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Ser-Surveying of Caprine Q-Fever (*Coxiella burnetii*) in Milk

Ali A. Shati [1], Hala SR. AL-Taee [2], Hayder D. Saleem [3]*

[1] Department of Animal Production, College of Agricultural, University of Wasit, Iraq
[2] Department of Microbiology, College of Veterinary Medicine, University of Wasit, Iraq
[3] Department of Density, Al-Manara College of Medical Sciences, Iraq
* *Corresponding author Emails*: hydrsalim@gmail.com

Abstract

Dairy domestic animals are the primary source of Q-fever disease that caused by *Coxiella burnetii* to both animals as well as humans. Although, Q-fever having usually mild symptoms or occur asymptomatically, subclinical complications are the main risks in humans and great economic losses among small ruminants have confirmed. This study was performed to investigating of prevalence of *C. burnetii* in goats of Wasit province (Iraq) during March to April / 2022. Off totally 186 milk samples collected from lactating goats, 26.34% animals were seropositives. Titers of seropositives showed a significant increase ($P<0.029$) in moderate (63.27%) infection when compared to weak (28.57%) and severe (8.16%). Among positive and negative goats, the findings of clinical examination revealed no significant differences ($P>0.05$) in body temperature, pulse and respiratory rates. Regarding case history data, significant increases were identified in positive goats with abortion (38.78%), low milk production (57.14%) and in study animals housed and/or pasteurized with sheep and cattle (63.27%). Concerning the demographic risk factors, findings of age were reported an increasing in positivity of *C. burnetii* in goats aged ≥ 4 years (40.43%) when compared to ≥2 - <4 years (22.5%) and <2 years (3.85%). In insignificant variation ($P\leq0.053$) in positivity was seen throughout study areas; Al-Numaniyah (19.35%), Al-Hai (26.44%), and Al-Kut (27.94%). In conclusion, *C. burnetii* is relatively high in distribution among study goats and, humans consumed milk and/or their non-pasteurized derivatives of might increasingly be exposed to great risks of infection.

Keywords: Q fever, Caprine, ELISA, Lactating, Does.

Introduction

Q-fever is one of the most serious and infectious diseases which caused by *Coxiella burnetii*, strict intracellular highly infectious bacterium that confirmed using the phylogenetic analysis to be close-related for *Legionella* and *Francisella* rather its relation to *Rickettsia* (1-3). There are 2 forms of *C. burnetii*, large intracellular and reproducible variant exists in host macrophages and monocytes, and small cell extracellular variant sheds in secretion and excretion of diseased host in particular milk (4). The last form is highly resistance for harsh environmental conditions as well as for several commonly known disinfectants due to spore forming (5). The disease infecting wide ranges of animals (wild and domestic) such as ovine (sheep), caprine (goat), bovine (cattle) and camelide (camels) which consider as the main reservoirs for the bacterium and the source of Q-fever for human (6). Epidemiological studies have demonstrated that *C. burnetii* having two complicated patterns of transmission involved circulation of organism between animal and ectoparasite, as well as occurrence in domestic ruminants independent of the wild animal cycle (6-8). Once domestic ruminants are infected, the pathogen resides in uterus, placenta, supramammary lymph nodes and mammary glands, and sheds in subsequent parturition and lactation (9). Direct contact with infected animals,

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ingestion the dairy products (milk and cheese), inhalation of infected aerosols or indirectly by contaminated fomites represent the main source for disease to human (10).

In domestic animals, *Coxiella* can cause severe economic loss due to infertility, abortion, stillbirth, birth of weak offspring and neonatal mortality (4, 11). However, most of these infections are asymptomatic or subclinical; therefore, the only way for confirmation of infection is based on advanced laboratory diagnosis (12). In veterinary medicine, routine diagnosis by isolation is not carried out because of zoonotic nature of *Coxiella*, in addition to requirement of BSL3 laboratories confinement and high level of expertise, and long-term of cultivation (13). In laboratory, different advanced techniques were modified such as immunofluorescence assay, complement fixation test, and enzyme-linked immunosorbent assay (ELISA). The last assay is preferred as reliable for demonstrating anti-phase II or both anti-phases (I and II) developed against infection, as well as for its convenience in large scale screening schedules (14, 15).

