Original Article

Prevalence and Identification of Infectious Abortion Pathogens in Sheep Flocks of North Khorasan, Iran

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ABSTRACT

Abortion has been identified as a primary factor contributing to reproductive losses in small ruminant flocks worldwide. Infection with the agents including Toxoplasma gondii, Campylobacter spp., Chlamydia abortus, and Coxiella burnetii frequently occurs worldwide. In Iran and its neighboring countries, Brucella melitensis has been identified as the predominant cause of abortion. It has been demonstrated that other abortifacient agents, such as C. abortus and C. burnetii, are prevalent among sheep flocks as well. The present study sought to investigate the presence of the most common abortifacient pathogens in sheep in North Khorasan, Iran. The samples were obtained from 133 aborted sheep fetuses. Subsequently, the presence of pathogens was assessed through the implementation of ELISA, conventional PCR, and bacteriological examination. The identified pathogens encompassed Escherichia coli, B. melitensis, Salmonella spp., C. burnetii, Campylobacter spp., Leptospira spp., Listeria monocytogenes, Toxoplasma gondii, Border disease virus, and Blue Tongue virus. The utilization of bacteriological culture techniques resulted in the isolation of E. coli (9%) and B. melitensis (12%). The following pathogens were identified in fetal serology: C. burnetii (2.5%), T. gondii (12%), B. duncani (3%), and B. abortus (9%). B. melitensis (12%), Salmonella (8.5%), Campylobacter spp. (1.7%), Leptospira spp. (2.5%), Chlamydia abortus (25.6%), C. burnetii (14.5%), and T. gondii (6.8%) were detected by PCR. C. abortus was the most prevalent pathogen detected by PCR (25.6%). The present results demonstrated that the studied sheep flocks are infected with the most significant abortifacient pathogens, thereby underscoring the necessity for further investigations to identify abortion causes based on different geographical regions using simple and sensitive methods. The prevalence of ovine abortion is influenced by epidemiological and risk factors, which necessitate further investigation.

Keywords: Abortion; Brucellosis; *Chlamydia Abortus*; Sheep; Small Ruminant.

1. Introduction

The small ruminant industry plays a significant role in livestock production, particularly in the production of sheep meat, which is the most popular meat consumed by the Iranian population (1). The primary objective of ovine rearing in Iran is the production of meat, with an annual output of 265,000 tons (2). The potential profitability of meat production in this country is significant. Nonetheless, the species may face potential threats from various factors, including reproductive failure, which has the capacity to significantly diminish both meat and milk production. Abortion has been identified as a significant contributing factor to reproductive setbacks in flocks of small ruminants, encompassing both infectious and non-infectious underlying causes. An array of agents, comprising bacteria, viruses, fungi, and protozoa, have been identified as potential contributors to abortion. Furthermore, non-infectious etiologies such as trauma, elevated body temperature, concentrated densities, toxicities from plant origins, and nutrient deficiencies are prevalent (3). Brucella melitensis is the principal infectious agent responsible for economically significant cases of abortion in Iran and various other countries (4). Furthermore, global instances of abortion caused by other pathogens, including Toxoplasma gondii, Campylobacter spp., Chlamydia abortus, and Coxiella burnetii, are frequent (5). It is noteworthy that viral agents such as the Blue Tongue Virus, Border Disease Virus, and Akabane virus are prevalent in regions where small ruminants are commonly used as a source of meat (5). In order to consider preventive measures and reduce economic losses, it is necessary for each country to estimate the presence of abortifacient agents in different geographical areas. In the domain of discerning and identifying the primary culprits behind the occurrence of miscarriages, the utilization of precise techniques boasting impeccable sensitivity and specificity assumes paramount significance. The present study sought to utilize ELISA, conventional PCR, and bacteriological examination to assess the presence of the most common abortifacient pathogens among sheep flocks in North Khorasan, Iran.

2. Materials and Methods

2.1. The structural Configuration of the Research Investigation

From 2016 to 2017, an investigation was conducted to ascertain the etiology of fetal losses in ovine flocks located in North Khorasan. The subjects of the study were sheep of the Moghani breed, and the cases examined were devoid of any placenta. A comprehensive examination of a total of 133 fetuses was undertaken, employing bacteriological culture, ELISA, and PCR methods in pursuit of identifying the precise causes of abortion. The examination protocol encompassed the evaluation of all 133 fetuses for the presence of BDV, BTV, and E. coli. Additionally, an assessment of other potential pathogens was conducted on 117 fetuses out of the original 133. The laboratory received the aborted fetuses while adhering strictly to the principles of cold chain logistics, ensuring proper preservation utilizing adequate ice pack distribution.

2.2. Sample Collection

The fetuses were necropsied in a sterile manner, and then, fetal fluids were extracted from the thoracic and pericardial cavities using sterile syringes for the ELISA test. A variety of parenchymatous tissues and fluids, including those from the liver, spleen, kidneys, lung, and abomasal contents, were obtained with the objective of subjecting them to microbiological culture and polymerase chain reaction (PCR) analysis.

