APPARENT PREVALENCE OF BRUCELLOSIS, Q-FEVER AND TOXOPLASMOSIS IN ABORTED GOAT'S AT NORTH SHOA, ETHIOPIA

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Abstract

Abortion imposes great economical loss in productivity and by product of small ruminants. The present study was conducted to determine the rate of abortion and apparent prevalence of Brucellosis, Toxoplasmosis and Q-fever in aborted goats from June 2015 to August 2019 in North Shoa, Ethiopia. During consecutive years of clinical case study 503 does were entered to mating, of which100 (19.5 %) animals aborted. There were significant differences (p=0.013) in abortion among parities. A total of 35 serum samples were collected from aborted does within six months periods. All samples were screened initially with Rose Bengal plate test (RBPT) for Brucellosis. All RBPT positive were further tested by i-ELISA. Also, serums were tasted to screen specific antibody against Q-fever and Toxoplasmosis using i-ELISA. Of total tested 64.7 % and 8.6 % of them were positive for Q-fever and Toxoplasmosis, respectively, but neither of them was positive for Brucellosis. The present clinical study revealed that abortion was the cause of kids' loss and serological investigation of antibody against Q-fever and Toxoplasmosis showed that the agents were the major causes of abortion. Even though there was no positive reactor does to brucellosis, the result must be interpreted with care since absence of evidence is not evidence of absence. Beside of this, some samples collected from active cases were sero-negative for neither of tested antibodies; it indicated there was other cause/s of abortion in the study site. These interesting findings deserve further detail study by using more sensitive diagnostic test in order to examine the full extent of the problem in small ruminant populations. Also, an awareness-raising campaign should be launched to educate farm workers and professionals about proper preventive and control measures for such zoonotic diseases.

Keywords: Abortion, Antibodies, Brucellosis, Ethiopia, Goat, North Shoa, Q-fever, Parities, Seroprevalence, Toxoplasmosis.

DOI: 10.21303/2504-5695.2022.002611

1. Introduction

Ethiopia is an agriculturally based country and owns a considerable number of small ruminants, which is estimated to be over 60 million heads of sheep and goats [1].

Despite these large-small ruminant population sizes, the country fails to optimally utilize these resources as the sector is suffering lower productivity due to various factors in which diseases stand front line [2]. One of the diseases that hamper the productivity of small ruminants is abortion, which has major economic and public health impacts [2]. Reproductive diseases that result in abortion impose great economical loss in productivity and by product of small ruminants [2].

The common diseases that cause abortion both in goats and sheep in sub-Saharan countries are Brucellosis, Campylobacteriosis (Vibriosis), Chlamydiosis, Foot and Mouth Disease (FMD),

Listeriosis, Q-fever, malnutrition, Nairobi Sheep Disease, Rift Valley Fever, Salmonellosis Trypanosomiasis [3, 4] and Toxoplasmosis [5, 6].

Non-infectious causes of abortion like poisoning, malnutrition, stress, inherited abortion, vitamin and mineral imbalances brings great loss in production of small ruminant [5].

Several diseases which infect small ruminants result in abortion or reduced fertility and some may also infect humans (zoonotic diseases) [2, 7].

Toxoplasmosis is zoonotic disease caused by an obligate intracellular parasite known as *T. gondii* [6]. It is the most prevalent parasitic infections in human and veterinary medicine and has negative impacts on public health and animal production. *T. gondii* is believed to be the most triumphant parasitic pathogen in large scale [6].

From wide range of farm animals, sheep and goats are more commonly infected with *T. gondii* than cattle and chicken. This parasite causes abortion and neonatal death in major monetary losses to sheep, goat and pig farming [8, 9]. This is more serious especially when primary infection occurs during pregnancy [10].

During the past decades, *Toxoplasmosis* was reported in different parts of Ethiopia; viz. 35 % in Debre Birhan and surrounding areas [11], 19.7 % in East and West Shewa Zones, Oromia Regional State [12], 15.1 % in sheep and goats slaughtered for human consumption in Central Ethiopia [13], 70.83 % in Menz and Horo areas [14] and 10.25 % in small ruminant in Abergele and Ziquala, Amhara Region [15].

