water, or pasture, which increased the risk for exposure of livestock to the parasite (Stelzer *et al*. 2019).

Our study found that exposure to *Brucella* spp. significantly increases the risk of abortion in sheep and goats, which is not surprising (Poester *et al*., 2013). There may be a high level of abortion in the “abortion storm” in the newly infected flocks (Ali *et al*., 2019). Nevertheless, it did not find any significant association between the occurrence of abortion and infection of *C. burnetii*, *C. abortus,* and *T. gondii*. This result agrees with the report from Gebremedhin *et al*. (2013), Gebretensay *et al*., (2019), and Tesfaye *et al*., (2020) who reported that evidence of *C. burnetii*, *C. abortus*, and *T. gondii* infection did not associate with flock level abortion. However, many studies have demonstrated the role of *C. burnetti*, *C. abortus,* and *T. gondii* in sheep and goat abortion (Givens and Marley, 2008; Hazlett *et al*., 2013). The absence of the association between those pathogens with sheep and goat abortion in this study might be related to maternal serum samples may yield positive results without the presence of abortion since the development of immunity within the flock prevents subsequent abortions (Givens and Marley, 2008; Menzies, 2011, 2012; Borel *et al*., 2014). Moreover, the presence of at least one of the four infectious agents under investigation in the flock could significantly increase both the flock level of abortion prevalence and the number of abortions in the flock. This indicated the involvement of multiple infectious agents in sheep and goat abortion which put cumulative effects on small ruminant abortion in different agroecology and production systems of Ethiopia. In agreement with the present findings, Bisias *et al*. (2010), Mahboub *et al*. (2013), and Benkirane *et al*. (2015) reported the important role of multiple pathogens as causal agents of abortion in sheep and goat flocks. The results from the household survey also highlighted infectious diseases were the highest priority problem perceived and a major concern for the producers as potential causes of sheep and goat abortion. This might correspond to an abortion incidence in June in lowland pastoral agroecology and production system in which grazing pasture is a relatively good but still higher proportion of abortion in this agroecology and production system.

4.5 Conclusion

The role of management, agroecological and infectious disease factors on sheep and goat abortion were obvious in this study and our findings highlight the multifactorial nature of the problem. The findings also emphasize the potential for substantial improvement in reproductive loss from abortion by improving management and health practices that fit the respective agroecological zones. This requires integrated approaches that improve the nutritional state of pregnant dams through targeted supplementary feeding, abortion management through appropriate biosecurity practices, and vaccination programs for major infectious causes of abortion and herd health management through better veterinary services. To make sure interventions are sustainable and can be scaled, recognizing the farmer/pastoralist indigenous knowledge and participating them in the process as partners, creating an enabling environment to engage private sectors as service providers, building strong partnerships with key stakeholders, and integrating other productivity improvement technologies are important strategies.

4.6 Ethics Statement

This study was approved by the Addis Ababa University, College of Veterinary Medicine and Agriculture Animal Research Ethics Review Committee (Certificate Ref. No: VM/ERC/05/08/11/2018). Before conducting the research, farmers were informed about the purpose of the study, and consent was sought from the household representative. Written informed consent for participation was not required for this study in accordance with the national legislation and institutional requirements.

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Chapter 5

Seroprevalences of multi-pathogen zoonotic abortion-causing agents in small ruminant and associated risk factors in three production system in Ethiopia

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Abstract

A cross-sectional study design was used to estimate the seroprevalence of abortion-causing pathogens and associated risk in sheep and goat in three production systems of Ethiopia between July 2018 to February 2019. A total of 1,402 serum samples were collected and tested for the presence of antibodies for four abortion-causing pathogens (*C. burnetii, Brucella spp., C. abortus,* and *T. gondii)* from five districts which represent three agroecologies in Ethiopia. From the total 1,402 serum samples tested, 231(16.48 %), 95 (6.78%), 124 (8.84%), and 137(11.42%) of them were seropositive for *C. burnetii, Brucella* spp*., C. abortus,* and *T. gondii,* respectively. The results of final multilevel mixed-effects logistic regression analysis showed that production system (OR=0.02; 95% CI=0.00%-0.21%; P=0.001) and measure taken on frequently aborted dams (OR=0.65; 95% CI=0.41%-1.04%, p=0.075) were important predictors of *C. burnetii* infection. Agroecology and production system, housing type for the night and birth product disposal practices significantly affect odds of *Brucella* spp infection. The presence of the dog in the household and housing type for the night identified as potential risk factors C. *abortus* infection. Moreover, the seroprevalence of *T. gondii* was significantly affected by age of the animals, flock mix, and grazing pastureland ownership. This study found co-infections of abortion pathogens where *C. abortus* (86.84%) showed the highest level followed by *Brucella* spp. (78.34 %) and *C. burnetii* (72.72%). Future studies, which aimed at identifying and characterization several possible abortion pathogens and their public health and socioeconomic impact should be done.

Keywords: Abortion; Co-infection; Goat; Multi-pathogens; Risk factors, Sheep

5.1 Introduction

Abortions in sheep and goat significantly impact food security and livelihoods of rural smallholder Ethiopian farmers as it decreases the potential number of replacement stocks for the flock and milk production and increase the number of unproductive females being maintained for long periods in the flock (Ali *et al*., 2019).

Abortion is usually a complex and multifactorial phenomenon where both non-infectious (nutritional, environmental, physical, toxic, and chemical) and infectious agents (viral, bacterial, fungal, and protozoal) can play a role (Díaz-Cao *et al*., 2018; Hazlett *et al*., 2013; Silanikove, 2000) C*oxiella burnetii (C. burnetii)*, *Chlamydia abortus (C. abortus),* and *Brucella* spp., *Leptospira* spp., *Campylobacter* spp., *Listeria* spp. and *Toxoplasma gondii (T.gondii)* are identified as most common of infectious causes of sheep and goats abortion. All of those pathogens are also zoonotic which can affect the health of the public besides their economic impact (Ganter, 2015; Lejeune & Kersting, 2010). Various reports showed that *C. burnetii, C. abortus, Brucella* spp. and *T. gondii* are prevalent and widely spread in all livestock production systems in Ethiopia (Gumi *et al*., 2013; De Vries *et al*., 2014; Gebremedhin and Tadesse, 2015; Tadesse, 2016; Tulu *et al*., 2018; Gebretensay *et al*., 2019; Thomas *et al*., 2019; Tesfaye *et al*., 2020) and remain an important public health threat to livestock producers in Ethiopia (WHO, 2009; Gebreyes *et al*., 2014).

Aborting animals shed large quantities of infectious agents through abortion foetus, placenta, uterine fluids, and vaginal discharges (Givens & Marley, 2008, Menzies, 2011). In addition, those pathogens can shade at the time of birth of healthy offspring from asymptomatic dams (Holler, 2012, Berri *et al*., 2002). Farmers get infected whilst assisting with the animal delivery and exposure to the contaminated environment from birth products (Klous *et al*., 2016). These need carefully designed, empirical one health research to untangle the complexity and enhance the development of appropriate control strategy to decrease the economic and public health impact of abortion-causing agents. To this end, it is important to determine the infection status of zoonotic abortion-causing agents targeting multiple etiological agents and associated risk factors across different production systems, which can initiate further studies. Therefore, the objectives of this study were to estimate the seroprevalence of multi-pathogen zoonotic abortion-causing agents in small ruminants and associated risk factors in three production systems in Ethiopia.

5.2 Material and Methods

5.2.1 Study design and areas

A cross-sectional study design was used to estimate the seroprevalence of abortion-causing pathogens and associated risks in sheep and goats in three production systems of Ethiopia between July 2018 to February 2019. Two districts from lowland mixed crop-livestock production system, two districts from lowland pastoral production system, and one district from highland mixed crop-livestock production system were selected from Amhara, Oromia, and Southern Nation, Nationality and People (SNNP) (Fig.1) regions, respectively. The sites were selected based on agroecology and production systems, their potential for sheep and/or goat production, accessibility, and the importance of sheep and goats to household livelihoods (Haile *et al*., 2019). In Ethiopia, the livestock production system was categorized based on the degree of integration with crop production, level of input, agro-ecology, relation to land and type of commodity to be produced, and mobility (Gizaw *et al*., 2010).

Abergelle and Zequala districts in the Wagihimira zone of the northern part of the Amhara region were selected to represent the lowland mixed crop-livestock production system. Sazba and Belteharf villages from Abergele and Bilaqu and Tsitsika villages from Zequala were selected from this production system. The altitude of these study villages range 1348 to 1486 meters above sea level (masl) and received mean annual rainfall ranged 647 and 732 mm. The annual mean temperature varies from 22 to over 24°C. This production system is dominated by livestock production but practiced in proximity to and perhaps functional association with cropping farming.

The productivity of crop farming is low in the area due to the shortage of rain. The livestock production activities are dominated by goats. The major feed resources for livestock in this production system are communal natural pastures, crop residues, crop stubbles, hay, browse plants, and weeds.

Yabello and Elwaya districts in the Borena zone from the southern part of the Oromia region were selected to represent the lowland pastoral production system. Derito and Dida-Yabello villages from Yabello and Adegalchet and Chari villages from Elwaya were selected from this production system. The altitude of these study villages ranges from 1181 to 1588 masl and received mean annual rainfall ranged 493 and 625 mm. The annual mean temperature varies from 20 to over 22°C. This production system is characterized by sparsely populated pastoral rangelands, where the subsistence of pastoralists is mainly based on livestock and livestock products. Livestock husbandry in this system is dominated by goats, cattle, sheep, and camels. Pastoralists in this production system take advantage of the characteristic instability of rangeland environments through strategic mobility and fencing of communal land.

Doyogana district in Kembata Tembaro zone from the SNNP region represented a highland mixed crop-livestock production system. Ancha Sadicho, Hawara Arara, and Gomora Gawada villages were selected from the district. The altitude of Doyogana is 2616 masl and received a mean annual rainfall of 1,275 mm and an annual mean temperature of 15°C. A mixed crop-livestock production system drives the predominant economic activities in this production system. Livestock husbandry and rain-fed cropping are closely interlinked to gain complementary benefits from an optimum mixture of crop and livestock and spreading income and risks over both crop and livestock production. Natural pastures, crop residues, and crop stubbles are used as major livestock feed.

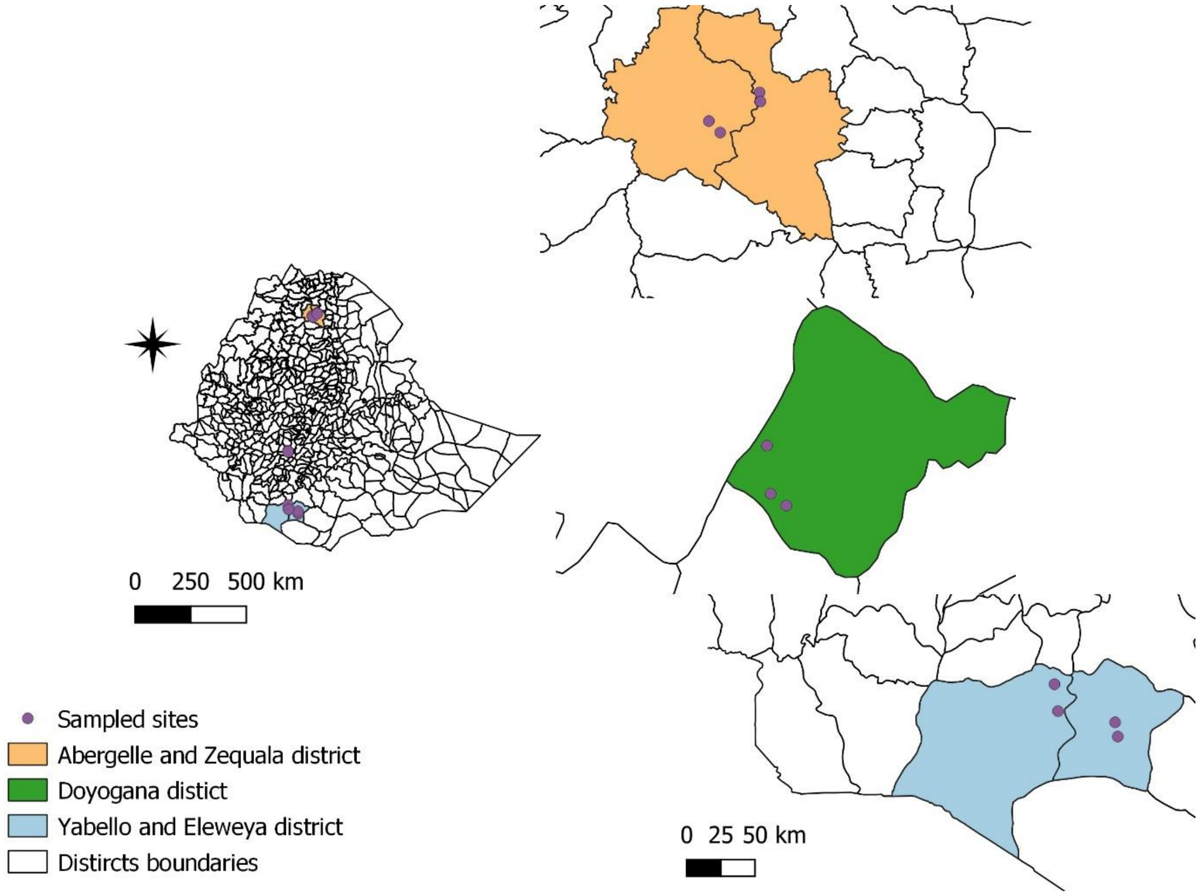

Figure 5.1 Map of study locations

5.2.2 Target and study population

The target population comprised of livestock farmers and their sheep and goat populations kept under extensive management system in three production systems of Ethiopia. Animals included in the study were selected from sheep and goat populations who were involved in the household survey. In addition, sheep and goats kept by the research center for breeding purpose were also included in the study. There are no vaccination strategies directed against targeted pathogens in Ethiopia. Therefore, none of the studied animals were vaccinated against any of the diseases tested.

5.2.3 **Sample size determination and sampling**

Sample size to determine the seroprevalence of *C. burnetii*, *C. abortus*, *Brucella spp*., and *T. gondii* in sheep and goat was calculated using web-based Epidemiological Calculators (AusVet, 2017) by setting 50% of the expected individual prevalence of each pathogen at 95 % a confidence level with 5% precision. Accordingly, a minimum of 384 animals were required for the study. However, considering that animals within the same households tend to have more similar disease outcomes due to their close contact and similar management level, the total sample size was adjusted for clustering at the household level using the formula described by Dohoo *et al*. (2009):

$$n' = n(1 + p(m - 1))$$

where

n' is the new sample size,

n is the original sample size estimate,

p is the intra-cluster correlation coefficient

m is the number of ewes /does sampled per flock.

The intra-cluster correlation coefficient *(P)* for the majority of infectious diseases is usually between 0.05-0.2 (Otte and Gumm, 1997). Accordingly, a value of 0.2 was taken for the initial sample size calculation. We planned to sample an average of 8 animals (5 goats and 3 sheep) from each household. Hence, a minimum of 922 small ruminants was required for the study. However, the sample size was increased to 1,226 to allow for poor quality samples, drop-outs and to increase precision. Blood samples were collected from a total of 154 households selected randomly selected households. In addition to these, 178 blood samples were collected from sheep and goats kept by agricultural research centers. Therefore, a total of 1,402 blood samples were collected and tested for the presence of antibodies for four abortion-causing pathogens.

5.2.4 Data collection

Data on animal management-related factors included farming system, flock type, flock size and presence of other livestock in the households), grazing, pastureland ownership, housing type, breeding buck/ram ownership, and biosecurity practices (routine manure cleaning, birth products disposal practice, action on frequently aborting dam, dog and cat access to the flock) were collected through an interview during blood samples collection.