In last decade, although *C. burnetii* had received more attention as a potential bioterrorism agent with occurrence many frequent outbreaks in different countries, information on the prevalence of Q fever in Iraq remains low (6, 16, 17). Hence, main objective of this work was identification of prevalence of Q-fever in lactating goats of some areas in Wasit province / Iraq, using ELISA with investigation the association of positivity to case history data, vital signs, and demographic risk factors.

Materials and methods

Ethical approval

This study approved by the Scientific Committee of Department of Microbiology, College of Veterinary Medicine / University of Wasit, and Department of Veterinary Public Health, College of Veterinary Medicine / University of Wasit, Iraq.

Study animals

Totally, 186 lactating goats were selected randomly from many regions related to the main districts in Wasit province / Iraq; during March to April / 2022. Under aseptic conditions, 10 ml of milk were collected directly from the quarters of each study animal into a glass tube, and transferred cooled using an icebox. In the laboratory, all samples were mixed gently by the vortex, centrifuged at 7000 rpm for 15 minutes, and then, the supernatant of each sample was pipetted into another glass tube while the sediment was discarded. Again, the tubes of supernatant were re-centrifuged at 4000 rpm for 5 minutes, and the clear layer (serum) of supernatant was pipetted into 1.5 ml eppendorf tube that labeled and kept frozen until be examined by serology.

Case history data regarding occurrence of mastitis, abortion, frequent diarrhea, and recurrent diarrhea as well clinical examination of vital signs (temperature, pulse and respiratory rates) with recording information of demographic risk factors (region and age) were carried out in this study.

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Serology

Following the manufacturer instructions of the ELISA kit (*Sunlong Biotech, China*), milk sera of study goats were tested. Initially, the frozen sera were thawed using water bath at 37°C, ELISA kit contents warmed at room temperature, and the Sample Diluents in addition to Washing Buffer were prepared. The steps of testing included briefly adding 50 µl of Positive and Negative Control in duplicate in the first wells and adding 10 µl of serum sample with 40 µl of Sample Diluent to other microplate wells except the blank well (last well). The microplate was incubated in the incubator (37°C / 30 minutes) and washed five times with the diluted Washing Buffer. A total 50 µl of HRP-Conjugate was added to all wells except the blank well, incubated (37°C / 30 minutes), and washed five times with the diluted Washing Buffer. A total 50µl of each Chromogen A and Chromogen B was added to each microplate's well except the blank, incubated (37°C / 15 minutes), and immediately, a total 50µl of Stop Solution was added to all wells. Then, the optical density (OD) was measured at 450nm using the microplate ELISA reader (*BioTek, USA*), OD value of the blank was set as zero, test validity determined, and CUT OFF was calculated. Samples were considered positive if their OD ≥ CUT OFF. Additionally, the positively infected does were divided into three categories according to their ODs; severe, moderate, and weak.

Statistical analysis

The collected data and results were tabled, figured, and analyzed using the Microsoft Office Excel (2013) and GraphPad Prism (6.0.1). Chi-square (x^2) and Odd tests were applied to detect significant differences in prevalence of positivity and to estimate the association of positive results to values of vital signs, case history data, and demographic risk factors. Variation between values were considered significant at $P<0.05$. Mean, standard error, and range were expressed as M, SE, and R, respectively.

Results

Of 186 samples of milk testing using ELISA, 49 (26.34%) were the total seropositives (Figure 1). Titers of seropositives were showed a significant variation ($P<0.05$) in their values. There were significant increases ($P<0.05$) in number of moderately [31/49 (63.27%)] infected does when compared to weakly [14/49 (28.57%)] and severely [4/49 (8.16%)] infected animals (Figure 2). The values (M±SE) of ODs were 0.275 ± 0.01, 0.451 ± 0.013, and 0.735 ± 0.036 for weakly, moderately and severely infected goats respectively at a significance of $P<0.0001$ (Figure 3). In comparison negative study goats, the results of seropositives showed that there no significant differences ($P>0.05$) in values of body temperature, pulse and respiratory rates (Table 1).

