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Evaluation of *Coxiella burnetii* Excretion in Parturition Discharge of Goats With Full Term Delivery Using PCR Method

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ABSTRACT

Background: *Coxiella burnetii* the causative agent of Q fever and one of the most important abortifacient agents in goats, is a gram negative bacteria. During an outbreak of coxiellosis, the infected animals shed *Coxiella burnetii* through vaginal discharges, feces, milk and the urine. The present study focused on the presence of *Coxiella* in the parturition discharges of goats with full-term delivery.

Methods: A total of 25 goats in their first parturition and 25 other goats in their second parturition with no clinical signs of infection caused by *Coxiella burnetti*, were randomly selected and examined. The vaginal swab samples which had been collected from the animals were extracted and tested by PCR.

Results: In the 25 goats that had parturated for the first time, 19 positive samples were detected (76%). Eighteen samples that had been collected from other goats in their second parturition ,had *C.burrenti* DNA in their vaginally swabs (72%). The infection rate in goats that had partuated for the first time ,was higher than the goats in their second parturition, had though the difference was not significant.

Conclusion: According to the high prevalence of *Coxiella burrentii*,the risk of abortion in goats and also as coxiellosis is a zoonotic disease, it is necessary to pay more attention to the bacterium and to conduct appropriate strategies for prevention of disease**.**

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Introduction

Coxiella burnetii can survives in arthropods hosts and primary spreads to ruminants (1). The bacterium infects it's hosts asymptomatically. though as it has a tropism to reproductive organs, (2) it may cause abortion, infertility or birth of weak offspring in sheep and goats (3).

The disease which is named Q fever, is more important in caprine flocks than sheep and may affect approximately 90% of them (4). Moreover, goats have identified as the source of outbreaks of Q fever in the human. These animals show abortion more than other susceptible species (5) Also up to 70-90% of them may lose their fetuses (6).

During an outbreak of coxiellosis, there is an extensive excretion of *Coxiella* in the animal housing which is highly contaminated (7). So the bacterial agent may persist in a flock for at least 2 years and some animals shed the bacterium in 2 consecutive parturitions (8, 9).

The infected animals shed *C. burnetii* through their vaginal discharges, feces, milk and urine for weeks after abortion or normal parturition (4, 6). It has been reported that up to 95 days post parturition, *C. burnetii* is excreted via feces and vaginal mucus (10). In addition, pregnant infected goats can shed the bacterial agent even for weeks before parturition through feces (11). Alvarez-Alonso *et al*. (2018) showed the vaginal discharges as the most important rout of excretion of *C. burnetii* and the longer prolong of shedding of the bacterium in comparison with other routs (4).

The importance of goats in spreading of *Coxiella* is more than other ruminants (7) so the presence of these animals among other ruminants may accelerate the rate of infection and economic losses due to abortion. Traditionally, in Iranian small ruminant flocks, a significant population of goats live along with sheep which are potential risk of infection for humans and other animals. Moreover, recently the number of industrial flocks which raise saanen and alpine goats has been increased in Iran, while their infection rate with *C. burnetii* is not clear.

Q fever is endemic among sheep and goat flocks in our country (12) and based on recent research it has high prevalence specially among goats (13).

It is obvious that aborted animals shed *Coxiella* via vaginal discharges, but the knowledge about the role of non-aborted goats in shedding, maintenance and transmission of *C. burnetii* is not clear in Iran. The aim of current investigation was to identify the presence of *C. burnetii* in the parturition discharges of animals which have no clinical signs.

Materials and Methods

Collection of samples

A total of 50 Saanen and Alpine goats in a farm in Tehran were chosen, 25 were in their first pregnancy and the others were in their second pregnancy. The animals were healthy and clinically had no signs of coxiellosis. All the goats had no history of abortion and had been vaccinated against *Brucella melitensis*. Vaginal swabs in normal saline had been taken from the goats within 24 hours of parturition.

Conventional Polymerase Chain Reaction

DNA was extracted from the vaginal samples using CinnaGen DNA extraction kit. Then PCR was conducted using Forward and reverse primers for amplification of 687-bp fragment of the IS1111 gene of *C. burnetii* including TATGTATCCACCGTAGCCAGTC and

CCCAACAACACCTCCTTATTC were used respectively.

PCR was performed using 2 µM of each primer, 200 µM of each dNTPs, 1.5 mM $MgCl_2$ and 0.3 U Taq DNA polymerase. The final volume of reaction mixture was amounted to 25 µl including 20 µl master mix and 5 µl template DNA.

Amplification was carried out in the automated DNA thermal cycle using the following cycling parameters: Denaturation at 94 °C for 30s, subsequently 5 cycles of 66-61°C (in each step, the temperature dropped by one degree) for 60s and 72°C for 60s, then the second denaturation at 94°C for 30s, subsequently 30 cycles of 61 °C for 30s and 72 °C for 60s. The final extension was performed at 72 °C for 10 min.