2.3. ELISA

Sera from aborted fetuses were subjected to testing for antibodies against C. burnetii, C. abortus, and T. gondii using a commercial indirect ELISA kit from IDvet, France. Infections with BDV and BTV were examined using a competitive ELISA kit from the same manufacturer, following their instructions.

2.4. PCR Assay

A CinnaGen DNA extraction kit was utilized to extract DNA from both the abomasal contents and the homogenized tissues. The detection of B. melitensis, Salmonella, Campylobacter spp., C. abortus, C. burnetii, T. gondii, L. monocytogenes, and Leptospira spp. was conducted through conventional PCR using methods that had been previously established and genus- and species-specific primers that had been cited in the literature.

2.5. Bacteriological Procedure

Consequently, bacteriological procedures were performed for the detected agents, based on the positive results of the PCR. However, the procedure of isolation was generally conducted for the purpose of diagnosing every involved pathogen. Subsequent to initial plating onto blood agar and MacConkey agar (Merck, Germany), the homogenized tissues were incubated aerobically and anaerobically for 48 hours at 37°C in pairs of plates. Following a 24-hour incubation period, an inspection of the bacterial growth was conducted, and colonies of interest were subsequently subjected to a series of biochemical tests. These tests were based on colony morphology and gram staining results, with the objective of obtaining and identifying pure bacterial cultures.

3. Results

The utilization of diverse diagnostic methodologies in this study yielded the identification of various pathogens, including C. abortus, E. coli, B. melitensis, Salmonella, Campylobacter, Leptospira, C. burnetii, T. gondii, BDV, and BTV, in the aborted fetuses (see Table 1 for details). T. gondii (12%), BTV (9%), BDV (3%), and C. burnetii (2.5%) were identified in fetal serology. The following were detected by PCR: C. abortus (25.6%), C. burnetii (14.5%), B. melitensis (12%), Salmonella spp. (8.5%), T. gondii (6.8%), Leptospira spp. (2.5%), and Campylobacter spp. (1.7%). Furthermore, positive results from the polymerase chain reaction (PCR) for B. melitensis, Salmonella spp., Campylobacter spp., Leptospira spp., L.

monocytogenes, and E. coli were utilized for bacteriological examination. B. melitensis (12%) and E. coli (9%) were isolated using bacteriological culture media. C. abortus was identified as the most prevalent agent in 25.6% of the studied fetuses. It was observed that all PCR-positive results were not consistent with the ELISA method and bacteriological procedures (Table 1). Furthermore, Brucella isolates in culture from fetuses were also confirmed by PCR, with all 15 samples yielding positive results.

4. Discussion

The present study identified some of the most prevalent abortifacient pathogens in sheep and identified C. abortus as the primary agent, accounting for 25.6% of cases (Table 1). C. abortus has been identified as the primary cause of abortion in various countries and poses a significant global challenge to the reproduction of small ruminants. This gram-negative bacterium has been identified as the causative agent of economic losses across Europe, North America, and Africa, accounting for 45% of ovine abortions in the UK (14). In their 2022 study, Esmaeili et al. observed bacterial involvement in 46.9% of small ruminant abortion cases in Iran, with C. abortus, B. melitensis, and C. burnetii notably implicated, particularly in instances where abortion rates exceeded 10% (15). The present data, when considered in conjunction with the preceding report, indicate that enzootic ovine abortion is an endemic disease in our nation (15). Given the direct correlation between infection rates and flock hygiene, educational initiatives should be implemented to mitigate the incidence of new infections, particularly in rural areas where rearing systems are less sophisticated. Fetal serology has been demonstrated to be a valuable diagnostic tool for the identification of Chlamydia-specific antibodies. It is evident that maternal antibodies are incapable of reaching the fetus through the placenta, while fetal antibodies are derived from uterine exposure. Consequently, the detection of any antibody titer is presumed to indicate the presence of the abortifacient chlamydial species, such as C. abortus. A comparable study conducted in Northern Ireland in 2001 revealed the presence of anti-Chlamydia antibodies in 70 out of 417 fetal blood and thoracic fluid samples (16). In the present study, while PCR revealed a high rate of fetal infection with C. abortus (Table 1), no antibody reaction was detected in the samples. This finding underscores the necessity of employing more sensitive methodologies with suitable samples. The Iran Veterinary Organization has initiated a national plan to address brucellosis, recognizing its significant role in small ruminant abortions in the country. As part of this plan, the reduced-dose Rev-1 vaccine against B. melitensis has recently been excluded, while concerns persist about the limited duration of immunity provided by the full-dose Rev-1 vaccine compared to the reproductive lifespan of ewes. Despite the implementation of vaccination programs, B. melitensis continues to pose a significant challenge to small ruminant flocks. Current data demonstrate that the bacterium is detected in 12% of aborted fetuses, with both polymerase chain reaction (PCR) and isolation methods employed in the diagnostic process (Table 1). In the present study, a polymerase chain reaction (PCR) analysis was conducted, which revealed C. burnetii as the second most frequently detected bacterium (14.5%). Mostafavi et al.'s 2019 study emphasizes the public health significance of Q fever, caused by C. burnetii, in different regions of Iran, often resulting from direct or indirect contact with ruminants (18). In the absence of vaccination, the implementation of strict biosecurity measures is imperative for the control of coxiellosis (19). As indicated by the findings presented in Table 1, the polymerase chain reaction (PCR) method demonstrated heightened sensitivity in comparison to the enzyme-linked immunosorbent assay (ELISA). Furthermore, the presence of the C. burnetii antibody was detected in a mere 2.5% of the fetal samples examined. The utilization of serological methodologies may result in the underestimation of infected cases, given the constrained validity of these approaches in detecting C. burnetii and the potential variability in the sensitivity of commercially available ELISA kits. Salmonella spp. was identified through the polymerase chain reaction (PCR) in

Table 1. The identified pathogens in the aborted fetuses based on the applied methods.