5.2.5 Serum sample collection and processing

About 6-8ml blood was collected from the jugular vein into 10ml sterile plain vacutainer tubes. Individual animal biodata was gathered during sample collection. The tubes were then labeled with a unique identification number and kept protected from direct sunlight. The samples were placed in the slant position until the blood was clotted, and sera were separated. The sera were separated from clotted blood after centrifugation at 1500 g for 10 min at Yabello Pastoral and Dryland Agriculture Research Center, Sekota Dryland Agriculture Research Center, and Wolayita Sodo Regional Veterinary Laboratory. The serum was transferred into a sterile cryovial tube bearing the identification number and transported to the National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia, for laboratory analysis. The samples were transported to the laboratory at +4 using a portable fridge, plugged into the car, and then stored at -20°C until analyzed.

5.2.6 Laboratory analyses

Commercial enzyme-linked immunosorbent assays (ELISA) were used to detect antibodies against *C. burnetii, Brucella spp., C. abortus,* and *T. gondii* at NAHDIC. For *C. burnetti* the Antibody Test Kit, (IDEXX® Switzerland AG, CH-3097 Liebefeld-Bern Switzerland), for Chlamydia, the *C. abortus* Antibody Test Kit (IDEXX® Switzerland AG, 3097 Liebefeld-Bern Switzerland), for

Brucella spp, Svanovir TM Brucella-Ab c-ELISA test kits (Svanova Biotech, Uppsala, Sweden) and for Toxoplasma the *T. gondii* Antibody Test Kit, (IDEXX® Switzerland AG, 3097 Liebefeld-Bern Switzerland) were used. The test procedure, computation of the sample to positive ratios, and final interpretation of the results were performed following the protocols provided by the respective kit manufacturers. Briefly, sera samples, negative and positive controls were diluted at 1:400 using wash solution. One hundred µL of pre-diluted negative and positive control and samples were added into a microtiter plate and incubated for one hour at 37°C. The plate was then washed 3 times and 100µL of the conjugate was added to each well. The plate was incubated at +37°C for 1 hour after the microplate was sealed using plate covers to avoid any evaporation. The plates were again washed 3 times. Finally, 100µL of the substrate was added to each well and incubated at 18–26°C for 15 min. Then 100µL of stop solution was added to each well and the result was read at a wavelength of 450nm. The OD of the positive control ($PC\bar{x}$) and the OD of the samples (sample A_{450}) are corrected by subtracting the OD of the negative control ($NC\bar{x}$). Sample to the positive ratio (S/P %) was computed as $\frac{100 \times sample\ A450 - NC\bar{x}}{PC\bar{x} - NC\bar{x}}$. An animal was considered to be infected when the serum presented an S/P% ≥30 for *Brucella* spp., ≥40 for *C. burnetii,* and *C. abortus,* and ≥ 50% for *T. gondii.*

5.2.7 Data management and analyses

The data cleaning and statistical analyses were conducted in STATA 15.1 (Stata SE/15.1, Stata Corp., College Station, TX, USA). The outcome of interest was the proportion of seropositive animals of each studied pathogen in sheep and goat flocks. Cross tabulations and frequency tables were used to describe proportions by all variables under consideration.

Unconditional association between potential risk factors and outcomes of interest was tested in univariable models. The maximum likelihood method was used to estimate parameters describing the relationship between predictor variables and outcomes of interest. Odds ratio (OR) was used to measure the effect of various predictors on the outcomes of interest. A multilevel mixed-effects logistic regression model was fitted to account for the clustering of the animal within villages and households. Variables showing an unconditional association with the outcome were selected for multivariable analysis, using $p < 0.2$ as the criterion for inclusion. The likelihood ratio test was used to evaluate the significance of variables. $P \leq 0.05$ was considered significant.

5.3 Results

5.3.1 Characteristics of examined animals

A total of 1,402 serum samples (980 goats and 422 sheep) were collected from 133 sheep and 90 goat flock from154 smallholder farmers and 3 sheep and 2 goat flocks from 3 government research center breeding farms. All examined animals were kept under traditional extensive management systems and therefore fully dependent on grazing land, with overall limited external input. The examined animals were reared in three production systems: namely lowland MCL (560), lowland pastoral (608), and highland MCL (234). The median size of goats and sheep flock was 13 and 5 animals, respectively. The majority (1,176/1402, 83.88%) of the sampled animals were female which has a median parity of 7 (min =1, max=12). Of the total female animals, 1,177(19.37%) have a history of abortion, and 37 (16.74%) of them aborted two or more times.

5.3.2 Seroprevalence of multi-pathogens

From the total 1,402 serum samples tested, 231(16.48 %), 95 (6.78%), 124 (8.84%), and 137(11.42%) of them were seropositive for *C. burnetii*, *Brucella* spp., *C. abortus*, and *T. gondii*, respectively. The serological results disaggregated by the animal species production system are presented in Table 5.1. The highest seroprevalence was found for *C. burnetii* with 19.7% (95% CI=17.3-22.3) in caprine species, followed by *T. gondii* with 16.8% (95% CI=12.7-21.6) in ovine species. Whereas *Brucella* spp. showed the lowest seroprevalence was among caprine species (4.0%, 95% CI: 2.8-5.4).

Table 5.1 Seroprevalence of multi-pathogen in sheep and goat managed under three production systems in Ethiopia.

Tested pathogens	**Animal species**	**Lowland MCL, %(CI)**	**Lowland pastoral, % (CI)**	**highland MCL, % (CI)**	**Overall, %(CI)**	**p-value**
C. burnetti	Ovine (422)	26.7 (14.6-41.9	16.8 (11.1-23.9)	0.9 (0.1-3.1)	9.0 (6.5-12.2)	0.000
	Caprine (980)	19.6 (16.3-23.3)	19.8 (16.3-23.7)	-	19.7 (17.3-22.3)	0.946
Brucella spp.	Ovine (422)	0.0 (0.0)	4.9 (6.8-30.7)	20.9 (15.9-26.7)	13.3 (10.2-16.9)	0.000
	Caprine (980)	1.9 (0.9-3.5)	6.2 (4.2-8.8)	-	4.0 (2.8-5.4)	0.001
C. abortus	Ovine (422)	2.2 (0.1-11.8)	7.7 (3.9-13.3)	13.7 (95.5-18.8)	10.4 (7.7-13.7)	0.030
	Caprine (980)	10.5 (8.0-13.5)	5.6 (3.7-8.1)	-	8.2 (10.1-6.5)	0.005
T. gondii	Ovine (292)	6.7 (1.4-18.3)	15.4 (9.9-22.4)	23.1 (15.4-32.4)	16.8 (21.6-12.7)	0.040
	Caprine (908)	5.6 (3.7-8.2)	13.6 (10.6-17.0)	-	9.7 (11.8-7.9)	0.000

5.3.3 Risk factors for multi-pathogen exposures

Variables related to the individual animal tested, production system, self-reported small ruminant management, and biosecurity practices were included in univariable analysis. Variable with $p<0.25$ in univariable logistic regression analyses were retained for final multilevel mixed-effects logistic as potential predictors for seroprevalence of tested pathogens. The results of the final multilevel mixed-effects logistic regression analysis used village and household as random effects are presented in Table 5.2. The result indicated that production systems and measures taken on frequently aborted dams were important predictors of *C. burnetii* infection. Animals in lowland mixed crop-livestock agroecology and production system and households keeping frequently aborted animals in their flocks have 4.16 (OR=0.02; 95%CI=0.00-0.21; p=0.001) and 1.72 (OR=0.65; 95% CI=0.41-1.04, p=0.075) times higher odds of infection than the animals in highland MCL production system and in households who sell frequently aborted dams, respectively. However, the odds *Brucella* spp. infection was significantly higher in small ruminants kept in highland MCL (OR=7.29; 95% CI =2.51-21.17; p=0.00) and lowland pastoral (OR=5.45; 95% CI=2.10-14.19; p=0.001) agroecology and production system than in lowland MCL agroecology and production system. Moreover, animals spending the night in traditional sheep houses had 2.30 (OR= 2.30, 95% CI =1.02-5.19; p=0.046) times higher odds of *Brucella* spp. infection than those spending the night in none shelter paddock. Unexpectedly, the odds of *Brucella* spp. infection was significantly higher by 4.76 (OR=0.21; 95% CI=0.08-0.59; p=0.003) times in animals owned by households who bury/burn birth products than those fed to dogs. Regarding *C. abortus* infection, the presence of the dog in the household decreased the odds of infection by 1.72 times (OR=0.58; 95% CI =0.35-0.98; p=0.04). Nevertheless, the odds of infection were significantly higher (OR=1.70, 95% CI=1.05-2.76; p=0.032) in animals who spent the night in the traditional sheltered house than those who spent the night in none shelter paddock. The seroprevalence of *T. gondii* was significantly affected by age of the animals, flock mix, and grazing pastureland ownership. The odds of infection were higher in adult animal (OR=3.5 ., 95% CI=1.27-9.62; p= 0.015), in animal belongs to sheep only flocks (OR=13.08-0.007; 95% CI, 2.05-83.47; p= 0.007) and animal grazed in both communal and private pastureland (OR= 0.36; 95% CI= 0.14-0.94; p=0.038).

Table 5.2 Multivariable mixed effect logistic regression analysis for risk factor associated with *C. burnetii*, *Brucella* spp., *C. abortus* and *T. gondii* in sheep and goat in three agroecologies and production systems

Predicators	**Categories**	**Odd Ratio**	**95%CI**	**P-value**
C. burnetii				
Agro-ecology and production system	Lowland MCL	1		
	Lowland Pastoral	0.65	0.41-1.04	0.075
	Highland MCL	0.02	0.00-0.21	0.001
Action on frequently aborting dams	keep in the flock	1		
	sell	0.58	0.36-0.95	0.029
	slaughter	0.70	0.26-1.88	0.475
Brucella spp.				
Agro-ecology and production system	Lowland MCL	1		
	Lowland Pastoral	5.45	2.10-14.19	0.001
	Highland MCL	7.29	2.51-21.17	0
Housing /shelter at night	none shelter paddock	1		
	traditional house	2.30	1.02-5.19	0.046
Birth products disposal practice	bury/burn			
	feed to dog	0.21	0.08-0.59	0.003
	disposed to environment	0.48	0.21-1.09	0.081
C. abortus				
Housing /shelter at night	none shelter paddock			
	traditional house	1.70	1.05-2.76	0.032
Dog access to the flock	not at all	1		
	yes, neighbor dog	0.95	0.49-1.84	0.88
	yes, own dog	0.58	0.35-0.98	0.04
T. gondii				
Animal age	young	1		

	adult	3.5	1.27-9.62	0.015
Small ruminant flock mix	goat only	1		
	sheep only	13.08	2.05-83.47	0.007
	mixed	6.94	1.38-34.99	0.019
Grazing land	privately owned only	1		
	communal only	0.62	0.24-1.59	0.32
	Both	0.36	0.14-0.94	0.038

5.3. 4 Evidence of Co-infections

The proportion of co-infection with different pathogens among small ruminant flocks is demonstrated in Table 5.3. The most common co-infections were *Brucella* spp-*T. gondii* (positive rate: 26/61, 59.09%) and *C. abortus*-*C. burnetii* (positive rate: 41/77, 53.25%) whereas the least common co-infections were *C. burnetii*-*Brucella* spp. (positive rate: 27/101, 26.73%) and *T. gondii*-*Brucella* spp. (positive rate: 26/94, 27.66%). Overall, accumulative co-infections for *C. abortus* (86.84%) showed the highest level followed by *Brucella* spp. (78.34 %) and *C. burnetii* (72.72%) (Fig.5.2). Two co-infections with *C. abortus* had the highest positive rate (52.63%), followed by three co-infections with *Brucella* spp. (31.67%). Similarly, the highest level accumulative co-infection was recorded for *C. abortus* (30.65%) at the animal level followed by *Brucella* spp. (29.47%) and *T. gondii* (29.2%) (Fig. 5.3).

Table 5.3 Evidence of co-infection by each positive pathogen among sheep and goat flock in Ethiopia

Pathogen (No. of positive samples)	***C. burnetii***	***Brucella* spp**	***C. abortus***	***T. gondii***
C. burnetii (101)	N/A	27 (26.73 %)	41 (40.59 %)	47 (48.96 %)
Brucella spp (61)	27 (44.26%)	N/A	30 (49.18%)	26 (59.09%)
C. abortus (77)	41 (53.25%)	26 (38.96%))	N/A	31 (50%)
T. gondii (94)	47 (50%)	26 (27.66%)	31 (32.98%)	N/A

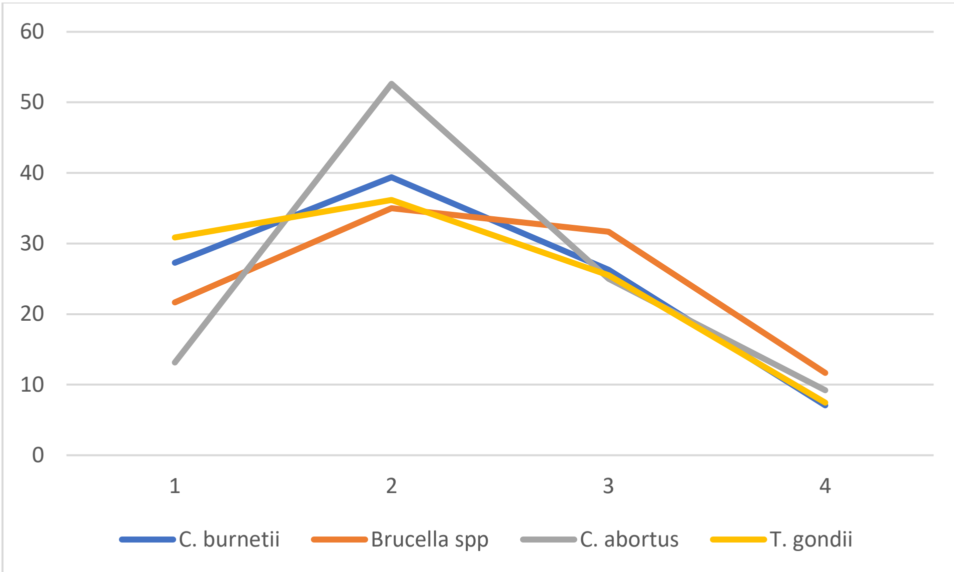

Figure 5.2 Co-infection trend of four tested pathogens among sheep and goat flocks from three agro-ecology and production systems