Q-fever is a zoonotic disease caused by *Coxiella burnetii*. Livestock (cattle, sheep, camels and goats) are the main reservoirs of infection to humans [16, 17]. It is also known as an occupational disease of veterinarians, farmers and abattoir workers [18].

Coxiella burnetii, the causative agent has been isolated from ticks. Q-fever is frequently misdiagnosed by physicians [16]. It is endemic, both in livestock and humans in North and Sub-Saharan Africa [19–21].

In Ethiopia, the existence of antibody against *C. burnetii* was reported in goats and sheep slaughtered at Addis Ababa abattoir, and its peri-urban zone [22, 23].

The diagnosis of *Coxiella burnetii* infection in animals is of great importance not only to identify the infected flocks but also to determine the risk of disease transmission to humans [24, 25].

Brucellosis is a disease of animals, especially livestock (cattle, goats, sheep, camels and pigs), but also wild animals [26].

It is caused by bacteria of the genus *Brucella spp.* In livestock, it is primarily a reproductive disease characterized by late abortion, retained fetal membranes, Orchitis and impaired fertility [27].

B. melitensis is considered to have the highest zoonotic potential, followed by *B. abortus*, and *B. suis* [28].

In Ethiopia, serological studies on brucellosis have been carried out in small ruminant; viz. zero serorevalence in Menz and Horo areas [14], in Abergele and Ziquala, Amhara Region [15], 9.6 % in Southeast Pastoral Livestock [29], and 1.55 % in Yabello districts [30].

During the Per-parturient period, the massive multiplication of *C. burnetii* and *Brucella melitensis* occurs within trophoblast cells, causing necrotic suppurative placentitis, which ultimately leads to pregnancy failure in the form of abortion, stillbirth, premature delivery and birth of weak offspring [31, 32].

There were frequent challenges of abortion, stillbirth, retained placenta, premature birth and delivery of weak kids in different goat breeds in Ataye Boer Nucleus Site; Debre Birhan Agricultural Research Centre, Ethiopia [33].

Also full-term kids were weak, with low body weight and high mortality [34]. Therefore the aims of the present study were to determine the rate of abortion and apparent prevalence of Brucellosis, Toxoplasmosis and Q-fever in the study flocks kept in North Shoa, Ethiopia.

2. Materials and Methods

2. 1. Description of study area

The study was conducted at the on-station Boer×Central Highland Goat cross-breeding program carried out at Ataye Research site, Debre Birhan Agricultural Research Center, Ethiopia. The site is located in central Ethiopia and the climate is characterized by bimodal rainfall consisting of the long rainy season (June-September), short rainy season (February-May), and dry season (October-January) [35]. The site's geographic coordinate reference is 10°35′ N latitude and [15]°93′ E longitude and is located at 1481 m above sea level altitude (**Fig. 1**).