Pathogens	Number of fetuses	Agent isolation	ELISA	PCR
Escherichia coli	133	12 (9%)	-	-
Brucella melitensis	117	15 (12%)	-	15 (12%)
***Salmonella* spp.**	117	0	-	10 (8.5%)
***Campylobacter* spp.**	117	0	-	2 (1.7%)
Listeria monocytogenes	117	0	-	0
***Leptospira* spp.**	117	-	-	3 (2.5%)
Chlamydia abortus	117	-	0	30 (25.6%)
Coxiella burnetii	117	-	3 (2.5%)	17 (14.5%)
Toxoplasma gondii	117	-	14 (12%)	8 (6.8%)
BDV+	133	-	4 (3%)	-
BTV++	133	-	12 (9%)	-

+: Border Disease Virus, ++: Bluetongue virus, -: The test wasn't performed, 0: The test was done and the result was negative.

8.5% of the samples examined in this study. Given its association with abortion and lamb mortality, it is imperative to incorporate preventive measures and carrier identification into the abortion control plan (20). We then proceeded to examine the PCR-positive samples by bacteriological procedures (Table 1). The negative result obtained from the isolation method indicated that PCR is a more sensitive method, despite the fact that isolation has high specificity and is generally considered a gold standard. Consequently, negative results from bacteriological samples must be confirmed by molecular methods. The association between infection with Leptospira spp. and reproductive failure in small ruminants, particularly goats, is well-documented (21). In the present study, polymerase chain reaction (PCR) analysis detected the bacterium in 2.5% of the samples, while Campylobacter spp. was not isolated through bacteriological procedures. However, DNA from the bacterium was detected in only 1.7% of the fetuses, indicating a lower rate of fetal infection with the bacterium. A similar phenomenon was observed in an abortion outbreak in Iran in 2022, where Campylobacter spp. was isolated in 3.3% of fetuses. This occurrence was less frequent when compared to other bacterial abortifacients, including C. abortus, C. burnetii, and B. melitensis (22). In the present study, E. coli was isolated from 9% of fetal samples. However, the bacterium is often neglected in abortion cases. The seroprevalence of T. gondii infection was found to be 12%. However, DNA from T. gondii was identified in 6.8% of the fetuses. As the parasite is lethal to ovine fetuses and has zoonotic potential, determining the infected animals is crucial. Currently, there is no approved vaccine available in Iran. In such circumstances, the implementation of strategies is imperative to prevent exposure of small ruminants to stray cats and to mitigate the likelihood of cats accessing the animals' feed (23). Border disease (BD) is recognized as endemic among small ruminants in Iran; however, there is a paucity of studies focused on the detection of BDV in sheep and goats. The results of the study indicated that 3% of the fetuses were infected with BDV. Given ELISA's sensitivity and value for virus detection in fetal fluid (24, 25), the present study emphasizes the assessment of seroprevalence in other regions of Iran. Furthermore, an analysis was conducted to ascertain the prevalence of BTV among the flocks, and the virus was detected in 9% of the fetuses. A previous study conducted by Esmaeili et al. in 2022 reported BTV in 1.9% of aborted fetuses in Iran (15). The present study employed a combination of PCR, ELISA, and bacterial isolation methodologies to investigate the etiology of abortion in fetal tissues and fluid. However, the identification of L. monocytogenes was not successful; consequently, it is necessary to utilize a variety of samples, including placenta from additional aborted animals. Given the zoonotic nature of many abortion causes of small ruminants; vaccination programs must be implemented as a primary strategy. Furthermore, given that the majority of sheep in Iran are managed under extensive farming systems, the absence of adequate educational resources among farmers can compromise the efficacy of any abortion control initiative. The present study demonstrated the necessity for further investigation, which can be accomplished through the utilization of straightforward and sensitive methodologies such as molecular tests. Moreover, additional epidemiological and risk factor research is necessary to further elucidate the etiology of ovine abortion.

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Authors' Contribution
Study concept and design: H. E and H.GH
Acquisition of data: H. E, P.B and M.SH
Analysis and interpretation of data: H. E.M.SH and M.H
Drafting of the manuscript: H.E and M.H
Administrative, technical, and material support: P.B and M.SH
Study supervision: H. E and H.GH

Ethics
The authors of this study affirm that all ethical standards were upheld in the preparation of the submitted article.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Data Availability
The data produced and/or analyzed during the present study can be obtained from the corresponding author upon request.

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