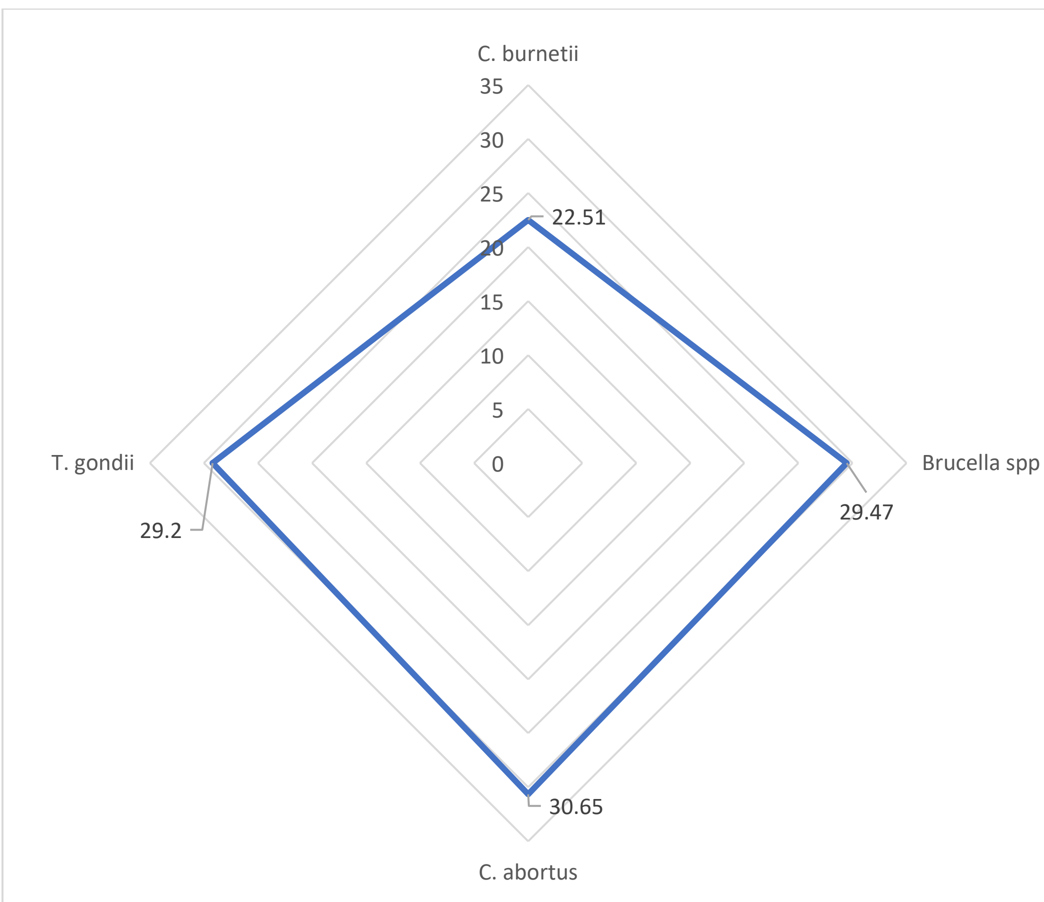

Figure 5.3 Accumulative co-infection of four tested pathogens among sheep and goats from three agro-ecology and production systems

5.3. 5 Association of history of abortion with seropositivity

Of 228 female animals with history of abortion 49 (21.49%; 95% CI=16.34- 27.39), 14 (6.14%; 95% CI =3.39-10.08), 18 (7.89%; 95% CI =04.745 12.19) and 22 (10.23% ; 95% CI= 6.14-14.24) of them were positive for *C. burnetii, Brucellas* spp*., C. abortus* and *T. gondii* antibodies (Fig. 5.4). Moreover, exposure to multiple infections was detected from 15 (6.58%; 95% CI=3.72-10.61) dam with a history of abortion. The history of abortion was not statistically associated with the seroprevalence of any of the tested pathogens.

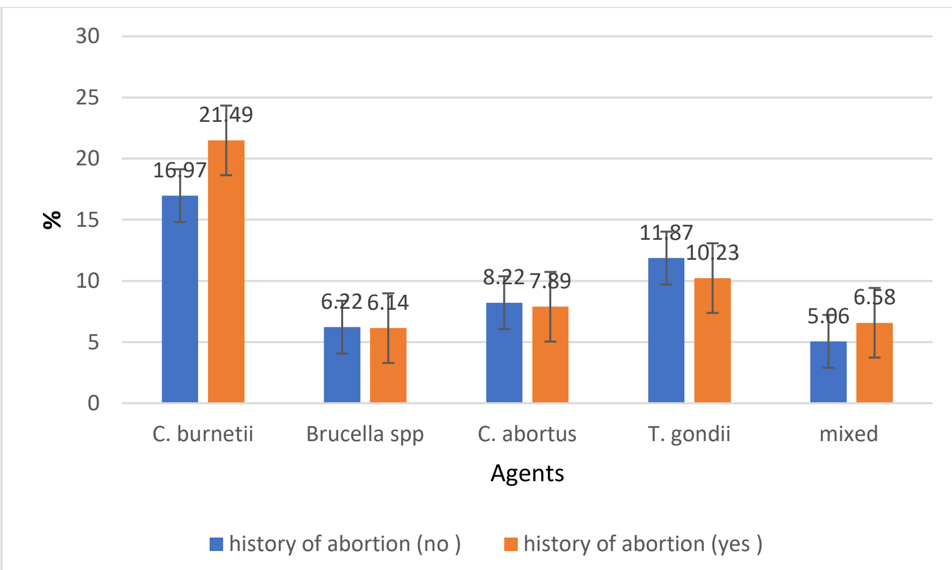

Figure 5.4 Seroprevalence of four tested pathogens among aborted and non-aborted sheep and goats from three agro-ecologies and production systems

5.4 Discussion

This study identified the exposure of four zoonotic abortion-causing agents in small ruminants across three production systems and associated risk factors. Four major abortion-causing agents: *C. burnetii, Brucellas* spp*. C. abortus* and *T. gondii* were targeted for this study. This study found a 16.48% overall prevalence of anti-*C. burnetii* antibody which agrees with the report from southern Italy (Villari *et al*., 2018) and Kenya (Muema *et al*., 2017). However, these finding is higher than those reported from Turkey (Karagul *et al*., 2019), Greece (Pape *et al*., 2009), Brazil (de Souza *et al*., 2018) and Egypt (Klemmer *et al*., 2018) and Cote d'Ivoire (Kanouté *et al*., 2017). On contrary, it is lower than those reported in different agroecology and production systems of Ethiopia (Gebretensay *et al*., 2019; Gumi, 2013; Ibrahim *et al*., 2020.; Tesfaye *et al*., 2020) and elsewhere in the world such as Iran (Ezatkhah *et al*., 2015), Jordan (Obaidat *et al*., 2019), Gambia (Klaasen *et al*., 2014) and Saudi Arabia (Jarelnabi *et al*., 2018).

Our study found a higher prevalence of *C. burnetii* infection in goats than sheep ($P<0.05$). Similar reports from different countries also showed a higher infection rate in goats than sheep (Anastácio *et al*., 2013; Filioussis *et al*., 2017; Klaasen *et al*., 2014; Muema *et al*., 2017). This might be due genetic susceptibility of goat to *C. burnetii* than sheep (Filioussis *et al*., 2017; Klaasen *et al*., 2014) Moreover, a higher seroprevalence of *C. burnetii* infection was recorded in lowland than highland agroecology. In the lowland parts of Ethiopia, the population of the goat is higher than from the highland of Ethiopia, which might be attributed to this difference. Moreover, the dry weather condition in the lowland of Ethiopia due to a shortage of rainfall might facilitate the air-borne transmission of Q fever in animals over long distances (Jones *et al*., 2006; Yohannes, 2018). *C. burnetii* can survive in harsh conditions and travel several km downwind under dry and dusty conditions caused human and animal outbreaks (Clark & Magalhães, 2018) (This study found keeping frequently aborted animal in the household increase the likelihood of exposure by 1.72 times than those sold out). This might be related to long time shading of the agent through feces, milk, and urine after abortion (Canevari *et al*., 2018; Esmaeili *et al*., 2019; Klaasen *et al*., 2014; Mohabati Mobarez *et al*., 2020).

From the result of this study, 6.78% of sheep and goat showed evidence of *Brucella* spp. infection which agrees with the seroprevalence of *Brucella* infection in northwestern Ethiopia (Ferede *et al*., 2011), Northeastern (Ashenafi *et al*., 2007) Southern (Dabassa *et al*., 2013), and Southwestern parts of Ethiopia (Tulu *et al*., 2020). It also agrees with the report from Cameron (Awah-Ndukum *et al*., 2018) and the Kingdom of Saudi Arabia (Shabana & Krimly, 2020). However, the findings from this are lower than the report from Nigeria (Salihu *et al*., 2010), Egypt (Selim *et al*., 2018), Ethiopia (Berhe *et al*., 2007; Edao *et al*., 2020; Teshome *et al*., 2018). Nevertheless, it is higher than the report from different parts of Ethiopia (Ashenafi *et al*., 2007; Megersa *et al*., 2012; Sintayehu *et al*., 2015). The result of risk factor analyses indicated that agro-ecology and production system, housing for overnight, and birth products disposal practice were the most important risk factors for *Brucella* spp. seropositivity The risk of infection significantly increased in small ruminants raised in lowland pastoral (OR=5.45) and highland MCL (OR=7.29). This might be related to the mixing of different livestock species and variation in management and farm biosecurity systems. Keeping small ruminant in the traditional house during the night increase the odd of *Brucella* spp. by 2.3 times. This might be related to the high level of confinement within limited space in a traditional house and poor ventilation system in traditional sheep houses (Abera *et al*., 2019).

Regarding Chlamydial infection, the overall prevalence (8.84%) recorded in the current study agrees with the report from in northern Namibia (Samkange *et al*., 2010), Syria (Roukbi *et al*., 2016), Algeria (Karim *et al*., 2017) China (Hu *et al*., 2018) and Slovak Republic (Cisláková & Halanova, 2008). However, it is lower than those reported in the highland sheep of Ethiopia (Gebretensay *et al*., 2019), Jordan (Al-Qudah *et al*., 2004), and in the North-West of Algeria (Karim *et al*., 2017). The result obtained from this study showed a higher seroprevalence of chlamydial infection rate than finding from the Kingdom of Saudi Arabia (Shabana & Krimly, 2020) and Korea (Oh *et al*., 2017). Risk factors analyses result showed that housing at night and dog access to the flock significantly affect the *Chlamydia* spp. infection rate in a small ruminant population. Confining small ruminants in the sheltered traditional house increase the odds of infection by 1.70 (OR=1.7, 95% CI: 1.05-2.76, P= 0.032). This might be related to confining animals with limited space and ventilation, which can compromise the health and welfare of the animal. Poor ventilation and limited space per animal facilitate the spread of infectious agents among the animals (Al-Khaza'leh *et al*., 2020). A higher risk of *Chlamydia* spp. infection of animals in those having a dog in their household might be related to the possible role of the dog in preventing the spread of the agent by eating the abortion material, which can otherwise stay a long time in the environment. However, the role of the dog in preventing *Chlamydia* spp. has not been described in the body of scientific literature.

The overall seroprevalence (12%) of *Toxoplasma* infection in sheep and goats reported in this study agree with the report from Central Iran (Rasti *et al*., 2018) and Myanmar (Bawm *et al*., 2016). However, it is higher in Egypt (Rouatbi *et al*., 2019). In contrary, the seroprevalence reported in this study is lower than the prevalence reported from different parts of Ethiopia (Gebremedhin *et al*., 2013; Gebretensay *et al*., 2019; Tilahun *et al*., 2018), Northern Greece (Tzanidakis *et al*., 2012), Morocco (Rouatbi *et al*., 2019), Pakistan (Khan *et al*., 2017), Iran (Rasti *et al*., 2018) and Sudan (Atail *et al*., 2017). From mixed effect models, age of the animal, mixing of small ruminants with other animals, and grazing practice were the most important risk factors for *T. gondii* infection. The presence of significantly higher *T. gondii* infection adult age group in small ruminant is in agreement with previous reports by the different scholars (Arwa Lachkhem & Wahiba Sakly, 2015; Gebremedhin *et al*., 2013). Tegegne *et al*. (2016) also found similar results in sheep and goats in Southwestern Ethiopia. The possible explanation is that as the animal age increases there is an increased risk of acquiring this infection through ingestion of oocysts from contaminated environments (Lindsay & Dubey, 2020; Pereira *et al*., 2021). It also suggests that the infection of *T. gondii* in small ruminants is predominantly postnatally acquired.

Interestingly, the risk of *T. gondii* infection was increased significantly when sheep and goats grazed on privately owned pasture than grazed on both communal and private-owned grazing pasture. As a matter of fact that many farmers were forced to keep their animals on privately owned pastureland due to the shrinkage of communal grazing areas as a result of the expansion of cropping and land grabbing for cultivation (Benin & Pender, 2001; Kebede *et al*., 2019). This resulted in frequent grazing of animals in the same plot for a long time leading to an increased chance of animal exposure to infectious agents discharged into the environment following abortion or lambing/kidding of infected dam. From the result of this study, herding sheep and goats together with other animal species significantly increased the odds of infection than herding goat alone or sheep and goat. Since toxoplasmosis shared among livestock species (Givens and Marley, 2008), keeping more animal species at the household level may increase animal density and chance of contact between animals, thus facilitating cross-transmission between livestock species which increase the chance of acquiring infection (Buxton *et al*., 2007; Gebretensay *et al*., 2019).

Our findings did not find a significant association with the history of abortion for the last one year with seropositivity for any of the tested pathogen. The possible justification for this is that positive serology for an animal might indicate previous exposure to a specific agent and it might not have the role in current abortion (Holler, 2012, Menzies, 2011).

The finding of this study demonstrated the evidence of multiple pathogens infection in small ruminants, which showed plenty of co-infections. This showed there is the involvement of multiple infectious agents, which can pose considerable risks on the naive population that can significantly increase the incidence of abortion in the flock. This study agrees with the findings of Bisias *et al*. (2009), Mahboub *et al*.(2013), and Benkirane *et al*. (2015) who reported the important role of multiple pathogens as causal agents of abortion in sheep and goat flocks. Infected animals shed large quantities of infectious agents during abortion through aborted fetus, placenta, uterine fluids, and vaginal discharges (Givens & Marley, 2008, Menzies, 2011) or shade asymptomatically those pathogens at the time of birthing of healthy offspring (Holler, 2012, Berri *et al.*, 2002). Therefore, those agents confer health risks to communities besides the economic impact imposed due to abortion (Pospischil *et al.*, 2002; Ganter, 2015; George's, 2012). Prevention and control strategy of small ruminant abortion targeting major causes of abortion in addition to management intervention is one of the most powerful approaches to reducing the impact of pathogens in small ruminant abortion. These also call for continued public education programs to raise the awareness of the communities towards the proper installation of personal protection measures and appropriate disposal of abortion materials to limit the spread of those pathogens.

5.5 Conclusion

Our study has demonstrated the evidence of four infectious zoonotic abortion-causing agents and their potential risk factors in small ruminants in three agroecologies and production systems. From the finding, those infectious diseases might have a role in the suboptimal reproductive performance of small ruminants from reproductive failure and pose a public health risk for the communities. Future studies aimed at identifying and characterization of several possible abortion causing pathogens and their public health and socioeconomic impacts should be done.

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Chapter 6

Knowledge, Attitude, and Practices to zoonotic disease risks from livestock birth products among smallholder communities in Ethiopia

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Abstract

Many causes of abortion in livestock are due to zoonotic pathogens that pose serious infection risks for humans. The study presented here aimed at understanding knowledge, attitudes, and practices (KAP) on zoonotic risks from livestock birth products among rural communities in Ethiopia. From July 2018 to February 2019, a cross-sectional study was conducted with 327 randomly selected farmers and pastoralists in five districts in three regions in Ethiopia. The structured questionnaire consisted of 48 items to evaluate knowledge (24), attitude (9), and prevention practices (15) related to zoonotic disease risks from livestock birth products. A unidimensional two-parameter logistic (2-PL) Item Response Theory (IRT) model was used for zoonotic disease risk KAP scale construction and evaluation. The 2-PL IRT model was fitted to determine the probability of a person appropriately respond an item with a provided zoonotic disease KAP level. We then examined differential item functioning (DIF) concerning five important covariates. The attitude subscale had the highest total mean score (37.3, ± 28.92 %) and the knowledge subscale had the lowest mean score (22.4, ± 33.6 %) among the three subscales. The mixed model regression analysis indicated that region was the only apparent factor explaining differences in zoonotic diseases knowledge, attitude, and practice total mean scores. The knowledge and attitude subscales had good internal consistency with a Cronbach's α at 0.83 and 0.81, respectively, whereas the practice subscale had lower internal consistency with 0.51. There was a positive association between responding to knowledge questions correctly and a positive attitude (r2= 0.44, $p<0.0001$) and self-reported good practice ($r^2 = 0.307$, $p<0.0001$). The differential item functioning test showed that 19 of 37 (51.35%) and 12 of 37 (32.43%) items of the retained KAPs survey items had non-uniform and uniform DIF linked to at least one covariate respectively and all the covariates were related with DIF in at least one item. This study found substantial knowledge gaps, a low level of the desired attitude, and high-risk behavioral practices regarding zoonotic diseases from livestock births products. Consequently, livestock keepers are likely to be exposed to pathogens and thus these practices are an important contributing factor for zoonotic disease infection in people.