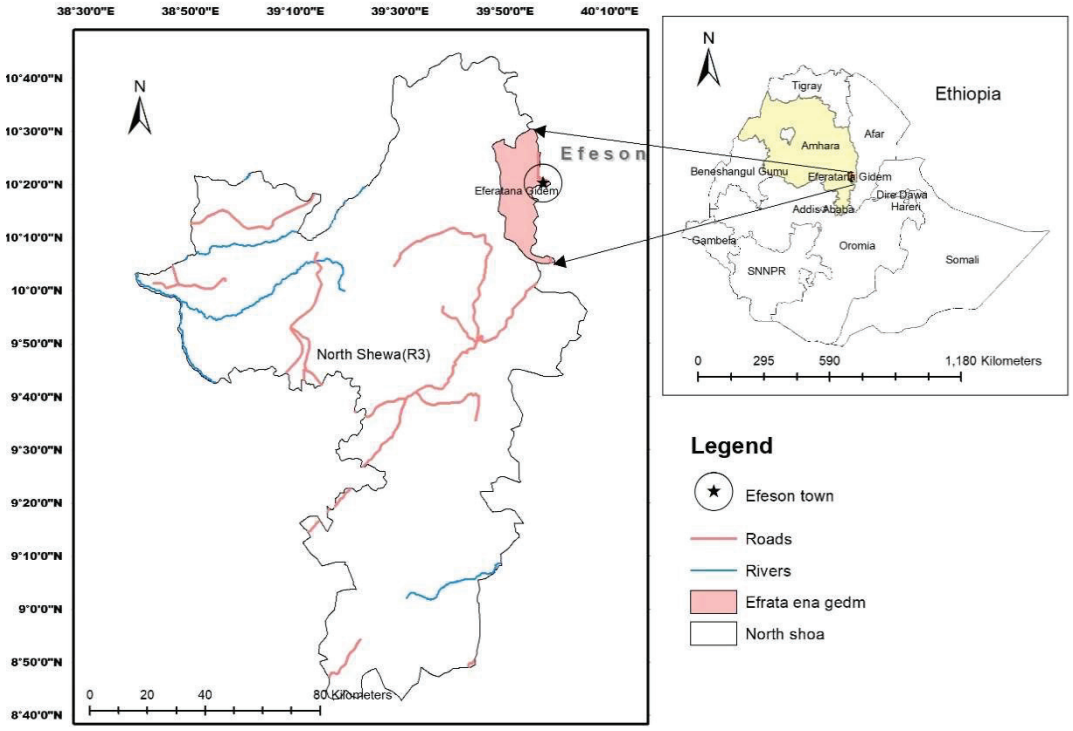

Fig. 1. Map of the study areas

The genotypes of the goats evaluated were pure Boer, Central Highland Goat, and 50 % Boer (pure Boer cross with Central Highland Goat). A total of 503 goats were used to assess the rate of abortion from June 2015 to August 2019. Flocks were reared with two categories of feeding management i.e. intensive and semi-intensive. Pure Boer goats were managed under intensive management system and 49 % Boer and Central Highland Goat were managed under semi-intensive system with grazing and a supplement. The supplement includes *adlibitum grass* hay, chopped pasture (Napier grass, *Desmodium species* and vetch) and commercial concentrate supplement based on their body weight [33].

Animals were treated using anthelmintic drugs that include albendazole, tetramizol, oxyclozanide, ivermectin and triclabendazole. The drugs were applied in three rounds per year in October, February and June following manufacturers' recommendations and deworming months were selected based on the epidemiological cycle of targeted parasite groups and the laboratory findings. Also animals were sprayed against ectoparasites by using diazinon and amitrazine and regular vigilance was performed to ensure feeding, herd health care, proper breeding, and cleanliness of the farm [33].

They were also vaccinated against major infectious diseases which include ovine pasteurellosis, sheep and goat pox, peste des petitis ruminants' /PPR/ small ruminant plague and contagious caprine pleura pneumonia. Regular vigilance was performed to ensure feeding, herd health care, proper breeding, and cleanliness of the farm.

2. 2. Study design and sample collection

Longitudinal clinical case study on abortion was conducted and diagnosis was based on clinical and post-mortem examination. Aborted animals were grouped based on breeds (Pure Boer, 49 % Boer and Central Highland Goat) and round of parity (1st, 2nd, 3rd, 4th and >=5th).

Blood samples were collected directly from the jugular vein of each of the aborted does by using sterile vacutainer tubes and needles for each animal and about 5 ml of blood was drawn. Each sample from each animal was labeled by using codes describing the specific animal. The tubes were kept overnight at a room temperature to allow clotting. Next morning the serums were separated from the blood and collected into 1.8 ml cry vial and were preserved at –20 ºC in deep refrigerator until they were processed.

