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


## Abstract

Abortion is one of the main causes of reproduction losses in small ruminant's flocks in the world. Infection with the agents including *Toxoplasma gondii*, *Campylobacter* spp., *Chlamydia abortus*, and *Coxiella burnetii* frequently occurs worldwide. *Brucella melitensis* is the most important cause of abortion in Iran and its neighbors. Other abortifacient agents such as *C. abortus* and *C. burnetii* are prevalent among sheep flocks as well. The present study aimed to investigate the presence of the most common abortifacient pathogens in sheep in North Khorasan, Iran. The samples were collected from 133 aborted sheep fetuses. Then, using ELISA, conventional PCR and bacteriological examination the presence of pathogens including *Escherichia coli*, *B. melitensis*, *Salmonella* spp., *C. burnetii*, *Campylobacter* spp., *Leptospira* spp., *Listeria monocytogenes*, *Toxoplasma gondii*, Border disease virus and Blue Tongue virus were assessed. Using bacteriological culture, *E. coli* (9%) and *B. melitensis* (12%) were isolated. *C. burnetii* (2.5%), *Toxoplasma gondii* (12%), Border disease virus (3%) and Blue Tongue virus (9%) were identified in fetal serology. *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 frequent pathogen detected by PCR (25.6%). The present results showed the studied sheep flocks are infected with the most important abortifacient pathogens which emphasize the demand for more investigations for the detection of abortion causes based on the different geographical regions using simple and sensitive methods. Epidemiological and risk factors contribute in ovine abortion is further necessary.



**Keywords:** Abortion, Brucellosis, *Chlamydia abortus*, Sheep, Small ruminant.

## 1. Introduction

Small ruminant industry has a huge proportion in livestock production especially sheep meat which is the most popular species to consume among Iranian people (1). The main principal goal of ovine rearing in Iran is meat production and Iran produces 265,000 tons of meat annually (2). This potential of meat production is profitable for this country. However, it may be threatened by several factors including reproduction failure which considerably reduces both meat and milk production.

Abortion stands as a prevalent catalyst for reproductive setbacks in flocks of small ruminants, encompassing both infectious and non-infectious underlying causes. An assortment of agents encompassing bacteria, viruses, fungi, and protozoa may lend themselves to the occurrence of abortion. Additionally, non-infectious causes such as trauma, elevated body temperature, concentrated densities, toxicities from plant origins, and nutrient deficiencies are prevalent (3).

*Brucella melitensis* represents the principal infectious agent responsible for economically significant cases of abortion in Iran and various other countries (4). In addition, global instances of abortion caused by other pathogens, including *Toxoplasma gondii*, *Campylobacter* spp., *Chlamydia abortus*, and *Coxiella burnetii*, are frequent (5). Notably, 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).

Every country needs to estimate abortifacient agents in different geographical areas to consider preventive measures and reduce economic losses. In the realm 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 aimed to use 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

In the time span of 2016-2017, an investigation was conducted to ascertain the etiology behind fetal losses in sheep flocks situated in North Khorasan. The examined sheep belonged to 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. These examinations included testing all 133 fetuses for the presence of BDV, BTV, and *E. coli*. Furthermore, an assessment of other potential pathogens was carried out in 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 aseptically and then, fetal fluids were aspirated from thoracic and pericardial cavities using sterile syringes for ELISA test. Various parenchymatous tissues and fluids, such as the liver, spleen, kidneys, lung, and abomasal contents, were acquired with the intention of subjecting them to both microbiological culture and polymerase chain reaction analysis.

### 2.3. ELISA assay

Sera from aborted fetuses were tested for antibodies against *C. burnetii*, *C. abortus*, and *T. gondii* with a commercial indirect ELISA kit from IDvet, France, and 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 used 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 previously established methods and genus- and species-specific primers cited in the literature.

### 2.5. Bacteriological procedure:

Based on the PCR-positive results, bacteriological procedures were performed for the detected agents. Nevertheless, the procedure of isolation was generally conducted to diagnose every involved pathogen. Following 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. After 24 hours of incubation, bacterial growth was inspected, and colonies of interest were subjected to appropriate biochemical tests based on colony morphology and gram staining results to obtain and identify pure bacterial cultures.

## 3. Results:

Regardless of the diagnostic methods used in this study, the pathogens including *C. abortus*, *E.coli*, *B. melitensis*, *Salmonella*, *Campylobacter*, *Leptospira*, , *C. burnetii*, *T. gondii*, BDV and BTV were detected in the aborted fetuses (Table 1). *T. gondii* (12%), BTV (9%), BDV (3%) and *C. burnetii* (2.5%) were identified in fetal serology. *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%) were detected by PCR. PCR-positive results of *B.*

*melitensis*, *Salmonella* spp., *Campylobacter* spp., *Leptospira* spp., *L. monocytogenes*, and *E. coli* were also used for bacteriological examination. *B. melitensis* (12%) and *E. coli* (9%) were isolated using bacteriological culture media. *C. abortus* was the most frequent agent in the studied fetuses (25.6%). All the PCR-positive results were not positive in the ELISA method and bacteriological procedures (Table 1). Brucella isolates in culture from fetuses were also confirmed by PCR, and all 15 samples were positive in PCR.