Keyword: Livestock Birth products; Differential item functioning; Ethiopia; KAPs; IRT; Zoonotic diseases

6.1 Introduction

Livestock is important for the livelihoods of rural households in Ethiopia and contributes to food security and improves the income and wellbeing of farmers and pastoralist families (Randolph *et al*., 2007; Wodajo *et al*., 2020). Nevertheless, keeping livestock can also be a source of infection for humans (Klous *et al*., 2016). In rural parts of Ethiopia, almost all household members have daily direct contact with animals and are involved in different stages of the animal production cycle (Kinati *et al*., 2018). Therefore, they likely face daily exposure to zoonotic pathogens. Close interaction between livestock and humans also occurs due to the proximity of livestock to living accommodation or even shared housing during severe weather conditions (WHO, 2009). Furthermore, limited public and livestock health services in the areas and poor zoonotic disease prevention practices increased the risk of households' exposure to those agents and pose a significant public health threat to the farming community (LeJeune and Kersting, 2010).

Abortion in livestock can be caused by zoonotic pathogens and affects the individual animal and overall herd productivity (Narrod *et al*., 2012). In addition to a significant economic impact on the rural economy, many of the infectious agents that cause animal abortion have the potential to cause serious illnesses in people - in particular *Toxoplasma gondii*, *Brucella* spp, *Chlamydophila* spp., *Campylobacter* spp., *Salmonella* spp, *Listeria* spp., *Leptospira* spp. and *Coxiella burnetii* (the cause of Q-fever) (Givens and Marley, 2008; Ganter, 2015). Animal to human transmission mainly results from direct contact with infected materials such as uterine discharge, aborted foetus, and placenta. Human infection occurs while assisting with animal delivery and while caring for the newborn, or through dealing with abortion cases or exposure to a contaminated environment with abortion products (Menziess, 2011; Holler, 2012). Animal birth products from an infected dam carry a large number of these pathogens, which can be a source of infection for an entire household because of their close contact with livestock in unsanitary conditions (George's, 2012; Borel *et al*., 2014)

There is increasing evidence that zoonotic causes of animal abortion are prevalent and widely spread in all livestock production systems in Ethiopia (Gumi *et al*., 2013; De Vries *et al*., 2014; Gebremedhin and Tadesse, 2015; Tadesse, 2016; Tulu *et al*., 2018; Gebretensay *et al*., 2019; Thomas *et al*., 2019). However, the prevention and control of these zoonotic diseases remain largely neglected in most African countries. This is largely related to poor understanding of the problem, non-existence of appropriate diagnostic facilities and trained human power, and lack of suitable and long-lasting zoonotic disease prevention and control strategies (WHO, 2009; Gebreyes *et al*., 2014).

The absence of rigorous zoonotic disease prevention and control programs poses a high risk to vulnerable poor rural livestock producers and livestock products consumers along with the value chain. Lack of awareness, combined with poverty, means that risky behaviours related to animal management and abortion material handling and disposal persist (Gebreyes *et al*., 2014). Lack of knowledge implies the need for carefully designed, empirical research that considers the animal, environment, and human health domains in a One Health approach to untangle the complexity and enhance the development of practical health education guidelines and best prevention practices for the design of veterinary public health intervention..

A zoonotic diseases control plan largely depends on public health awareness that can help in lowering the risk of infections along with livestock production and processing value chain. Changing behavioural practices of high-risk groups through better public health education is critical to contain the spread of zoonotic infection from animal to the human population in resource-scarce settings (WHO, 2012; Gustafson *et al*., 2015). The evaluation and description of KAP among farmers and pastoralists concerning zoonotic diseases risk from livestock birth products can help in designing and implementing effective zoonotic disease prevention and control strategies in the livestock population and in developing and executing more efficient community-based health education for livestock producers. Therefore, this study aimed at understanding the knowledge, attitudes, and practices (KAP) on zoonotic risks from livestock birth products among three rural communities in Ethiopia.

6.2 Material and Methods

6.2.1. Study design and setting

A cross-sectional study design was used to collect information from July 2018 to February 2019 in five locations (districts) in three regional states in Ethiopia, namely, Amhara, Oromia, and Southern Nation, Nationality and People (SNNP) (Fig.6.1). The study sites were purposively selected to represent different agroecological conditions and production systems. Sites in the SNNP region represented the highland agro-ecology, whereas sites in Oromia and Amhara regions represented the lowland agro-ecology. Mixed crop-livestock production is the predominant economic activity in Ancha district in SNNP, and in Abergelle and Zequwala districts in the Amhara region. Livestock keeping as the predominant economic activity was the case in Yabello and Eleweya districts in the Oromia region. In each of the five districts, two *kebeles* (= smallest administrative unit in Ethiopia) were selected, of these one *kebele* was a site included in the research of the CGIAR research program (CRP) on Livestock and had seen previous livestock value chain interventions, and one *kebele* had not previously seen any livestock interventions. The CRP Livestock intervention *kebeles* had received animal health intervention such as vaccination and community-based internal parasite control program and training on herd health management (control of respiratory and reproductive diseases).

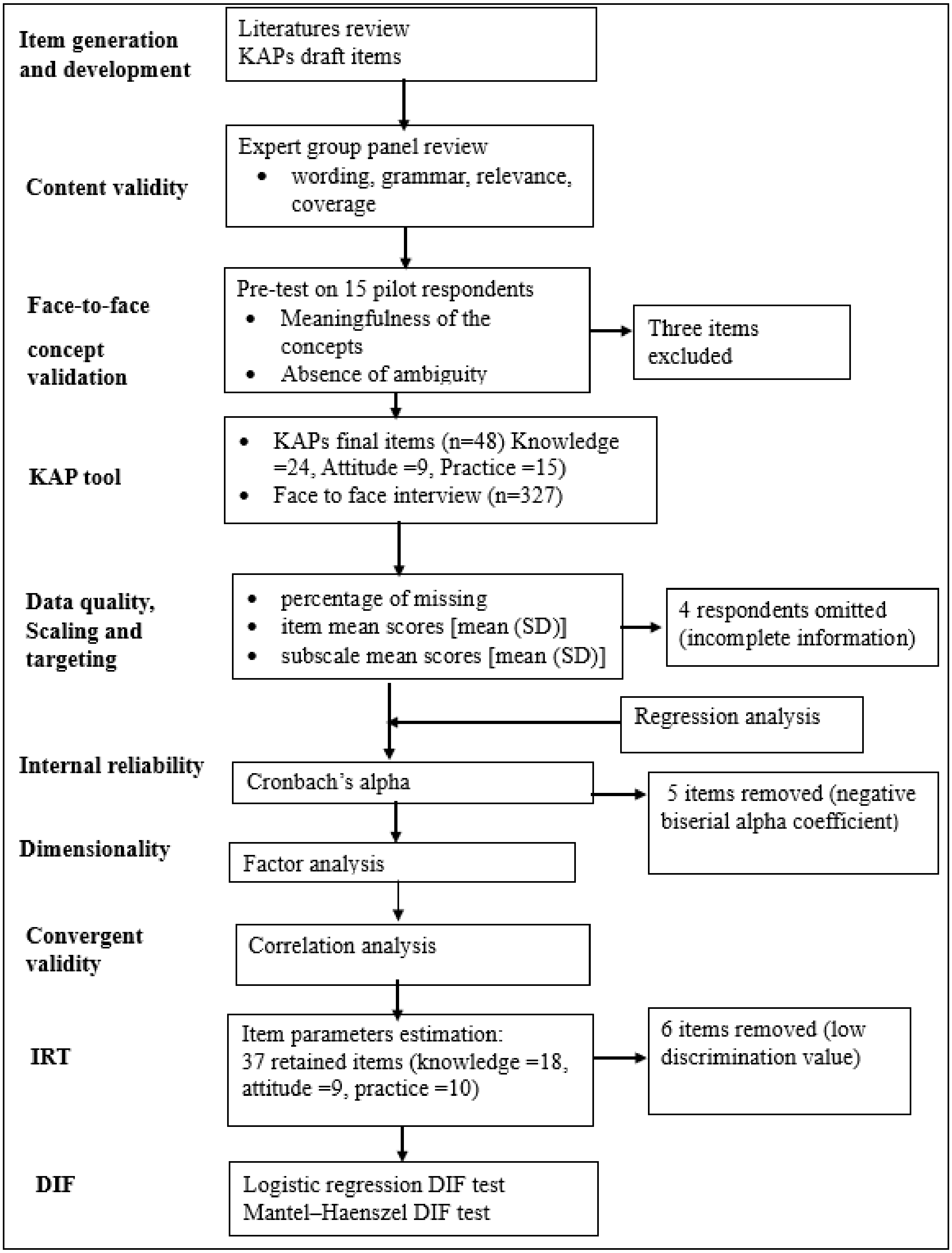

Figure 6.1 Study flowchart

6.2.2 Participants

A total of 327 randomly selected participants were interviewed in the 10 targeted *kebeles.* The required sample size was calculated using an expected Cronbach's α of 0.7 with a significance level = 0.05; confidence interval of 95%; 24 response items (for knowledge sub-scale); and an expected dropout rate (incomplete information rate) of 10% (Bonett, 2002; Arifin, 2018). The calculated sample size was equally distributed among the *kebeles*. Lists of potential participants in each *kebele* were obtained through local field researchers of CRP Livestock intervention *kebeles* and from key informants in the non-CRP Livestock *kebeles* one day before the survey. From these lists, households were selected randomly using the random function in Excel. Facilitators then contacted the household heads, asked for their willingness to participate, and planned the timing of the interview. Interviews were made in a place where both the interviewer and the participant felt comfortable and consent from all participants was obtained before the interview.

6.2.3 Survey instrument

A questionnaire was developed to measure participants' knowledge about zoonotic diseases, attitudinal barriers related to zoonotic disease risk and prevention methods from livestock birth products, and practices used to prevent zoonotic disease risks from livestock birth products. The demographic questionnaire included information on gender, age, education, primary livelihood activity, ethnicity, marital status, intervention status, and residential area, as well as on animal abortion history in the flock.

The first author conducted a thorough review of the literature to generate items for the KAP survey. Then, to confirm the content validity of the survey tool, a panel of five experts (two veterinary public health specialists, two epidemiologists, and animal production and health expert) evaluated the questionnaire in terms of wording, grammar, relevance, and coverage. The survey tool was tested on 15 farmers who were not included in the study population. The pilot study helped to assess the face validity of the items and to understand the meaningfulness of the concepts in the studied population. The difference and clarity of respondents' answers for each item were also evaluated. The survey tool was updated based on the feedback received during the pre-test.

The final version of the KAP tool comprised 48 items in three subscales (Table 6.1); 24 items measuring zoonotic disease knowledge, 9 items measuring zoonotic disease risk attitude, and 15 items measuring zoonotic disease prevention practices. Of the 24 knowledge subscale items, 20 items had dichotomous responses (correct or incorrect) and 4 items were initially open-ended questions and then restructured into "correctly named" vs "not named correctly". Items related to attitude were measured on a Likert scale ranging from 1 to 5 (1=strongly disagree to 5=strongly agree), with higher scores indicating the most desired attitude. All items in the practice subscale had dichotomous ("success" vs. "failure"). The questions were coded using Epi Info™ 7.2.1.0 software and copied onto Galaxy Tab A (2016) for digital data collection.

Table 6.1 KAPs items description

Item no	Item content	Response
Knowledge subscale items		
K1	When animals are sick in your flock, you can get the same sickness.	Correct/incorrect
K2	Many animal diseases can be transmitted from animals to humans	Correct/incorrect
K3	Identified the name of three diseases correctly that transmitted from animals to humans	Name /not name
K4	Identified the name of only two diseases correctly that transmitted from animals to humans	Name/ not name
K5	Identified the name of only one disease correctly that transmitted from animals to humans	Name/not name
K6	Please list at least one symptom for anyone zoonotic disease in animals	Correct/incorrect
K7	Animal disease can be transmitted via different routes	Correct/incorrect
K8	Eating uncooked meat can transmit diseases from animals to you	Correct/incorrect
K9	Drinking raw milk can transmit diseases from animals to you	Correct/incorrect
K10	Close contact with sick/dead animal can transmit diseases to you	Correct/incorrect
K11	You can get an infection from an environment contaminated with the secretions of sick animals	Correct/incorrect
K12	An insect bite can transmit animal diseases to you	Correct/incorrect
K13	Animal bites can transmit diseases to you	Correct/incorrect
K14	Animal abortion can cause a serious economic and public health problem	Correct/incorrect
K15	Abortion in animals can be caused by agents that spread between animals	Correct/incorrect

K16	Infectious diseases that cause abortion in animals might cause abortion in humans	Correct/incorrect
K17	Abortion causing agents can pass to you through different routes	Correct/incorrect
K18	Name at least one abortion-causing agent that is transmitted from animals to humans	Name /not name
K19	Assisting animals during parturition with bare hand exposes you to diseases	Correct/incorrect
K20	Assisting new-borns right after delivery exposes you to diseases	Correct/incorrect
K21	Any contact with aborted materials can expose you to diseases	Correct/incorrect
K22	Collecting aborted fetuses and placenta with bare hand exposes you to diseases	Correct/incorrect
K23	Disposing aborted fetuses into the environment can spread the diseases	Correct/incorrect
K24	Animal abortion can be prevented	Correct/incorrect
	Attitude subscale items	
At1	Some animal diseases are dangerous for people	SDA/ DA/ N/ A/ SA
At2	Diseases that cause animal abortion are serious and need the highest consideration	SDA/ DA/ N/ A/ SA
At3	Assisting the animal in delivery with a bare hand can expose you to disease risks.	SDA/ DA/ N/ A/ SA
At4	Collecting the aborted fetuses and placenta with bare hands can expose you to disease risks	SDA/ DA/ N/ A/ SA
At5	Throwing aborted fetuses and placenta into the environment contribute to the spread of the diseases on your farm	SDA/ DA/ N/ A/ SA
At6	You are at risk of acquiring diseases from abortion-causing agents	SDA/ DA/ N/ A/ SA
At7	Many of the agents that cause abortion in animals have the potential to cause disease in people.	SDA/ DA/ N/ A/ SA
At8	The spread of animal abortion-causing agents to humans is preventable	SDA/ DA/ N/ A/ SA
At9	Animal health care providers can handle abortion outbreaks very well	SDA/ DA/ N/ A/ SA
Practice subscale items		
P1	Assist animal delivery with protected hands	yes=success / no=failure
P2	Wash hands with soap after assisting animal delivery	yes=success / no=failure
P3	Avoid any contact with aborted material	yes=success / no=failure
P4	Collect aborted fetus and placenta with protective wear	yes=success / no=failure
P5	Always cover hands while touching animal birth products	yes=success / no=failure
P6	Dispose aborted fetus and placenta properly (bury or burn)	yes=success / no=failure
P7	Remove retained placenta manually	yes= failure / no=success
P8	Assist new-born with protected hands	yes=success / no=failure
P9	Wash hands with soap after assisting new-borns	yes=success / no=failure
P10	Suck new-born's noses to remove mucus	yes=failure / no= success
P11	Remove manure from barn regularly	yes=success / no=failure

P12	Take different prevention measures to stop animal abortion outbreak	yes=success / no=failure
13	Report abortion outbreak	yes=success / no=failure
P14	Visit veterinary clinic in case of animal abortion	yes=success / no=failure
P15	Cull frequently aborting animals	yes=success / no=failure

SDA= strongly disagree; DA= disagree; N= neutral; A= Agree; SD= strongly

The interviews were conducted by four trained veterinarians and animal production experts from the National Agricultural Research System (NARS) who spoke the local language of the respective study sites. They received training on the survey instrument, interview approach, and digital recording of responses. The training ensured a common understanding of the meaning of each question and in what way to ask participants.

