



The First Evaluation of *Chlamydia abortus* Infestation in the Iranian Dromedary Camel Population

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ABSTRACT

Chlamydiosis is an important disease in sheep, camel, goats, cats, birds, and cattle, which is caused by different species of the genus *Chlamydia*. Chlamydiosis of ruminants is a zoonosis and is especially worrying for pregnant women in contact with animal shelters. Chlamydiosis in camels can cause abortion, cervical adhesion, ovarian hydrobursitis, and reproductive failure in male camels. *Chlamydia* in camels can remain asymptomatic for a long time. Infected camels may play an important role in the transmission of *Chlamydia* to other animal species. Chlamydiaceae family members are currently placed in one genus and seven species. Among these seven species, *Chlamydia abortus* is of special importance in camels. It is possible to detect and distinguish chlamydial species by PCR and specific primers. The present study is the first study of *Chlamydia abortus* in the Iranian dromedary camel, which is very important. A total of 100 blood samples with anti-coagulant were taken from apparently healthy male and female camels in the south of Kerman province. Next, DNA was extracted from each blood sample using a blood DNA extraction kit according to the manufacturer's instructions. PCR was performed using rOMP90_3 specific primer to evaluate the presence of *Chlamydia abortus*. None of the samples were positive for *Chlamydia abortus*. According to the results, it can be said that *Chlamydia abortus* is probably not common in camels in the south of Kerman province of Iran.

Keywords

Chlamydia abortus, PCR, Camel, Iran

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Abbreviations

PCR: Polymerase chain reaction
ELISA: Enzyme-linked immunosorbent assay
EDTA: Ethylene diamine tetraacetic acid

rOMP: Recombinant outer membrane protein
DNA: Deoxyribonucleic acid

Introduction

Chlamydiosis is an important disease with global distribution in animals, especially ruminants, which is caused by different species of *Chlamydia* genus. *Chlamydia* can be considered gram-negative bacteria without metabolic energy production mechanisms. Therefore, Chlamydia is an obligate intracellular parasite that must live inside the cell, where the host cell provides intermediate compounds rich in energy [1]. These bacteria are also called "energy parasites". One of the important consequences of chlamydiosis is abortion [2]. Chlamydia abortion occurs in the last 2-3 weeks of pregnancy. The fetus is born dead and the placenta is inflamed. Chlamydiosis of ruminants is a zoonosis, especially worrying for pregnant women in contact with animal shelters [3]. Studies in sheep and goats showed that infection is mainly transmitted through contact with abortion products, vaginal secretions, and aborted or dead fetuses. The same may be true for camels as well [4]. Chlamydiosis in camels can cause abortion, cervical adhesions, ovarian hydrobursitis, and reproductive failure in male camels [5–8]. Chlamydia in camels can remain asymptomatic for a long time [9], and infected camels may play an important role in the transmission of Chlamydia to other animal species [10]. The prevalence of camel chlamydiosis is higher in adult camels than in young camels and female camels than in male camels [5, 11–13]. Chlamydiaceae family members are currently placed in one genus and seven species. Among these seven species, Chlamydia abortus in camels is of special importance [14]. Using PCR, it is possible to detect and distinguish Chlamydial species by specific primers [15]. The present study is the first detection of Chlamydia abortus infection in the Iranian camel population by PCR, which is very important.

Results

In this study, based on the results of electrophoresis, all 100 blood samples tested were negative for Chlamydia abortus (Figure 1).

Discussion

Chlamydiosis is an important disease in a wide range of animals caused by different species of Chlamydia bacteria [2]. This disease in camels can cause various symptoms, including abortion, cervical adhesion, ovarian hydrobursitis, and reproductive failure [5–8]. Moreover, Chlamydia in camels can remain asymptomatic for a long time [9]. As a result, infected camels can transmit this bacterium to other animals [4]. The present study is the first evaluation of Chlamydia abortus in the dromedary camel population in Kerman, Iran, which is very important. None of the samples were positive for Chlamydia abortus. Therefore, it can be said that Chlamydia abortus is probably not common in camels in the south of Kerman province of Iran.

Studies similar to the current research have been conducted around the world. In a study conducted in Tunisia, blood and serum samples of 470 healthy dromedary camels from eight different provinces of Tunisia were collected to detect Chlamydia abortus. In the serological method (ELISA), 5.75% positive samples were detected, while no positive samples were observed in the molecular method (PCR) [16]. In another study, similar to the results of the present study, blood samples were collected from 82 camel herds (865 dromedary camels). Chlamydia abortus was detected in camel blood by ELISA at a very low percentage (2.5%) [17].