All sera were initially tested by Rose Bengal Test (RBPT). For RBPT, 30 µl of serum and 30 µl of antigen (Rose Bengal stained *B. abortus* antigen obtained from BIO-RAD, Marnes-la- Coquette, France) were mixed and rotated on a glass plate for 4 minutes. Sera with no visible agglutination were recorded as negative, while sera showing agglutination were considered positive. For further analysis, all RBPT-positive sera were tested by using i-ELISA kits for Brucellosis. ELISA kit was obtained from ID Screen® Brucellosis Serum Indirect Multi-species (ID Vet innovative diagnostic Grabels, France). The sensitivity and specificity of the ELISA test kit as provided by the manufacturer was 100 % and 99.74 %, respectively. The tests were performed according to manufacturer's instructions [36].

All samples were tested against specific antibodies to Q-fever by using indirect enzyme-linked immunosorbent assay (I-ELISA) test using microtiter plates pre-coated with the *C. burnetii* phase I and II strains (ID Screen® Q Fever Indirect Multi-Species, IDvet®). Positive and negative control sera were included in each plate. The sensitivity and specificity of the ELISA test kit as provided by the manufacturer was 99 % and 98 %, respectively. The test was conducted according to the manufacturer protocol.

Also the samples were tested by using indirect ELISA test using ID Screen Toxoplasmosis Indirect Multispecies manufactured by ID vet, France with wells coated with P30 *T. gondii* antigen to detect the anti-Toxoplasma antibody. The sensitivity and specificity of the ELISA test kit as provided by the manufacturer was 100 %. The samples were analyzed in Ethiopian Institute of Agricultural Research; National Agricultural Biotechnology Research Centre.

2. 3. Data management and statistical analysis

The data generated were entered and managed in MS excel work sheet. The data were checked manually for obvious inconsistencies, recording errors or missing data. Data collected were analyzed by using SPSS-20 software version. Descriptive statistics were used to determine the rate of abortion and seroprevalence of Brucellosis, Q-fever and Toxoplasmosis in goats. Univariable logistic regression analyses (LR) was also used to measure the level of association between the possible associated risk factors and clinical abortion. A significance level ($p<0.05$) and confidence level (95 %) was set to determine the presence or absence of statistically significant difference between the given parameters.

3. Results

During the study periods 503 does were entered to mating, of which 100 (19.5 %) animals aborted. Higher rate of abortion were recorded in the second parity and 50 % Boer goats with rate of 35.1 % and 22.8 %, respectively (**Table 1**).

Table 2 showed that the association between different risk factors and abortion. There were statistical significance difference in clinical abortion between 1st (28.1 %) and >=5th ([39].3 %) parities (p=0.045, OR=2.5). Even though there was no statistical difference between first and 2nd and 4th parities they were about 1.6 and 2 times likely to abort than first party, respectively. There was no statistical difference (p>0.05) in clinical abortion among different goat breeds.

In the present study a total of 35 serum samples were collected from does which aborted within six month periods and of which 23 (64.7 %) and 3 (8.6 %) animals were positive for Q-fever and Toxoplasmosis, respectively. But, all animals tested for Brucellosis were negative as illustrated in **Table 3**.

Table 1

The rate of abortion in different goat breeds and parities at Ataye Boer Goat Nucleus, Site North Shewa, Ethiopia

Variables		No. does entered to mating	No. aborted does	Abortion rate (%)
Breed	Pure Boer	104	22	21.2
	50 % Boer	197	44	22.8
	CHG*	212	33	15.6
Parities	1st	104	25	24.0
	2nd	77	27	35.1
	3rd	114	9	7.9
	4th	113	14	12.4
	>=5th	105	25	23.8
Total		503	100	19.5

Note: CHG=Central Highland Goat

Table 2

Univariable logistic regression analyses (LR) of risk factors (breed and round parties) and abortion at Ataye Boer Goat Nucleus Site North Shewa, Ethiopia

Risk factors		No. does conceived	No. does aborted (%)	*p*-value	OR	Confidence interval (95 %)
Breeds	Pure Boer*	56	22 (38.6)	*	*	*
	CHG	115	33 (23.7)	0.156	0.593	0.300–1.214
	50 % Boer	128	44 (35.2)	0.627	1.223	0.521–2.816
Parities	1st*	89	25 (28.1)	*	*	*
	2nd	64	27 (40.5)	0.054	1.958	0.985–3.894
	3rd	56	9 (15.8)	0.484	0.716	0.275–1.863
	4th	50	14 (27.5)	0.340	1.585	0.601–4.162
	>=5th	61	25 (39.3)	0.045	2.494	1.015–6.175
Total		324	100 (30.9)	–	–	–