6.2.4 Ethical approval and consent from participants

All the procedures for this study were conducted in accordance with a protocol approved by Addis Ababa University, College of Veterinary Medicine and Agriculture (VM/ERC/05/08/11/2018). The farmers/pastoralists were informed about the purpose of the study and the approximate time the interview will take, and their oral informed consent was sought before their participation in the survey.

6.2.5 Data Analysis

Descriptive statistics were used to summarise the demographic characteristics and item scores. The item mean scores were transformed to a 0–100 scale. Responses for attitude items were dichotomized into "undesirable" attitudes by combining "strongly disagree" and "disagree" responses and a "desirable" attitude by combining "strongly agree" and "agree" responses. "Neither disagree nor agree" responses were removed during the analysis. A mixed-effects linear regression model was fitted to predict the effect of participants' demographic characteristics on mean scores of knowledge, attitudes, and practices subscales using *kebele* as a random effect.

The internal consistency of the subscales was assessed by Cronbach's α coefficient, where a Cronbach's $\alpha \geq 0.7$ was considered acceptable. A value of Cronbach's $\alpha > 0.8$ was an indicator of good reliability and Cronbach's α between 0.7 and 0.8 indicated adequate reliability. Subscales with Cronbach's α values below 0.5 indicated unacceptable internal consistency (Bland and Altman, 1997; Tavakol and Dennick, 2011).

Unidimensionality of the three subscales was assessed using the sign of biserial correlations coefficient and confirmatory factor analysis. Negative point-biserial correlations identified potentially problematic items which were subsequently excluded from further analysis. Furthermore, the size of eigenvalues, scree plots, and the magnitude of item loading from the first factor from the factor analysis was used to evaluate the unidimensionality (Nguyen *et al.*, 2014; Zanon *et al.*, 2016).

Pearson's correlation coefficient was used to measure the relationship between subscales (Sheard, 2018). The absolute value indicated the strength of the relationship and the sign indicated a positive or negative relationship. Coefficient values between 0.8 to 1.0 indicated a very strong relationship, 0.6 to 0.8 indicated a strong relationship, 0.4 to 0.6 a moderate relationship, 0.2 to 0.4 weak relationship, and a value between 0 to 0.2 indicated a very weak to no relationship.

A unidimensional two-parameter logistic (2-PL) model was used to evaluate the probability of a person to appropriately respond to an item with a provided zoonotic disease knowledge, prevention practices level, and attitudes towards the risks. This model is represented by the following equation (Chernyshenko *et al.*, 2001):

$$Pij\ (ui = 1|\theta = t) = 1/1 + \exp[-1.7ai(t - bi)]$$

Where, a_i is the discrimination parameter for item i (i = 1, …, n), b_i is the difficulty parameter for item i, u_i is the response of the person with trait level θ (theta) to item i, and 1.7 is a scaling constant.

The item discrimination parameter (a_i) helps to determine whether the items appropriately distinguished farmers/pastoralists at different levels of zoonotic diseases, knowledge, prevention practices, and attitudes. Items with a_i values below or equal to 0.7 were removed from subsequent analysis due to low discrimination power.

Item characteristic curves (ICCs) were used to visualize and determine whether an item should be retained or removed. If the ICC of an item was too flat, it was removed because of low discriminatory power between KAPs levels. The item location parameter b_i determined the 50% probability of responding a given item correctly provided the respondent's level of the latent variable (theta θ). The unidimensional latent trait (latent variable) θ was used to assess the ability of a respondent. Predicted values of θ were calculated for every respondent based on their cumulative responses to the KAP questions. The transformed scale of θ had a mean of 0 and a standard deviation of 1 with an arbitrary range to cover the range of zoonotic diseases risk KAP from livestock birth products. The estimation of the parameters was repeated for each subscale after removing unfit items (items with inadequate discrimination).

A test information curve that graphically depicted the amount of information provided the sum of the item information functions at each value of theta for all items in each subscale were plotted.

Differential item functioning (DIF) analysis was performed on the retained items for each subscale to examine whether the items were answered in the same way across respondent groups. The five important subgroupings were: gender (female vs male); literacy level (never went to school vs went to school); primary activity (farmer vs pastoralist); age (less or equal to 35 years vs above 35 years) and intervention status (never seen CRP Livestock animal health intervention vs received CRP Livestock interventions).

Logistic regression was used to determine non-uniform DIF, that is, whether an item favours one group over the other for all values of the latent trait or only some values of the latent trait. Mantel-Haenszel Tests (MH), which calculated MH X^2 and odds ratio for dichotomously scored items, were used to test the presence of uniform DIF amongst the respondent groups. It examined whether an item responded in a better way by one respondent group relative to the other for all values of the latent trait. The statistical significance of the non-uniform DIF of the items was identified by the interaction term. A p-value of ≤ 0.05 was used in all analyses to determine the statistical significance. Data analyses were carried out using STATA software program version 15(Texas, USA).

6.3 Results

6.3.1 Characteristics of participants

A total of 323 adult respondents provided complete information during the interview. Table 6.2 presents the demographic characteristics of participants. The survey included 80 women (24.77%) and 243 men (75.23%). The mean age of the respondents was 39.5 years (±13.7), with a range from 18 to 85 years. About 60.37% and 39.63% of the participants were farmers or pastoralists, respectively. Agew (39.94%), Borena (39.94%), and Kenbata (20.43%) ethnic groups participated in the study. About 89.78% of the participants were married and 68.42% did not receive any formal education at school. The majority of the respondents (71.52%) reported animal abortion in their herd for the previous two years and 52.94% of respondents had received CRP Livestock animal health interventions.

Table 6.2. Socio-demographic characteristics of participants

Demography	**Category**	**N (%)**	**Mean (SD)**
Gender	Female	80 (24.77)	
	Male	243 (75.23)	
Age (year)			39.52 (13.68)
Livestock keeping type	MCL farmer	195 (60.37)	
	Pastoralist	129 (39.94)	
Ethnic group	Agew	128 (39.63)	
	Borana	129 (39.94)	
	Kenbata	66 (20.43)	
Literacy level	Never went to school	221 (68.42)	
	Went to school	102 (31.58)	
Marital status	Single	17 (5.26)	
	Married	290 (89.78)	
	Divorced/widowed	16 (4.95)	
CRP Livestock	Not received	152 (47.06)	
interventions	Received	171 (52.94)	

Herd abortion history*	No	92 (28.48)
	Yes	231 (71.52)

MCL= mixed-crop livestock
*Abortion history of the herd the last two years

6.3.2 KAP mean scores

The KAP survey included 48 items representing the three subscales knowledge, attitude, and practice to zoonotic disease risks from livestock birth products among smallholder communities in Ethiopia. The items scores were transformed to a 0–100 scale, and mean item scores for correct answers ranged from 0.31% (±5.6) to 65.42% (±47.6) for knowledge, 12% (±32.5) to 66.4% (±47.3) for attitude, and 4.3% (±20.39) to 90.4% (±29.5) for practice subscales (annexed as the supplementary table). Generally, the attitude subscale had the highest total mean score (37.3 ±28.92 %) and the knowledge subscale had the lowest scores (22.4 ±33.6 %) among the three sub-scales (Table 6.3). However, all subscales had a total mean score below 50%, which could be achieved by chance alone or by indicating misperceptions of zoonotic risks. Table 6.3 summarizes the total mean score of each subscale aggregated by socio-demographic characteristics. Univariable mixed-effect linear regression analysis showed that respondents from the Amhara region (Agew community) had a higher total mean score than respondents from Oromia Region (Borena community) (Coef=-15.65, P=0.00) and SNNP region (Kenbata community) (Coef. =-6.12, P=0.002) for knowledge subscale. However, respondents from SNNP presented a higher desired attitude total mean score compared to respondents in Oromia (Coef = -17.83, P=0.00) and Amhara (Coef. =-6.10, P= 0.153). The practice total mean score was significantly higher for the SNNP respondents than in Oromia (Coef=-11.06, P=0.00) and Amhara (Coef=-11.84, P=0.00).

ble 6.3. Knowledge, attitude, and practices regarding zoonotic risk compared with socio-demographic variables among communities in Ethiopia

Demography	**Category**	**N**	**Knowledge**			**Attitude**			**Practice**		
			mean	Coef.	p-value	mean	Coef.	p-value	mean	Coef.	p-value
	Overall	323	22.42			37.3			36.20		
Ethnic group	Agew	128	29.92			40.74			38.20		
	Borana	129	14.28	-15.65	0.00	29.01	-12.3	0.001	27.14	-11.0	0.00
	Kenbata	66	23.80	-6.12	0.040	46.84	6.10	0.149	50.04	11.84	0.00
Gender	female	80	22.09			38.63			38.14		
	male	243	22.53	-0.30	0.854	36.86	-2.47	0.506	35.57	-2.58	0.091
Age	<=35	155	21.13			34.49			36.03		
	>35	168	23.62	0.68	0.627	39.89	3.83	0.233	36.36	-0.08	0.925
Literacy level	never went to school	221	21.78			36.20			33.73		
	went school	102	23.82	1.35	0.449	39.68	0.35	0.925	41.57	-1.83	0.126
CRP livestock Intervention	control	152	21.14			35.99			36.18		
	Intervention	171	23.56	0.93	0.595	38.46	1.56	0.649	36.23	-0.84	0.480

6.3.3 Internal consistency

Cronbach's α was calculated for each subscale after removing all items with a negative biserial coefficient (5 items). The knowledge and attitude subscales had good internal consistency reliability with Cronbach's α of 0.83 and 0.81, respectively. The practice subscale had a Cronbach's α of 0.51 and thus lower than the minimum acceptable value of 0.7, indicating that this subscale showed inadequate internal consistency reliability.

6.3.4 Correlation between knowledge, attitude, and practice

Through correlation analysis, Pearson's correlation coefficient (*r*) indicated that there was a moderate positive association between responding correctly in the knowledge section and having the desired attitude (r2= 0.44, p<0.0001). There was a positive but weak relationship between correctly responding in the knowledge section and self-reported good practice (r^2= 0.307, p<0.0001). Good practices were also positively associated with the desired attitude (r^2= 0.18, p<0.0001).

6.3.5 Item parameter estimates

Factor analysis showed that all subscales were sufficiently unidimensional for the application of unidimensional IRT analysis. A 2PL IRT model was fitted to the data with the marginal maximum likelihood method to estimate the probability of correctly answering an item as a function of the person's ability parameters. The item characteristic curve (ICC) for each item was checked to determine whether an item should be retained or removed. If an item presented a very flat 2PL ICC between -4 to 4, it was removed due to low discrimination power between KAP levels. Accordingly, 6 items were removed from further analysis. The model was then refitted with the remaining items. The items retained for each subscale can be considered as an evaluation scale that measures zoonotic diseases knowledge, attitude, and prevention practices level of the individual respondent. Parameter estimates of retained items for each subscale are presented in Table 4. Parameter estimates were obtained for 37 items with adequate discrimination power. The mean discrimination parameters for knowledge, attitude, and practice subscales were 2.35 (SD=1.90), 4.94 (SD=4.86), and 1.06 (SD=0.36), respectively. Items in each subscale have a wide range of difficulties and discrimination power. The item with the highest discrimination power (a_i= 6.81) in the knowledge subscale was the item *'animal diseases can be transmitted to you and your family through different routes'*. The item '*many of the agents that cause abortion in animals have the potential to cause same in people'* in the attitude subscale had perfect discrimination power (a_i =14.76). In the practice section, the item *'do you visit the veterinary clinics in case of abortion'* had the highest discrimination parameter (a_i=1·65).

Mean difficulty parameters for subscales ranged from 0.38 (0 .862) to 1.82 (SD=1.57). The attitude section had the easiest items while the knowledge questions were harder. The mean of the practice section location parameters was 1.14 (SD= 2.12). Difficulties expressed through the location parameters ranged from -0.231 to 4.58 for knowledge items; -0.93 to 1.13 for attitude items; -2.76 to 3.52 for practice items.

Among the knowledge items, the easiest was *'when animals are sick in your flock, you might get the same sickness*' and the most difficult was '*insect bite can transmit animal diseases to you'*. The attitude item with the lowest location (difficulty) parameter was *'diseases that cause animal abortion are serious and need the highest consideration'*, and the item with the highest location parameter power was item '*many of the agents that cause abortion in livestock have the potential to cause same disease in people'*. *'Washing hands with soap after assisting animal delivery'* was the easiest practice item, whereas *'avoiding any contact with aborted materials'* was the hardest, respectively the least common practice for the farmers/pastoralists to implement.

Table 6.4 Discrimination and difficulty parameter from the Item Response Theory analysis of the KAP Scale.

Items	Items contents	Discrimination	Difficulty
Knowledge subscale			
K1	When animals are sick in your flock, you can get the same sickness.	1.17	-0.23
K2	Many animal diseases can be transmitted from animals to humans	4.07	-0.17
K3	Identified the name of three diseases correctly that transmitted from animals to humans	1.19	4.47
K4	Identified the name of only two diseases correctly that transmitted from animals to humans	1.76	1.56
K5	Identified the name of only one disease correctly that transmitted from animals to humans	4.64	-0.03
K6	Please list at least one symptom for anyone zoonotic disease in animals	2.32	0.71
K7	Animal disease can be transmitted via different routes	6.82	0.14

K8	Eating uncooked meat can transmit diseases from animals to you	4.37	0.15
K9	Drinking raw milk can transmit diseases from animals to you	1.37	1.47
K10	Close contact with sick/dead animal can transmit diseases to you	1.77	0.81
K12	Insect bites can transmit animal diseases to you	0.96	4.58
K13	Animal bites can transmit diseases to you	1.41	2.30
K16	Infectious diseases that cause abortion in animals can also cause abortion in humans	1.35	2.23
K17	Abortion-causing agents can pass to you through different routes.	1.03	1.89
K18	Identify at least one abortion-causing agents correctly that is transmitted from animals to humans	5.82	2.79
K21	Any contact with aborted materials can expose you to diseases	0.87	3.14
K22	Collecting aborted fetuses and placenta with bare hand exposes you to diseases	0.73	4.34
K23	Disposing aborted fetuses into the environment can spread diseases	0.79	2.53
	Knowledge subscale average	2.35	1.81
Attitude subscale			
At1	Some animal diseases are dangerous	0.95	-0.78
At2	Diseases that cause animal abortion are serious and need the highest consideration	0.82	-0.93
At3	Assisting the animal in delivery time with a bare hand can expose you to disease risks.	5.23	1.07
At4	Collecting the aborted fetuses and placenta with bare hands can expose you to disease risks	5.76	1.08
At5	Throwing aborted fetuses and placenta into the environment contribute to the spread of the diseases in your farm	10.43	1.01
At6	You are at risk of acquiring diseases from abortion causing agents	2.62	0.99
At7	Many of the agents that cause abortion in animals have the potential to cause some diseases in people.	14.77	1.13
At8	The spread of animal abortion-causing agents to humans is preventable	3.62	0.31
At9	Animal health care providers can handle abortion outbreaks very well	0.90	-0.39
	Attitude subscale average	5.01	0.39
Practice subscale			
P1	Assist animal delivery with protected hands	0.76	3.29
P2	Wash hands with soap after assisting animal delivery	0.71	-2.77
P3	Avoid any contact with aborted material	0.76	3.52
P4	Collect aborted fetus and placenta with protective wear	1.62	2.46
P5	Always cover hands cut while touching animal birth products	1.32	2.63

P6	Dispose aborted fetus and placenta properly (bury or burn)	0.89	2.43
P9	Wash hands with soap after assisting new-born	0.77	-1.07
P13	Report abortion outbreak	1.20	0.38
P14	Visit veterinary clinic in case of animal abortion	1.66	1.36
P15	Cull frequently aborting animals	0.93	-0.75
	Practice score average	1.06	1.15

Fig 6.2 presents the predicted θ scores for each respondent compared with the probability of correct responses in the respective KAP subscale. There was a difference in zoonotic diseases knowledge, attitude, and risk prevention practice level among respondents. The probability of someone answering all correct ranged between knowledge of the level of -1.1 to 2.6, attitude level of -1.2 to 1.8, and practice level of -1.45 to 2.56 (Fig 6.2).