Statistical analysis

The *C.burnetii* shedding with regard to the first or second pregnancy of the goats was statistically analyzed using Chi-squared test. $P \leq 0.05$ was considered significant in the SPSS software version 19.

Results

In the 25 goats that had parturated for the first time, 19 positive samples which harboured *C.burnetii* DNA in their vaginal swabs, were detected (76%). Eighteen samples had been collected from the other goats with second parturition (72%).

The infection rate in the goats that had parturated for the first time was higher than the goats in their second parturition, though the difference was not significant ($P > 0.05$)

Table 1. Positive goats according breed and parturition**.**

Breed	First parturition	Second parturition
Alpine N(%)	9 (36%)	8 (32%)
Saanen N(%)	10 (40%)	10 (40%)

Discussion

The present study evaluated the excretion of *C. burnetii* in parturition discharges of clinically healthy goats without the history of abortion for the first time. Our results showed high infection of the studied goats, so that among 50 animals, 74% of them had *C. burnetii* in their vaginal discharges (Table 1).

Other studies that had been done in Iran also showed high infection rate of small ruminants. In the study of Nokhodian *et al*., (2017) in Iran, the seroprevalence of *Coxiella* among ruminants and goats was 27% and 33% respectively (14). In the recent study in 2020 in northwest of Iran, the prevalence of the bacterium in goat's milk was 16.6% (15). In addition, Mostafavi *et al*., (2019) showed that Q fever is a public health issue in different parts of our country (16).

A meta-analytic study in 2017 in Iran, revealed that the seroprevalence of coxiellosis among the sheep and the goat flocks were 96.07% and 93.42%, respectively (1). Ezatkhah *et al*. (2015) reported that the seroprevalence of coxiellosis among small ruminants in southeast counties of Iran was between 17.1% to 39.2% (18).

The present study proved that infected non-aborted goats had a predominant role in the spreading of *C. burnetii* for long time in a flock. They could excrete the bacterium which is the key factor of maintenance of the infection among animals, asymptomatically (10). de Cremoux *et al*,

(2012) detected 10^6 *C. burnetii* in vaginal swabs of 30.8% of goats with full-term delivery (19). In the study of de Oliveira *et al*. (2018), in 10.5 % of goat's placentas with healthy kids, *C. burnetii* was detected. As the bacterium didn't lead to abortion in this goats despite the placenta infection,The researchers concluded that a latent infection had occurred and there was a possibility of the excretion of the agent and contamination of the environment by these goats (20).

The results of Roest *et al*. (2012) study showed no association between *Coxiella* excretion and pregnancy outcome (abortion or live-born kid), though it is assumed that aborted goats shed more bacteria than full term goats (10). In contrast to the study of Canevari et al. (2018), that reported some of the full-term goats shed *Coxiella* in concentrations similar to the aborted goats (5).

It is evident that even if goats don't excrete *Coxiella* via placenta or birth fluids, there is a risk of execration via colostrum (21). Moreover, it had been reported that live kids from infected goats had DNA of *Coxiella* in their organs (10). So these cleanically healthy animals are the cause of persistence of Q fever in flocks. During the first pregnancy, when trophoblast cells arise, the cells can give the infection from these sources of persistent infection (21). The results of current study also emphasize that goats with-full term delivery should be considered in control programs of abortion cases due to *C. burnetiipresence*.

While most of the studies which were conducted in Iran, had focused on the bacterium shedding via milk and it's risk factors for human, in a way that other shedding ways such as vaginal route were ignored, the present study showed vaginal discharges as an important source of the bacterial shedding and environmental contamination. It seems that vagina and rectum are important sources of *C. burnetii*,and it should be noted that goats shed the bacterial agent more than sheep and for longer period (7)**.** When *Coxiella* is introduced in a flock, animal infection and shedding may continue for several years (22).

Bauer *et al*., (2020) found that the infected goats continued shedding the pathogen at the next lambing season (7). Accordingly, when Q fever appears in a goat herd, the controlling procedures should be maintained for a long periods of time (4). On the other hand, it should be noted that because the infectious abortifacient agents could be also detetcted in healthy animals, the isolation or detection of a bacteria is not necessarily considered as the only causative factor in abortion.

In the present study, although the infection rate was higher in goats which had parturated for the first time, though the difference was not significant (P >0.05). In contrast with our result, Esmaeili *et al*. (2019) found that younger goats shed *C. burnetii* significantly more than older ones through milk (13).

Conclusion

In conclusion, the high prevalence of *C. burnetii*, risk of abortion and also it's zoonotic potential, show the importance of the prevention of coxiellosis. As a result, appropriate measure should be taken to prevent coxiellosis. Based on the present results and other similar studies, special attention to asymptomatically infected goats and vaccination which has been shown to be one of the most effective ways to reduce the bacterium shedding and the incidence of abortion, are important strategies.

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Ethics approval and consent to participate

This study did not require an ethics license.

Conflict of interest

The authors declare that they have no conflict of interest.

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