In a study, serum and milk samples were taken

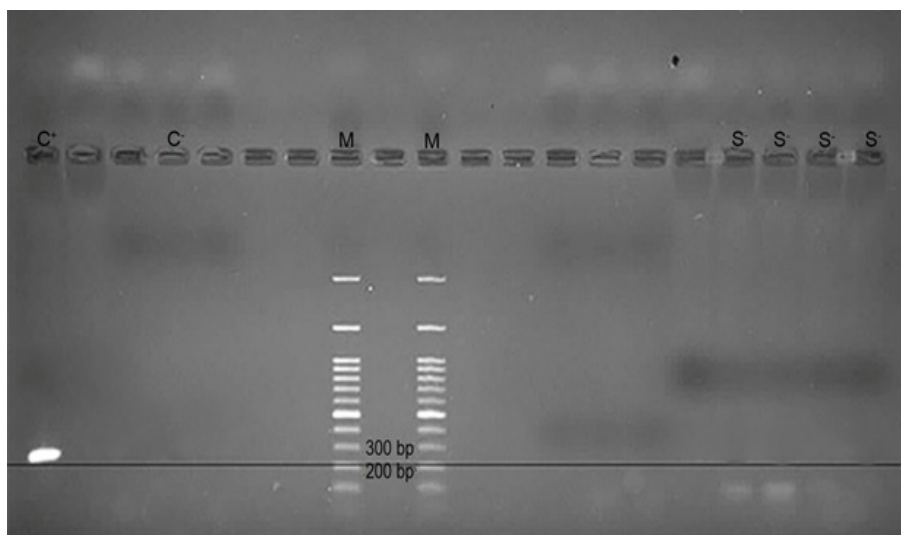


Figure 1.

Amplification of Chlamydia abortus gene using rOMP90_3 primers. Negative clinical samples (lanes 17-20), negative control (lane 4), positive control (lane 1), and molecular weight marker (lanes 8 and 10).

from 30 camels and 300 contact sheep belonging to six different farms in the western region of Saudi Arabia. In these farms, camels and sheep were kept together. Three of these farms had sheep abortions. Two methods, ELISA and PCR, were used in this study, showing that 18 camels and 142 sheep were positive with PCR, while 11 camels and 109 sheep were positive with ELISA. The findings showed that camels can be infected with Chlamydia, but most of the infected camels look healthy. Therefore, they can play an important role in the transmission of this infection to the animals in contact with them [18]. Among the reasons for the difference between the latter study and the current research, we can mention the type of sample, the way of keeping livestock, and the diagnosis method. In the mentioned article, the milk and serum samples were examined by molecular and serological methods, while in the present study, the study was performed exclusively on the blood samples of camels and only by molecular methods. In addition, the camels were next to the sheep in the field. It should also be noted that three of these sheep had abortions. In the present study, camels in the deserts were investigated. In another research, bursal tissue (n=5) and bursal fluid (n=6) samples were collected from 11 female camels with ovarian hydrobursitis. Real-time PCR was used for the initial detection of Chlamydia abortus in infected samples. The prepared samples were inoculated into embryonated chicken eggs. Subsequently, Giemsa staining and direct immunofluorescence were used to detect any chlamydial inclusions in infected yolk sacs. Next, the second real-time PCR was performed on infected yolk sacs. The Chlamydia abortus gene was found in 83.8% and 63.6% of infected bursa tissue and bursa fluid samples and infected yolk sacs, respectively. Moreover, all the yolk sac smears tested with direct immunofluorescence and Giemsa staining showed intracytoplasmic inclusion bodies [7]. The difference between the mentioned study and the present research is that in the above study, camels affected by ovarian hydrobursitis were evaluated, while in the present study, apparently healthy camels were assessed. Furthermore, in our study, the molecular technique was conventional PCR, while in this study, the molecular technique was real-time PCR. In another research, to evaluate the causes of abortion in Western camels, samples were taken from 34 camels older than 5 years and 19 camels younger than 5 years. Fifteen internal organs (liver, heart, lung, and spleen) from aborted camels and twenty vaginal swabs from aborted camels were collected for Chlamydia isolation through inoculation in embryonated chicken eggs. Chlamydia inclusion bodies were detected in 45% and 20% of vaginal swabs and internal organs, respectively [5]. The difference between the above study

and the current research results from several reasons. They studied the internal organs and vaginal swabs of aborted camels, while we used the blood samples of healthy camels. Moreover, in the mentioned study, Chlamydia isolation through inoculation in embryonated chicken eggs was performed, while in the current investigation, the molecular test was performed. In another research, 1560 sheep and goat blood samples were collected from 130 flocks in five Kajiado counties. The samples were tested by PCR, and Chlamydia abortus DNA was detected in 20.3% and 28.1% of sheep and goats, respectively [19]. The difference between our study and this study may result from different species studied.