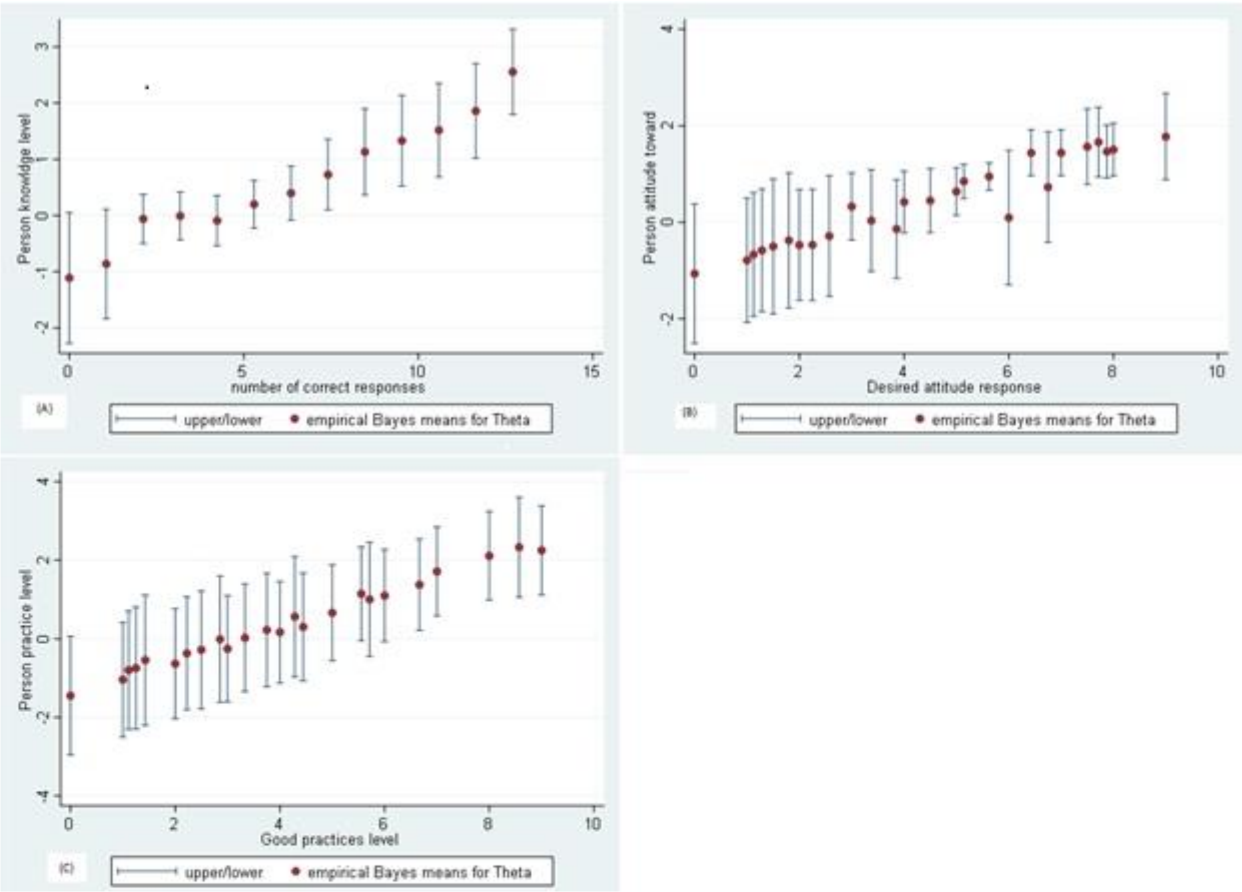

Fig 6.2. The plot of predicting subject scores (thetas) vs. the proportion of correct response of knowledge (A), attitude (B), and practice (C) subscales.

Fig 6.3 (A-C) presents the test information functions (solid lines) and standard errors (broken lines) to the knowledge(a), attitude (b), and practice(c) subscales. The KAP tool provided maximum information for respondents with θ between -0.8 to 0.8 for knowledge, 0.7 to 1.5 for attitude, and -0.7 to 2 for the practice subscale, respectively.

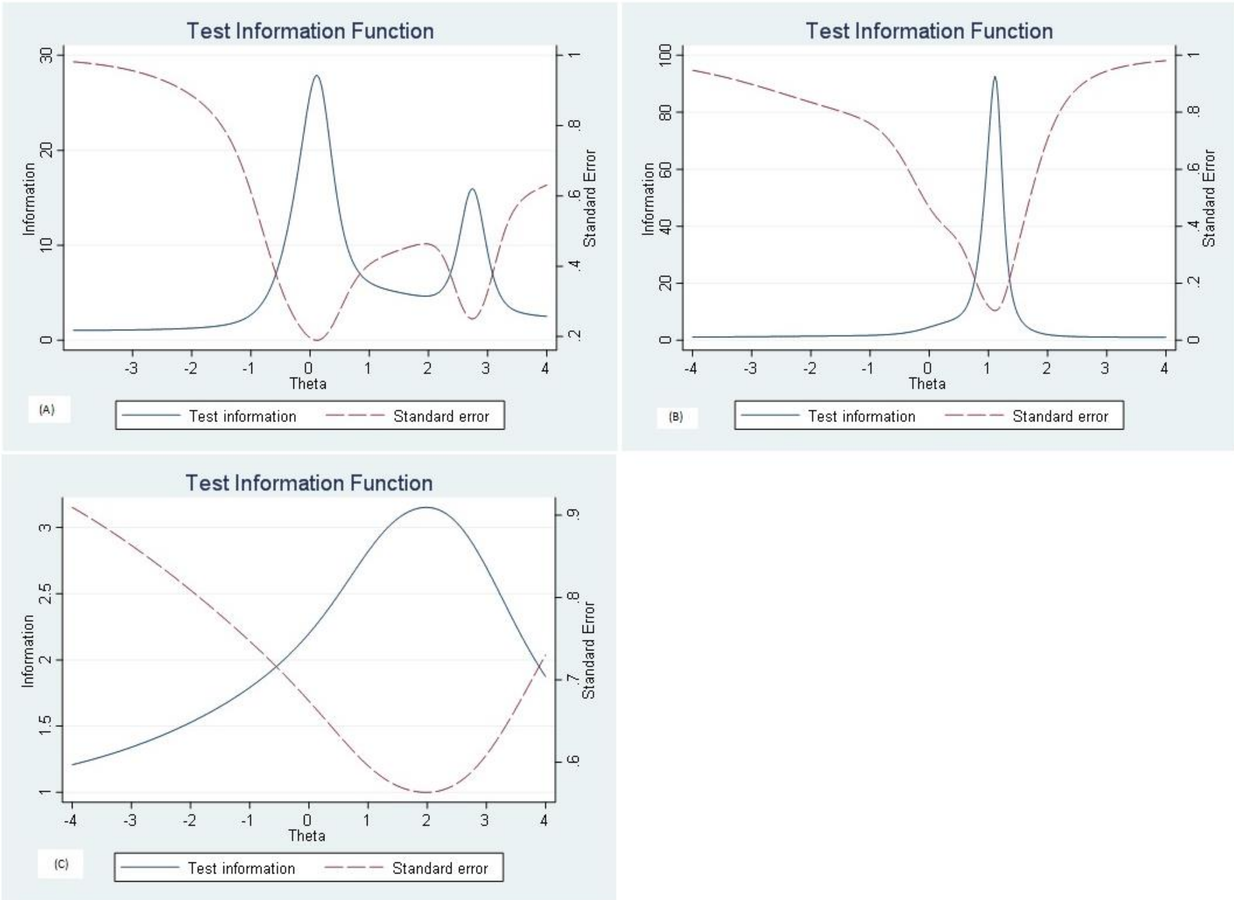

Figure 6.3Test information function for knowledge (A), Attitude(B) and Practice(C) subscales

6.3.6 Differential item functioning

We examined differential item functioning (DIF) in relation to gender, literacy, primary livelihood activity, age, and recent exposure to animal health-related interventions.

Table 6.5 presents items that had non-uniform DIF for each of the covariates based on a 5% significance level for KAP subscales. The results of the logistic regression analysis showed that one item had DIF associated with gender, six items related to age, one item associated with literacy level, three items associated with primary activity, and five items associated with intervention status for the knowledge section. For attitude, two items had DIF linked to gender, age, and primary activity, one item had DIF linked to intervention status, and none of the items had DIF-related literacy level. For the practice subscale, one of the items had DIF linked to gender and primary activity, two items had DIF related to age and intervention status, and none of the items presented DIF related to literacy level.

Table 6.5 Logistic regression DIF analysis findings for zoonotic diseases KAPs subscales

Item*	**Gender**		**Age**		**Literacy level**		**Activities**		**Intervention status**	
	X^2	**P-value**	**X^2**	**P-value**	**X^2**	**P-value**	**X^2**	**P-value**	**X^2**	**P-value**
Knowledge subscale										
K1	2.52	0.1127	0	0.9939	0.9	0.3428	22.5	0.00	0.13	0.7136
K2	0.18	0.6755	4.81	0.0283	1.24	0.265	0.42	0.5188	1.14	0.2846
K7	0	0.9972	8.8	0.003	9.71	0.0018	0.33	0.5642	0	0.9814
K9	2.51	0.1131	4.26	0.0391	1.45	0.2288	4.81	0.0283	3.99	0.0457
K10	1.36	0.2439	0.44	0.5067	0.01	0.9026	17.02	0.00	4.21	0.0403
K13	0.69	0.4055	5.39	0.0203	2.56	0.1099	0.03	0.8602	9.45	0.0021
K17	4.29	0.0384	0.28	0.5972	1.44	0.2304	0.32	0.5744	0.01	0.932
K21	0.19	0.6603	0.59	0.4419	2.18	0.1394	1.64	0.201	17.47	0.00
K22	0	0.9597	4.9	0.0269	0.07	0.7938	0.85	0.3569	0.3	0.5808
K24	2.21	0.1369	3.93	0.0474	0.02	0.8914	2.46	0.117	7.59	0.0059
Attitude subscale										
At2	6.72	0.0096	0.26	0.6088	2.63	0.105	2.77	0.0961	2.04	0.1528
At3	.	.	0.82	0.3658	2.51	0.1133	5.36	0.0206	0	0.9927
At6	0.04	0.8487	7.12	0.0076	0.29	0.5927	1.12	0.2892	5.65	0.0175
At8	7.48	0.0062	4.15	0.0415	0.01	0.9097	4.97	0.0258	3.6	0.0577
Practice subscale										
P6	3.15	0.0758	4.54	0.033	0.53	0.4648	.	.	0.63	0.4284
P9	0.04	0.8409	0.01	0.9147	3.32	0.0686	7.46	0.0063	9.31	0.0023
P13	0.75	0.3857	12.31	0.0005	0.04	0.8477	1.97	0.1599	0.17	0.6776
P14	8.59	0.0034	1.03	0.3102	2.11	0.1468	-	-	1.38	0.2406
P15	2.2	0.1382	1.83	0.1763	0.01	0.9089	0.15	0.694	4.08	0.0433

*items that had DIF for at least one covariate based on a 5% significance level were presented

Table 6.6 presents the items that had a uniform DIF for each of the covariates based on a 5% significance level. The Mantel-Haenszel DIF test for knowledge items showed that the item '*when animals are sick in your flock, you might get the same sicknesses*' answered in a better way by respondents' groups who never received formal education (odds ratio =2.7), pastoralists (odds ratio =2.1879) and respondents who have not involved in any intervention form CRP Livestock (odd ratio=3.57) compared to their counterparts. Crop-livestock farmers had 3.44 higher odds to identify at least one zoonotic disease correctly (item K5) and 2.87 and 2.56 higher odds to answer items related to animal disease transmission routes (K7) and eating uncooked meat can transmit diseases from animals (K8) correctly compared to pastoralists.

Female respondents, respondents with formal education, and respondents aged 35 or below had 2.94, 2.38, 3.12 times higher odds to respond correctly to the item '*drinking of raw milk can transmit diseases from animals to you*' compared to their counterparts. Being aged 35 years or less also increased the odds of responding correctly to the item *'close contact with the sick/dead animal can transmit disease to you'* by 2.04 times. Respondents who engaged in mixed crop-livestock farming had 2.21 higher odds to respond to the item *'animal bite can transmit diseases to you'*) correctly compared to pastoralists. Finally, respondents who never went to school had 3.05 times higher odds to correctly respond to the item *'abortion-causing agents can pass to you through different routes'* compared to those with formal education.

The Mantel-Haenszel DIF test for attitude items showed that only one item had a uniform DIF related to gender (Table 6.6). Female respondents had 5.88 times higher odds to have a positive attitude for the item *'animal health care providers can handle abortion outbreaks very well'*.

The results of the Mantel-Haenszel DIF test on practice items showed that women had 2.78 times higher odds to wash hands with soap after assisting newborns than men. Respondents aged 35 years or less and those who had been involved in CRP Livestock health intervention had 2.94 and 3.06 higher odds to remove manure from the barn regularly. Respondents who were older than 35 years of age and who were involved in mixed-crop livestock farming had 2.35- and 131.57-times higher odds to report an abortion outbreak than their counterparts, respectively.