*Note: * Reference category; CHG=Central Highland Goat*

Table 3

Apparent seroprevalence of Brucellosis, Q-fever and Toxoplasmosis in does at Ataye Boer Goat Nucleus Site North Shoa, Ethiopia

Types of Diseases	No. Animal Examined	No. Animal Positive	Apparent prevalence (%)
Brucellosis	35	0	0.0
Q-Fever	35	23	64.7
Toxoplasmosis	35	3	8.6
Total	105	26	24.8

4. Discussion

Abortion imposes great economical loss in productivity and by product of small ruminants. Clinical study on the prevalence of abortion in goat has been conducted at different times in various countries. The result of the present study conducted from purposively selected Ataye Boer Nucleus Site Debre Birhan Agricultural Research Centre, Ethiopia, showed an overall rate of clinical abortion in goats to be 19.5 %. This rate of clinical abortion indicated the occurrence and wide distribution of abortion causing infectious and/or non-infectious agents in goats in the study site.

During the past years, clinical analysis of reproductive diseases; abortion was reported in Ethiopia [15, 33, 37, 38].

The current study was comparable with 15.8 % reported in Northern Barind Tract [39] and 22 % reported in five upazillas of Mymensingh district in Bangladesh [40].

However, the clinical prevalence of the present study was higher than 1.4 % reported at the state veterinary hospital maiduguri, Nigeria [41].

The finding in this study was much lower than 42.7 % incidence of abortion in Black Bengal goats in Bangladesh [42]. Such inconsistency in the rates of abortion may be due to the variation in

the susceptibility of different breeds to abortion causing diseases, management practices, distribution and load of abortion causing agents, and measures taken to control the diseases.

The parity related clinical abortion in present study showed statistical significant difference (p<0.05) between parities, which disclosed that 5th parity were about 2.5 times more likely to be aborted as compared to first parities. The older the animals are the higher the risk of being infected to abortion causing agent compared to animals younger. In this regard, this study was consistent with that of the previous reports; findings [43] they reported that the age of the animals appeared to be the most significant risk factor for seropositivity of Q-fever. Similarly, other scholars reported that there is statistical difference for seropositivity of Toxoplasmosis and age of animals; adults were more prone to toxoplasmosis than young groups [23, 44, 45].

There was no statistical difference (p>0.05) in the rate of clinical abortion among different goat breeds. This similarity might be due to all breeds were herded together and in direct contact with each other. This result was coincided with the finding of other scholar [45]) who reported no statistical differences among different breeds of goats and sero-positivity of Toxoplasmosis.

Zero seroprevalence of Brucellosis in this study was comparable with other scholar findings; zero in livestock-wildlife interface areas of Zambia [46], 1.55 % in small ruminants in the Yabello district, Ethiopia [30], 2.7 % overall pooled prevalence of brucellosis in goats in Ethiopia [47], zero in goats [14], zero in small ruminant in Abergelle and Ziquala, Ethiopia [15]. However, the sero-prevalence result of the present study was much lower than of the previous findings; 9.6 % in Southeast Ethiopian pastoral livestock [29], 13.2 % in Somali region [48], 5.8 % in the pastoral region of Afar, eastern Ethiopia [49], and 12.4 % in Borena pastoralist [50].

Difference in the management system used in different countries, types of samples and laboratory test, sampling methods and/or absence of infected goat herds may attribute to such variations. In addition, those differences were might be due to variations of environment of study areas. *Brucella* transmission is favored by a more humid climate, which prolongs the survival of the bacteria in the environment [51].