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.

## 4. Discussion

The current study identified some of the most common abortifacient pathogens in sheep and identified *C. abortus* as the main 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 small ruminant reproduction. Responsible for economic losses across Europe, North America, and Africa, this gram-negative bacterium is accountable 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 current data in agreement with the previous report showed enzootic ovine abortion as an endemic disease in our country (15) and since the infection rate has a direct

relationship with the hygiene level of a flock, educational attempts should be considered to reduce the occurrence of new incidences of the infection especially in rural condition in which the rearing system is primitive.

Fetal serology can be helpful for the detection of Chlamydia-specific antibodies. As maternal antibody fails to reach to fetus through the placenta and fetal antibody is from uterus exposure, detection of any antibody titration presumably shows the abortifacient chlamydial species such as *C. abortus*. In a similar study in Northern Ireland in 2001, anti-Chlamydia-antibody was detected in 70 out of 417 fetal blood and thoracic fluids (16). In the current research, although PCR showed a high rate of fetal infection with *C. abortus* (Table 1), no antibody reaction was detected in the samples. This indicates the importance of using more sensitive methods with appropriate 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 vaccination efforts, *B. melitensis* remains a challenge for small ruminant flocks, with current data showing its detection in 12% of aborted fetuses using both PCR and isolation methods (Table 1).

In the current study, PCR analysis revealed *C. burnetii* as the second most frequently detected bacterium (14.5%). Mostafavi et al.'s 2019 study underscores the public health significance of Q fever, caused by *C. burnetii*, in different parts of Iran, often stemming from direct or indirect contact with ruminants (18). In the absence of vaccination, implementing strict biosecurity measures is essential for control of coxiellosis (19). According to table 1, we found the PCR method more sensitive than ELISA and antibody against *C. burnetii* was merely

detected in 2.5% of the fetuses. It indicates using serological methods may lead to missing many infected cases since their validity to detect *C. burnetii* is limited and available ELISA kits may have different sensitivities.

*Salmonella* spp. was detected exclusively through PCR in 8.5% of the samples in this study. Due to its association with abortion and lamb mortality, it is crucial to incorporate preventive measures and carrier identification into the abortion control plan (20). We examined PCR-positive samples by bacteriological procedures (Table 1). The negative result in the isolation method revealed that PCR is a more sensitive method though isolation has high specificity and is a golden standard. Accordingly, negative results from bacteriological samples need to be confirmed by molecular methods.

It is evident that infection with *Leptospira* spp is associated with reproductive failure in small ruminants especially goats (21). In the current study, PCR analysis detected the bacterium in 2.5% of the samples, while *Campylobacter* spp. was not isolated through bacteriological procedures. However, DNA of the bacterium was detected in only 1.7% of fetuses which showed a lower rate of fetal infection with the bacterium. Similarly, in an abortion outbreak in 2022 in Iran, *Campylobacter* spp. was isolated in 3.3% of fetuses and was less frequent compared to other bacterial abortifacients including *C. abortus, C. burnetii* and *B. melitensis* (22).

*E. coli* was isolated from 9% of fetal samples in the present study. Nevertheless, the bacterium is neglected in most of the abortion cases. We found the seroprevalence of infection with *T. gondii* was 12%. Nonetheless, DNA from T. gondii was identified in 6.8% of the fetuses. As the parasite is fatal for ovine fetuses and has zoonotic potential, determining the infected animals is crucial. There is no approved vaccine available in Iran. In this situation,

strategies including the prevention of exposure of small ruminants to stray cats and reducing the chance of cat's access to the animals' feed are essential (23).

Border disease is recognized as endemic among small ruminants in Iran, yet there is a scarcity of studies focused on the detection of BDV in sheep and goats. Our results showed 3% of the fetuses were infected with BDV. As ELISA is sensitive and valuable for virus detection in fetal fluid (24, 25), the present study emphasizes on more seroprevalence evaluation in other parts of Iran. We also studied the prevalence of BTV among the flocks and the virus was detected in 9% of the fetuses. A recent previous study conducted by Esmaeili et al. in 2022, reported BTV in 1.9% of aborted fetuses in Iran (15).

Using PCR, ELISA and bacterial isolation methods, the present study showed some of the leading causes of abortion in fetal tissues and fluid. However, we didn't identify *L. monocytogenes* so it is necessary to use a variety of samples including placenta from more aborted animals. Since many abortion causes of small ruminants are zoonotic, vaccination programs are required as a primary strategy. Moreover, considering that most of the sheep in Iran are kept in an extensive system, the lack of proper education among farmers can lead to failure in any abortion control plan. The present study showed the demand for more investigations which can be achieved by simple and sensitive methods like molecular tests. Besides, epidemiological and risk factors that contribute to ovine abortion are further necessary.

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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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