Table 6.6. Mantel-Haenszel DIF analysis findings for zoonotic diseases KAPs scales

	Gender		**Age**		**Literacy level**		**Activity**		**Intervention**	
Item*	**OR**	**P-value**	**OR**	**P-value**	**OR**	**P-value**	**OR**	**P-value**	**OR**	**P-value**
Knowledge subscale										
k1	0.83	0.7126	1.07	0.917	0.3735	0.0042	2.1879	0.0074	0.2803	0.000
k5	1.08	0.9236	0.82	0.7877	1.6914	0.3102	0.2975	0.0121	1.7457	0.2763
k7	0.94	0.8513	1.01	0.8282	0.6157	0.3625	0.348	0.0432	0.4814	0.2182
k8	1.12	0.9896	0.74	0.6061	0.7941	0.7564	0.3974	0.0459	1.2099	0.8042
k9	0.34	0.0121	0.42	0.0211	3.1236	0.0007	1.9411	0.1962	1.6575	0.2356
k10	0.95	0.9585	0.49	0.0428	1.6502	0.1701	2.5655	0.0632	0.9151	0.9179
k13	0.49	0.3424	0.22	0.0531	1.2365	0.9394	2.2198	0.0468	0.7863	0.8443
k17	1.50	0.4047	1.63	0.2567	0.3362	0.013	0.3624	0.0898	0.7835	0.5956
Attitude subscale										
At9	0.17	0.007	2.63	0.0583	0.9377	0.9137	0.99	0.8330	0.8695	0.9129
Practice subscale										
P9	0.36	0.0467	0.92	0.9464	0.5323	0.1606	2.0395	0.4775	1.1641	0.7594
P11	1.78	0.3963	0.34	0.0515	0.4043	0.2161	-	-	3.0654	0.0489
P13	1.74	0.2359	2.35	0.0184	1.8664	0.1981	0.0076	0.000	0.7085	0.3832

*items that had DIF for at least one covariate based on a 5% significance level were presented

6.4 Discussion

To the best of our knowledge, this is the first study attempting to describe the knowledge, attitude, and prevention practices of zoonotic disease risk from livestock births products in Ethiopia. The current study revealed overall low zoonotic disease knowledge, low attitude towards zoonotic disease risk, and common risk behaviours among smallholder farmers and pastoralists. Even though the majority of the respondents reported sheep and goat's abortion during the two years before the interview, the causes of abortion, their transmission modes, preventive actions, and their public health significance are rarely known and understood by farmers and pastoralists. Previous studies in Ethiopia (Legesse *et al*., 2018; Zewdie *et al*., 2018) and elsewhere (Ndengu *et al*., 2017; Zhang *et al*., 2019) have also described knowledge gap on the public health risk of zoonotic abortion-causing agents such as *Brucella*, *Leptospira*, *Toxoplasma*, *Chlamydophila* and *Coxiella*. This low level of awareness of zoonotic diseases among communities may be the consequence of low information and awareness about the burden and transmission of the disease among veterinary and public health professionals, inaccessibility of public health centres, and lack of trained manpower in health education (Schelling *et al*., 2005; WHO, 2009; Gebreyes *et al*., 2014; Ndengu *et al*., 2017). In developing countries such as Ethiopia, the lack of appropriate diagnostic tools for the diagnosis and detection of pathogenic agents limits reliable qualitative and quantitative information on the burden of zoonotic disease (Pieracci *et al*., 2016). This led to an underestimation of the burden and the impact of zoonotic diseases on the farming community among policymakers and donors, which in turn is an obstacle to develop and implement appropriate policies to assess and manage zoonotic diseases risks when there are other competing public health priorities (Belay *et al*., 2017). Generating reliable information on zoonotic disease burden needs to be given higher priority to increases the awareness level of government agents, funders, and other concerned bodies (WHO, 2009). Increased awareness will help to promote cost-effective integrated approaches to address these knowledge gaps to reduce the risk of zoonotic infection to livestock producers and livestock product consumers along with value within existing health and agricultural systems.

It is generally assumed that expanding community-based livestock health education and promotion activities has a key role in improving the awareness of livestock framers towards zoonotic disease risks. Consequently, the behavioral practices of the farmers improved and the likelihood of human exposure from livestock significantly decreased (WHO, 2012; Gustafson *et al*., 2015; Olalekan and Adebukola, 2015; Rahman *et al*., 2018). The Federal Ministry of Health of Ethiopia launched the Health Extension Program in 2003 to increase the knowledge and skills of communities and households to deal with preventable diseases and to promote health in rural villages of Ethiopia (Assefa *et al*., 2019). While this program brings in a significant difference in public health, prevention of transmissible diseases, family planning, and the hygiene and sanitation of the environment. (Admassie *et al*., 2009; Banteyerga, 2011; Seyoum *et al*., 2016; Assefa *et al*., 2019), there remain important gaps in zoonotic disease prevention. Integrating zoonotic disease control training into this program with clear linkages to livestock health management would allow conveying accurate public health information about the zoonotic diseases' risks to the local community in rural areas of Ethiopia. Development agents are well known by the local community and their massages are positively taken. Therefore, it is important to consider those agents for the conveyance of information related to zoonotic disease transmission routes and prevention methods and herd health and husbandry management practices for the community (Van Metre and Morley, 2015).

Incorrect perceptions and attitudes towards the prevention of zoonotic disease from animal birth products, such as assisting and dealing with animal abortion with bare hands and improper disposal of aborted fetus and placenta, strongly support the need for culturally appropriate health education in rural communities. Therefore, it is important to change the attitude of the community to improve their behavioral practice towards zoonotic disease transmission and prevention practices (Nijland *et al*., 2013). The finding of this study suggested that establishing a desired attitude on the impact of those diseases on the public health and their mitigation strategies among the community is vital to reduce the transmission of zoonotic agents from animals to humans.

The majority of farmers and pastoralists did not implement appropriate risk prevention practices. Comparable findings were also obtained from Pakistan (Arif *et al*., 2017), Tajikistan (Lindahl *et al*., 2015), and Egypt (Holt *et al*., 2011). Poor knowledge of animal owners could be one explanation for these high-risk behavioural practices.

Moreover, farmers and pastoralists do not use personal protective equipment when dealing with animal abortion due to the limited availability in their areas.

The mixed model regression analysis indicated an important difference in zoonotic diseases knowledge, attitude, and practices across regions. This might be related to the difference in availability and accessibility of public and animal health facilities in studied agroecologies. Mixed crop-livestock farmers tend to have a better understanding of the problems since they have relatively better access to information as a result of better infrastructure such as health centers and roads. Borana pastoral communities and their livestock have a high level of mobility, which hampers access to resources, health services, and information due to limited infrastructures in the area, their lifestyle, and community norms (Schellingt al., 2005). In contrast, crop-livestock mixed farmers have better access to veterinary services such as vaccination and health education through different campaigns implemented by different government and non-government agencies (Hooper, 2016).

In the present study, the IRT method was used to develop scales to evaluate zoonotic KAP items. IRT models characterize items with different difficulty levels and measure discriminatory power based on responses and identify items that are not appropriate to be included in the scale for evaluation (Lalor *et al*., 2016). In this study, all three subscales met the assumption that the underlying trait measured was unidimensional and all, except the practice subscale, showed good reliability with acceptable Cronbach's α values. Moreover, the knowledge and attitude subscales were positively correlated with the practice subscale. Knowledge and risk perceptions of zoonotic causes of abortion among high-risk community groups are crucial in influencing behavioural practices in preventing its transmission in animals and humans in communities. Effective public education for zoonotic diseases demonstrated a detectable positive effect on risk perception and possible actions that can be taken to safeguard human health through appropriate hygienic measures and preventive practices (Kansiime *et al*., 2014; Çakmur *et al*., 2015; Van Metre and Morley, 2015; Hasanov *et al*., 2018).

Our results found moderate to very high discrimination mean values of KAP subscales, which shows strong consistency between the items and the KAP levels of the respondents (Baker, 2001; Gorin *et al*., 2008). The difficulty parameter estimates showed that the attitude section had the easiest items, and the knowledge section was comparably more difficult. This result indicated that it can be possible to have a positive attitude towards zoonotic diseases risks with some level of knowledge on zoonotic diseases from livestock birth products. Items in the knowledge subscale required a higher level of knowledge to be answered correctly. This indicate that health education on prevention and control of zoonotic diseases was not provided for the animal owners or correct levels of knowledge on zoonotic diseases at livestock birth were not attained. These results provide vital information for future interventions and illustrate a critical need to improve farmers' and pastoralists' knowledge.

Predicted subject scores (θ) indicated that the probability of correct responses increased consistently with individual knowledge, attitude, and practice level. However, the attitude subscale needs additional questions to be able to better discriminate between attitude levels. The test information curve showed that the knowledge subscale questions provided more precise information within the medium knowledge scores. However, attitude and practice questions presented more precise information within the higher trait levels of farmers and pastoralists. The KAP scales developed in this study can thus be used in future studies, such as, for example, for the evaluation of interventions aimed at improving public awareness on zoonotic diseases from livestock birth and abortion.

The DIF analysis found that 19 of 37 (51.35%) and 12 of 37 (32.43%) items of the final version of the KAP survey differed depending on the subgrouping of the respondents. The presence of DIF in the majority of KAP questions in this study might be attributed to the lack of equal understanding of zoonotic disease risks and their perception of the risks and risk prevention practices among the respondents. It appears that there are discrepancies between the real risks associated with animal abortion and their perception by the public. This difference could be due to communication inequalities at the grassroots level.

Moreover, some respondent groups had a higher awareness regarding zoonotic diseases from livestock birth products, which might be attributed to their desire to obtain health information from media and professionals. Farmer and pastoralist perceptions about zoonotic disease risk from livestock are guided by socio-environmental barriers, beliefs, and may often be misinformed and incorrect (Arif *et al*., 2017; Chenais *et al*., 2017; Alhaji *et al*., 2018; Amenu *et al*., 2019).

The role and location of the households might determine livestock owners' awareness, disease identification skills, and preventive behavioural practices, which need attention during community health education program development. For example, women and men have different experiences and capacities in animal management and husbandry. Women are predominantly involved in milking and processing of milk as well as the care of sick animals and aborted animals (Yisehak, 2008; Nigussie *et al*., 2014; Kinati and Mulema, 2018). Similarly, women are responsible for milking in Borana pastoral communities (Amenu *et al*., 2019). Therefore, women involved in milking and milk processing daily have a better chance to observe foreign materials such as dungs or pus from animals with mastitis. This increases the concern of women towards milk hygiene and encourages women to boil milk before serving, which would help prevent most milk-borne diseases. This implies the need for community health education programmes that target specific groups such as youth, women, farmers, pastoralists, or community leaders. This in turn requires building the capacity of development agents, medical and veterinary doctors, and technical personals and calls for more efficient public health education at the grassroots level. (Gustafson *et al*., 2015; Van Metre and Morley, 2015). Appropriately prepared targeted public information materials such as pamphlets and posters can be used to communicate information on diseases to local communities and to encourage them to adopt better risk management practices (Hasanov *et al*., 2018). More promising to achieve lasting practice change, however, would be discussions with communities to clarify myths and find context-specific solutions that are accepted by the communities (Lemma *et al*., 2018; Mulema *et al*. 2020). Moreover, the utilization of local mass media such as radio to disseminate public information also plays a key role in continued efforts to prevent and control zoonotic diseases and promoting zoonotic control interventions (WHO, 2009; Abdi *et al*., 2015; Zhang *et al*., 2019).

4.2 Limitation of the study

Even though interviewers received training to ensure a common understanding of the meaning of each question and in what way to ask them, the questions may have been interpreted incorrectly by the respondents. Validity of the questions was ensured through expert consultation and pre-testing with a pilot group of farmers before the survey. Attempts were also made to make sure that the farmers and pastoralists understood all items correctly before they responded. The items included were based on expert knowledge and literature review but did not include in-depth qualitative research with communities at first. Adding this step may help to identify additional items to be added to the KAP tool in the future.

6.5 Conclusion

The present study evaluated the knowledge, attitudes, and prevention practices of zoonotic diseases from livestock birth products in rural Ethiopia. The KAP tool developed showed high validity and reliability. This study highlighted substantial knowledge gaps and high-risk behavioural practices towards zoonotic disease risks from livestock births products, which are an important contributing factor for zoonotic disease infection. Differential item functioning test showed that more than half of the items in the final version of the KAP scale had DIF related to at least one covariate, which indicated all items were not equally addressed by the respondents. The low mean scores recorded for all subscales and the presence of DIF in the majority of the items highlight the need for targeted community health education programmes to minimize the transmission of zoonotic pathogens from livestock birth products.

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Supplementary Table 6.1. Mean items scores of KAPs survey on zoonotic disease risks from livestock births

Item no	Item content	Mean (%)	SD (%)
Knowledge subscale items			
K1	When animals are sick in your flock, you can get the same sickness.	55.4	49.8
K2	Many animal diseases can be transmitted from animals to humans	57.0	49.6
K3	Identified the name of three diseases correctly that transmitted from animals to humans	52.0	50.0
K4	Identified the name of only two diseases correctly that transmitted from animals to humans	13.3	34.0
K5	Identified the name of only one disease correctly that transmitted from animals to humans	0.9	9.6
K6	Please list at least one symptom for anyone zoonotic disease in animals	28.8	45.3
K7	The animal disease can be transmitted via different routes	46.1	49.9
K8	Eating uncooked meat can transmit diseases from animals to you	45.8	49.9
K9	Drinking raw milk can transmit diseases from animals to you	18.0	38.4
K10	Close contact with sick/dead animal can transmit diseases to you	28.2	45.1
K11	You can get an infection from an environment contaminated from secretions of sick animals	1.9	13.5
K12	An insect bite can transmit animal diseases to you	1.9	13.5
K13	Animal bites can transmit diseases to you	7.4	26.3
K14	Animal abortion can cause a serious economic and public health problem	65.4	47.6
K15	Abortion in animals can be caused by agents that spread between animals	39.6	49.0
K16	Infectious diseases that cause abortion in animals might cause abortion in humans	8.7	28.2
K17	Abortion causing agents can pass to you through different routes	16.4	37.1
K18	Name at least one abortion-causing agents that are transmitted from animals to humans	0.3	5.6
K19	Assisting animals during parturition with bare hand exposes you to diseases	7.1	25.8
K20	Assisting new-borns right after delivery exposes you to diseases	4.3	20.4

K21	Any contact with aborted materials can expose you to diseases	8.0	27.2
K22	Collecting aborted fetuses and placenta with bare hand exposes you to diseases	5.0	21.7
K23	Disposing aborted fetuses into the environment can spread the diseases	13.0	33.7
K24	Animal abortion can be prevented	14.2	35.0
	Attitude subscale items		
At1	Some animal diseases are dangerous for people	65.4	47.6
At2	Diseases that cause animal abortion are serious and need the highest consideration	66.4	47.3
At3	Assisting the animal in delivery with a bare hand can expose you to disease risks.	15.6	36.3
At4	Collecting the aborted fetuses and placenta with bare hands can expose you to disease risks	15.2	36.0
At5	Throwing aborted fetuses and placenta into the environment contribute to the spread of the diseases on your farm	14.9	35.7
At6	You are at risk of acquiring diseases from abortion-causing agents	21.0	40.8
At7	Many of the agents that cause abortion in animals have the potential to cause disease in people.	12.0	32.5
At8	The spread of animal abortion-causing agents to humans is preventable	42.4	49.5
At9	Animal health care providers can handle abortion outbreaks very well	58.4	49.4
Practice subscale items			
P1	Assist animal delivery with protected hands	9.3	29.1
P2	Wash hands with soap after assisting animal delivery	85.8	35.0
P3	Avoid any contact with aborted material	8.0	27.2
P4	Collect aborted fetus and placenta with protective wear	5.0	21.7
P5	Always cover hands while touching animal birth products	5.9	23.6
P6	Dispose aborted fetus and placenta properly (bury or burn)	13.0	33.7
P7	Remove retained placenta manually	18.6	39.0
P8	Assist new-born with protected hands	67.7	46.9
P9	Wash hands with soap after assisting new-borns	39.7	49.0
P10	Suck new-borns noses to remove mucus	16.3	37.0

P11	Remove manure from barn regularly	64.0	48.1
P12	Take different prevention measures to stop animal abortion outbreak	90.4	29.5
13	Report abortion outbreak	4.3	20.4
P14	Visit veterinary clinic in case of animal abortion	67.4	46.9
P15	Cull frequently aborting animals	53.9	49.9

Chapter 7

General Discussion

7.1 Introduction

The main motivation for the studies presented in this Ph.D. dissertation was the increasing interest to initiate in designing empirical one health research to untangle the complexity and enhance the development of appropriate control strategy to decrease the economic and public health impact of abortion-causing agents. Such a strategy to increase flock reproductive performance provides unique opportunities for smallholder farmers using small ruminant production as instruments for sustainable development and pathways out of poverty (Desta *et al*., 2020; Kebede *et al*., 2019; Oseguera Montiel *et al*., 2014; Perry *et al*., 2018). The Ph.D. study was set with an overall objective of generating information on the cause, magnitude, risk factor of reproductive health problems, and public health risk of infectious causes of small ruminant abortion.

7.2 Methodological approaches

The overall approach of this study is interdisciplinary, involving methodologies from different scientific fields. A brief description of the methodologies used in this Ph.D. dissertations is presented in the following paragraphs.