The seroprevalence of 64.7 % of Q-fever in this study was comparable with 68.33 % in Menz and Horo areas, Ethiopian [14], 53.2 % in Southeast Ethiopian pastoral livestock [29]. However, the sero-prevalence result of the present study was higher than that of the previous reports; 7 % in nomadic pastoralists and their livestock in Chad [19], 24.2 % in Gambia [43], 14.3 % in Central African Republic [52] and 15.0 **%** in Punjab, Pakistan [53]. This variation in the prevalence of infection in different geographical areas might be associated with the farm hygienic measures, routine management practices and environmental factors such as vegetation, soil moisture and the presence of infected animals and vectors in the surroundings [54, 55]. Our study showed a higher seroprevalence in goats, which suggests that Q fever, is of considerable importance in the animal population in the study area. The highest seroprevalence observed in the study animals may be due to genetic susceptibility of goats to *C. burnetii*, higher prevalence of tick vectors to goats, grazing management where animals share common graze and watering point, agent could be transmitted though aerosol and/or prevailing climatic and weather conditions in the study site.

The seroprevalence of 8.6 % of Toxoplasmosis in this study was comparable with that of the previous reports; 10.25 % in small ruminant in Abergele and Ziquala, Ethiopia [15]. However, the seroprevalence of Toxoplasmosis demonstrated in this study is higher than that of the previous findings; 4.6 % from goats in Nigeria [56]. However, the sero-prevalence result of the present study was much lower than that of the previous reports; 35 % [11], 19.7 % [12], 15.1 % [13], 70.83 % [14] in Menz and Horo areas, 15.5 % [23], 54.2 % [44], 27.6 **%** [45], 24.1 % [57] using MDAT and 25.9 % using ELISA, and 74.8 % [58] in Ethiopia.

Also, the present finding was lower than other world; 26.8 % in Ghana [59], 31 % in Uganda [60], 27.9 % in Thailand [61] and 19.3 % in Tanzania [62], and as well as 58.4 % [63] and 43.3 % [64] in Egypt. The wide variation in the seroprevalence of Toxoplasmosisi infection seen between the present study and aforementioned studies might be due to difference in sample size, types of study design conducted, agro-ecology, climate, carnivorous density, farm hygienic practices, animal management, type of serological tests used and the cut-off value used [6, 65].

5. Conclusions

Based on clinical study, the present finding revealed that abortion was the major constraint of kid loss with 19.5 % rate of clinical abortion in goats. The significant risk factor associated with abortion is parity. Serological investigation of antibody against Q-fever and Toxoplasmosis showed that the agents were the major causes of abortion and endemic in the study site with seroprevalence of 64.7 and 8.6 %, respectively.

Even though there was no positive reactor does to Brucellosis, the result must be interpreted with care since absence of evidence is not evidence of absence, and even a single infected animal can serve as source of infection and contribute to spread of the bacteria within flock and to different areas. Beside of this, some samples collected from active cases were seronegative for neither of tested antibodies; it indicated there was other cause/s of abortion in the study site. Serological survey highlights the importance of clinical herd health management to avoid economic losses in animals.

These findings deserve further detail study by using more sensitive diagnostic test in order to examine the full extent of the problem in small ruminant populations and other animals, taking into account those infections with different causes of reproductive problems and to formulate appropriate disease control and prevention method so as to reduce the impact of the disease in animal production in Ethiopia. Also, an awareness-raising campaign should be launched to educate farm workers and professionals about proper preventive and control measures for diseases.

Conflict of interests

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

Acknowledgement

We would like to thank Ethiopian Institute of Agricultural Research; National Agricultural Biotechnology Research Centre for laboratory analysis. Also, we would like to express our sincere appreciation to Debre Birhan Agricultural Centre; Amhara Agricultural Research Institute for giving us all the necessary facilities for the completion of this work.

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Received date 03.08.2022
Accepted date 14.09.2022
Published date 30.09.2022

How to cite: *Alamerew, E. A., Yitagesu, E., Areaya, A., Aydefruhim, D. (2022). Apparent prevalence of brucellosis, Q-fever and toxoplasmosis in aborted goat's at North Shoa, Ethiopia. EUREKA: Life Sciences, 5, 28–37. doi: https://doi.org/10.21303/2504-5695.2022.002611*