Research on Chlamydia abortus in the camels of different regions of the world has been conducted by ELISA. In a study, 245 blood samples were collected from Abu Dhabi female dromedary camels, aged 5-8 years, with a history of reproductive failure, including repeat breeder and abortion. The samples were tested by ELISA. The overall prevalence of chlamydiosis was 19.59%. The results showed that chlamydiosis was common among camels in Abu Dhabi [20]. Another study was conducted on 245 dromedary camels (205 females and 40 males) in different regions of western Libya. The animals varied in age from <1 to 20 years and were sampled randomly from both housed and nomadic herds. Blood serum samples of camels were tested by ELISA. The results showed that out of 245 camels tested, 30 camels were positive. The prevalence of chlamydiosis in females (14%) was twice males (5%) [11]. In a study, blood samples were taken from 60 dromedary camels (38 females and 22 males) aged 5-12 years in Iraq. These samples were tested by ELISA. All the male camel serum samples were negative for the presence of antibodies against Chlamydia abortus, while 18 of the 38 (47.36%) female camel samples were positive [21]. In another study, serum was collected from 378 female Mijaheem camels in different age groups from different parts of Saudi Arabia. The samples were tested by ELISA. They found the prevalence of chlamydiosis as 10.05% [13]. In a study, 141 infertile male dromedary camels (4-20 years) were used. Antibodies against Chlamydia abortus were detected by ELISA. The incidence rate of Chlamydia abortus was 13.48%. It could be concluded that Chlamydia abortus may play a role in causing reproductive failure in male camels [8].

Conclusions

According to the results of the present study, Chlamydia abortus is probably not common in camels in the south of Kerman province. However, further studies should be conducted to provide better conclusions.

Materials and Methods

Sample collection

One hundred blood samples with EDTA, as an anticoagulant, were taken from the jugular vein of apparently healthy mature (with an average age of four years and above) male and female camels with an average weight of 370 and 300 kg, respectively, in the south of Kerman province. It should be mentioned that camels in the deserts and far from other animals were used.

DNA extraction

DNA extraction was completed using the commercial blood DNA extraction kit according to the manufacturer's instructions (Parstous, Iran). The quality and quantity of extracted DNA were assessed using a Nanodrop spectrophotometer (Epoch, BioTek Instruments Inc., USA).

Conventional PCR

Chlamydia abortus was confirmed using PCR with a specific primer pair rOMP90_3 (Metabion, Germany) to identify the relevant gene with a weight of 220 bp [22] (Table 1). The PCR reaction mix was at a final volume of 20 µl, containing 2 µl template DNA, 0.5 µl of each of *Chlamydia abortus* specific F and R primers (0.25

Authors' Contributions

M.P. carried out the laboratory works, data analysis, and wrote the manuscript. E.M. designed the study, supervised the project, revised the data analysis, supervised the laboratory works, and revised all parts of the manuscript. M.G. supervised the project and revised the data analysis.

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Competing Interests

We pronounce that we have no irreconcilable situation.

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Table 1.
The sequence of rOMP90_3 primers for the specific detection of *Chlamydia abortus*

Target	Primer name	Sequence(5-3)	Product size (bp)
rOMP90_3	rOMP90_3_F	5'-TTTTTCAGGATCCTATTGTCCTCCAGGCA -3'	220
	rOMP90_3_R	5'-GTGAATTCAGCATAAATAGCCCCG-3'	

µM), 7 µL of distilled water, and 10 µL of commercial master mix (Ampliqon, Denmark). *Chlamydia abortus* [23] and distilled water were also used as positive and negative controls, respectively. Subsequently, the samples were placed in a thermocycler (Biorad, USA) to amplify the target gene with the temperature program given in Table 2. PCR products were electrophoresed with a ladder (Ampliqon, Denmark) in agarose gel at a concentration of 1%. Following the electrophoresis, reading in the gel documentation system (Vilberlomat, France), photographing with the quantum capture software, and analyzing the results were performed.

Table 2.
Temperature program of thermocycler device to amplify the target gene of *Chlamydia abortus*

Number of cycles	Temperature (centigrade)	Time	PCR step
1	95°C	3 min	Initial Denaturation
	95°C	30 sec	Denaturation
35	60°C	30 sec	Annealing
	72°C	60 sec	Elongation
1	72°C	10 min	Final elongation

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