To develop a novel quantitative tool to determine goat annual reproductive performance index at the flock level, the reproductive performance of the flock was estimated based on the annual reproductive output, kidding interval, and annual reproductive wastage (Chapter 3). Flock level reproductive performance was estimated by combining data on reproductive performance traits collected from selected flocks in the drylands of Ethiopia. Principal component analysis was used both as reproductive measures dimensional reduction technique and to develop the final model to predict the reproductive performance scale. Then the final algorithm was developed to estimate the goat annual reproductive performance index. It can be a useful and objective tool to compare reproductive performance between breeds, management systems, agro-ecological zones, and interventions, especially in projects targeting dryland production systems.

To estimate the magnitude of abortion, the occurrence of at least one abortion in the small ruminant flock in the 12 months was obtained from three agroecology and production systems of Ethiopia (Chapter 4). To improve the overall financial performance of the flock and ensure the sustainability of smallholder sheep and goat production, it is important to identify and address the causes of sheep and goat abortion managed under an extensive management system. A perceived list of causes and seasonality of abortion was obtained from the producers. This follows a participatory research approach that has been widely acknowledged at remote locations; long-term herd monitoring is typically limited in its practical feasibility (Feldt *et al*., 2016). It yielded reliable estimates of reproduction data among the studied flocks and is comparable to those reported by various researchs conducted elsewhere in Africa (Gojam & Tulu, 2020; Hary *et al*., 2003; Otte & Chilonda, 2002; Wilson *et al*., 1989). A causal diagram was generated to identify causal relationships between the potential predictors and abortion in the flock. A zero-inflated negative binomial regression model was used to determine the associations between potential risk factors with the number of abortion cases in a sheep and goat flock. A random-effects regression model was used to account for the clustering of flocks within villages.

To determine the seroprevalence of abortion-causing pathogens and associated risks in sheep and goats in three production systems of Ethiopia, randomly collected and tested serum samples from small ruminant flocks were used (Chapter 5). The seroprevalence of four infectious abortion-causing agents (C. *burnetii*, *C. abortus*, *Brucella* spp., and *T. gondii*) and mixed infection were determined in this study. A multilevel mixed-effects logistic regression model was fitted to account for the clustering of the animal within villages and households.

To understanding knowledge, attitudes, and practices (KAP) on zoonotic risks from livestock birth products among rural communities, data were collected from randomly selected farmers and pastoralists in five districts in three regions in Ethiopia (Chapter 6). The structured questionnaire was used to collect the data. A unidimensional two-parameter logistic (2-PL) IRT model was used for zoonotic disease risk KAP scale construction and evaluation. The 2-PL IRT model was fitted to calculate the probability of a person correctly answers an item with a given zoonotic disease KAP level. Differential item functioning (DIF) analysis was performed on items for each subscale to examine whether the items were answered in the same way across respondent groups. Logistic regression was used to test for non-uniform DIF, that is, whether an item favours one group over the other for all values of the latent trait or for only some values of the latent trait. Mantel-Haenszel Tests (MH), which calculated MH X^2 and odds ratio for dichotomously scored items were used to determine whether an item exhibited uniform DIF between the observed groups, that is, whether an item answered in a better way by one group relative to the other for all values of the latent trait.

7.3. Synthesis of results

7.3.1. Goat annual reproductive performance index

Based on a developed algorithm to measure goat annual reproductive performance, the flocks were classified into good, moderately, and poor performing. Good performing flocks have higher scores for reproductive output measures, lower scores for reproductive wastage, and lower kidding interval. Many of the flocks were moderately affected by reproductive failures, consequently categorized as moderately performing flocks. The categorization allowed the identification of extreme flocks, good performers, or poor performer flocks. The presence of a larger number of moderately performed flocks gives opportunities for development partners and smallholders to upgrade those flocks with a moderate improvement of management practices, feed supplementation and health care management systems, evaluate the costs of poor reproductive management, and will be useful for economic models that aim to identify the most cost-efficient intervention option and monitor the impact of interventions.

7.3.2. Abortion and its cause in small ruminant in Ethiopia

This Ph.D. research demonstrated the multifactorial nature of abortion in small ruminant flocks where infectious diseases, extreme weather conditions, feeding, management practices, and biosecurity play a role in its occurrences (Chapter IV). There was significant variation in small ruminant abortion across agroecology and production systems and species. Overall, a higher percentage of abortions were recorded in lowland agroecology than highlight agroecology. It can be related with drylands agro-ecosystems where multiple stressors such as the cumulative effects of poor nutrition, excessive heat, and the need to walk long distances to source feed and water compromise the production and reproduction performance of small ruminants (Gaughan *et al*., 2019; Sejian & Maurya, 2013). The goat was considered as the most affected livestock species by abortion. The reason for this might be genetic factors, physiological or higher susceptibility to risk factors present (Mellado *et al*., 2004).

This thesis found that many small ruminant flocks tested positive for one or more abortion-causing agents (*C. burnetti*, *C. abortus*, *Brucella* spp., and *T. gondii*) (Chapter 4). There was also evidence of co-infection in the majority of tested flocks. This result indicated that multiple infectious abortion pathogens might play a significant role in small ruminant abortion. The final zero-inflated negative binomial regression analysis result indicated that spending the night in traditional sheep house', 'providing supplementary feed for pregnant dams', 'presence of other livestock species and dog in the household' had a marked effect on the rate of abortion in sheep and goat flocks. In addition, exposure of the flock to *Brucella* spp. or any one of the four tested infectious agents significantly increased the risk of abortion in the flock. From the finding of this study, abortion is a multifactorial problem by its nature that requires integrated approaches that improve the nutritional state of pregnant dams through targeted supplementary feeding, abortion management through appropriate biosecurity practices, and vaccination programs for major infectious causes of abortion and herd health management through better veterinary services.

The study to determine the seroprevalence and associated risk factors of common abortion-causing agents found the infection *C. burnetii, Brucella* spp., *C. abortus*, and *T. gondii* with different infection rates (Chapter 5). The study also found the co-infection of those pathogens at the flock and individual animal level. From the result multivariable mixed effect logistic regression analysis, it was evident that ago-ecology and production systems, flock management practices, flock composition and mix and biosecurity practice associated with the prevalence of above mention pathogens in small ruminants.

7.3.3. Community knowledge, attitude, and practice to zoonotic disease risks from livestock birth products in Ethiopia

In addition to identify abortion causes and determine the epidemiology of infectious causes of abortion, it is important to understand knowledge, attitude, and practice to zoonotic disease risks from livestock birth products to reduce the public health risk of those pathogens through carefully designed veterinary public health interventions. Chapter six of this thesis found huge knowledge gaps and high-risk behavioural practices towards zoonotic disease risks from livestock births products. The study found a positive association between responding to knowledge questions correctly and a positive attitude and self-reported good practice. Creating a positive attitude towards the prevention of the disease was one of the main factors identified to have a significant association with zoonotic diseases' prevention practice. Knowledge of factual risks and the public's perception of risks influenced individual decision-making on behavioral practices

(Múnera-Bedoya et al., 2017). This study implied that creating a positive attitude towards zoonotic disease transmission, prevention, and its public health importance among livestock owners is vital to prevent the spread of zoonotic disease from animals. Differential item functioning test showed that more than half of the items in the final version of the KAP scale had DIF related to at least one covariate, which indicated all items were not equally addressed by the respondents. This implies the need for community health education programmes that target specific groups such as youth, women, farmers, pastoralists, or community leaders. This in turn requires capacity building for extension officers, physicians, veterinarians, and technicians and calls for better extension services at the community level (Gustafson *et al*., 2015).

7.4 Policy implication

Since the production efficiency of a flock is directly related to the number of products obtained, reproductive efficiency is increasing the profitability and access to animal source food for rural poor households. Improving flock reproductive performance requires measurements of current performance and general flock management practices. Performance needs to be assessed not only on an individual animal basis but also at the flock and at general management level. Once the key performance indicators are defined, data collected at both the flock and doe level are required to allow the calculation of indices for a specific flock. A wide range of data must be collected to aid decision-making, given the complex interactions between genetics, physiology, nutrition, and management. The current traditional way of production system with limited or no input will not be able to meet the growing demand for livestock products in developing countries or will not provide products to be provided by producers who get supports. These resulted in an assumption that indigenous tropical small ruminant has a low genetic potential for meat and milk production. During the last decades, there were breeding strategies to change small ruminant productivity through crossbreeds with foreign germplasm or replace indigenous breeds with foreign breeds (Kosgey, 2004). However, their implementation has been unsystematic, resulting in a mosaic of genotypes. Nevertheless, community-based breeding programs, which aim to increase the performance of indigenous sheep and goat, have resulted in tangible outcomes by lamb and kid growth rate (Abegaz *et al*., 2014; Mekuriaw & Haile, 2014). These must be complemented with interventions that improve the nutritional state of pregnant dams through targeted supplementary feeding, abortion management through appropriate biosecurity practices, strategies that improve neonatal survival and growth, and vaccination programs for major infectious causes of abortion and herd health management through better veterinary services to increasing reproductive efficiency. On the other hand, it is important to create enabling environment to engage private sectors as service providers, building strong partnerships with key stakeholders and integrating other productivity improvement technologies to improve the overall performance of small ruminant sectors.

The evaluation and description of KAP among farmers and pastoralists concerning zoonotic disease risk from livestock birth products might therefore help in the development of evidence-based disease prevention campaigns and efficient veterinary public health interventions. The Health Extension Program launched in 2003 to increase the knowledge and skills of communities and households to deal with preventable diseases and to promote health in rural villages of Ethiopia has shown tangible positive impacts on community health, in disease prevention, family health, and environmental hygiene and sanitation (Admassie *et al*., 2009; Banteyerga, 2011; Seyoum *et al*., 2016; Assefa *et al*., 2019). However, this program misses zoonotic disease prevention through better herd health management, animal husbandry, and biosecurity. Therefore, integrating zoonotic disease control training into this program with clear linkages to livestock health management would allow conveying accurate public health information about the zoonotic diseases' risks to the local community in rural areas of Ethiopia.

7.5 Future research outlook

This Ph.D. study provided an important index to measure goat annual reproductive performance in the dry land of Ethiopia. There is a need to validate and possibly to re-calibrate the index and its cut-offs and to adapt the approach for other production systems in different agroecology. It is also important to implement research that evaluates the effect of integrated intervention packages that improve feed efficiency, reproduction, parasites, and disease control to support decision-makers.

The present study provides insight into the magnitude and causes of abortion in smallholder systems in Ethiopia by testing for several possible agents at the same time. To get a comprehensive picture of the impact of reproductive failures on small ruminant flock dynamics, it is important to implement a longitudinal study that monitors the incidence and associated risk factors over time. It is also vital to capture costs associated with abortion and the cost-effectiveness of intervention and control programs.

To ensure that intervention strategies such as vaccines or antibiotics are successful, accurate diagnosis of abortive pathogens is critical (Holler, 2012). This Ph.D. study tried to detect common causes of abortion in small ruminants through serological assay. Even though serological analysis of dam serum remains the preferred option in many diagnostic laboratories to test the presence of antibodies against various bacterial agents, it is difficult to establish a causal relationship with exiting abortion in the flock. Therefore, it is important to have accurate abortion diagnostic investigation through regular flock follow up and monitor to inform possible control options or research on management strategies to reduce reproductive losses. More research should be done looking at several possible pathogens at the same time and assess the socioeconomic impact caused by these pathogens. Since the majority of abortion-causing agents have a zoonotic potential, studies that investigate the public health impact of those diseases and intervention design improve the awareness of the community are very critical.

7.6 Limitations of the study

Even though this Ph.D. research tried to address important information gap on the magnitude and the causes of small ruminant reproductive failures, it is limited in its scope to cover in detail the following issues:

1. Due to time limitations, this Ph.D. thesis did not present the impact of various management factors, agroecology and production systems, and infectious diseases on neonatal loss. The result of the analysis on the effect of management factors on neonatal mortality will be published after the official defense of this thesis.

2. Eventhough a great deal of effort was exerted to detect infectious causes of abortion from samples of small ruminant abortion cases, it was impossible due to various reasons such as security, lack of information on abortion outbreaks.

3. This Ph.D. thesis determined antibodies against *C. burnetii*, *C. abortus*, *Brucella* spp., and *T. gondii*. There are other abortion-causing organisms such as *Listeria* spp., *Campylobacter* spp., *Leptospira* spp., and *Neospora caninum.* Due to financial and logistic limitations, the author was forced to prioritize diseases according to their burden on the small ruminant and human populations in Ethiopia. First, six pathogens including *Leptospira* spp. and *Neospora caninum* were listed by consulting existing literature and expert in the field. Unfortunately, we couldn't receive kits for *Leptospira* spp. and *Neospora caninum* testing regardless of our effort.

5. Looking at studies published over 20 years, we found 79 studies that could be included in meta-analyses for four abortion-causing agents. The majority of these are reported from European and Asian countries. Only very few articles were obtained from Africa and North and South America. Therefore, the findings may not necessarily be conclusive of the actual role of infectious agents for abortion in Africa in general and Ethiopia in particular.

7.6. Conclusions and Recommendation

Based on the main results of the research presented in this thesis, the following conclusions are drawn:

- ❖ From comprehensive meta-analysis done on data collated from different continents and demonstrated the important role of infectious agents in small ruminant abortion. Abortion in small ruminants is caused by multiple etiological agents in which some agents such as Chlamydia play a major role in both sheep and goat abortion. Overall, very few studies were found on this subject, especially for low- and middle-income countries, even though reproductive performance in these countries is lower.
- ❖ The developed G-ARPI index is an ideal management tool to help to evaluate the costs of poor reproductive management, which are often hidden and help to the development of economic models that aim to identify the most cost-efficient intervention option to increase reproductive performance.
- ❖ The role of management, agroecological and infectious disease factors on sheep and goat abortion were obvious in this Ph.D. study and our findings highlight the multifactorial nature of the problem.

- ❖ This Ph.D. study has demonstrated the evidence of four infectious zoonotic abortion-causing agents and their potential risk factors in small ruminants in three agroecology and production systems. From the finding, those infectious diseases widely distributed across different agroecologies might have a role in the suboptimal reproductive performance of small ruminants from reproductive failure and pose public health risks for the communities.
- ❖ This Ph.D. study highlighted substantial knowledge gaps and high-risk behavioral practices towards zoonotic disease risk from livestock births products, which are an important contributing factor for zoonotic disease infection.

From the above main conclusions, the following recommendations are forwarded:

- ➢ Accurate and timely diagnosis of abortion pathogens through the appropriate collection and submission of abortion samples along with a complete case history is essential to reduce the impact of abortion in small ruminant productivity.
- ➢ More research should be done looking at several possible pathogens at the same time and assess the socioeconomic impact caused by these pathogens.
- ➢ Future activity should focus to validate and possibly to re-calibrate the index and its cut-offs and adapting the approach for other production systems in different agro-ecologies.
- ➢ Integrated approaches that improve the nutritional state of pregnant dams through targeted supplementary feeding, abortion management through appropriate biosecurity practices, and vaccination programs for major infectious causes of abortion and herd health management through better veterinary services should be implemented to reduce reproductive loss.
- ➢ To make sure interventions are sustainable and can be scaled, recognizing the farmer/pastoralist indigenous knowledge and participating them in the process as partners, creating an enabling environment to engage private sectors as service providers, building strong partnerships with key stakeholders, and integrating other productivity improvement technologies are important strategies.
- ➢ A future study aimed at identifying and characterization several possible abortion pathogens and their public health and socioeconomic impact should be done.

- The low mean scores recorded for all subscales and the presence of DIF in the majority of the items highlight the need for targeted community health education programs to minimize the transmission of zoonotic pathogens from livestock birth products.

7.7 Reference

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