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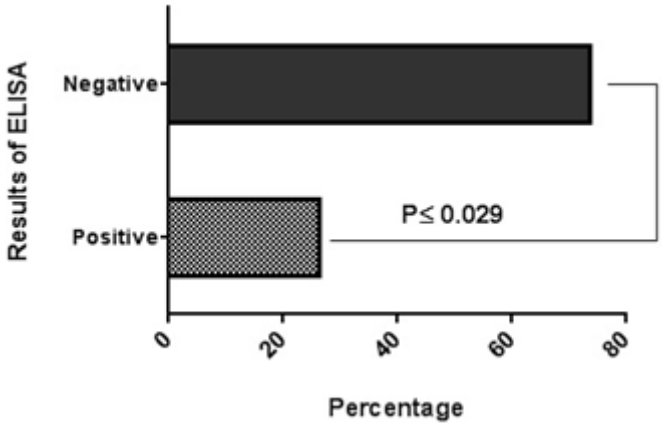

Figure (1): Total results for testing totally milk samples of 186 lactating goats by ELISA

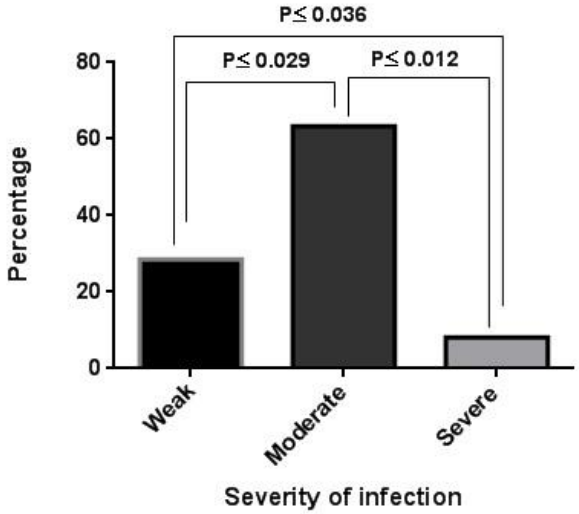

Figure (2): Number of weakly, moderately and severely infected does detected by ELISA

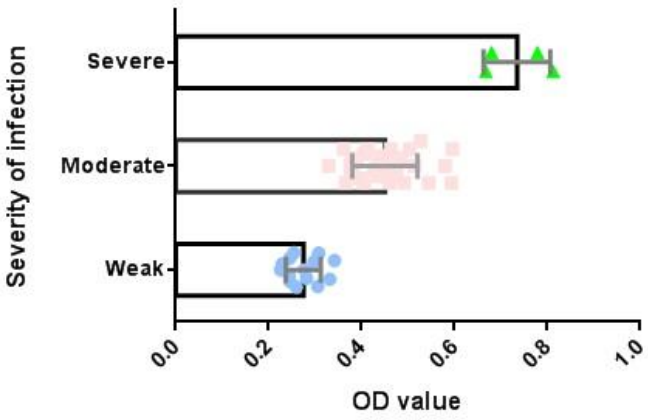

Figure (2): OD values among weak, moderate and severe infected does using ELISA

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Table (1): Results of vital signs among positive and negative study goats examined by ELISA

Factor	Positive	Negative	P-value
Temperature	39.92 ± 0.21 (37.81-40.13)	39.69 ± 0.17 (37.46-40.64)	0.059
Pulse rate	86.17 ± 2.05 (68-98)	84.91 ± 1.8 (64-113)	0.078
Respiratory rate	26.32 ± 1.53 (13-29)	26.07 ± 1.35 (11-32)	0.065

Values: M± SE (R), Significance * (P<0.05)

Regarding case history data of seropositive goats, the findings revealed a significant variation in their values (Table 2). However, significant increases (P<0.05) were identified in abortion (38.78%) group for reproductive status factor, low milk production (57.14%) for milking status factor, normal (77.55%) condition of physical status of feces, as well as in goat housing and pasturing with sheep and cattle (63.27%) in factor of presence / absence other domestic animals.

Concerning the findings of demographic risk factors, significant variation (P<0.05) was reported among the groups of age but not region factors (Table 3). For age factor, significant increases (P≤0.026) in prevalence of *C. burnetii* were observed in positive goats of ≥ 4 years (40.43%) when compared to ≥2 - <4 years (22.5%) and <2 years (3.85%). For region factor, no significant differences (P≤0.053) were seen between the areas of Al-Numaniyah (19.35%), Al-Hai (26.44%), and Al-Kut (27.94%).

Table (2): Results of case history data of 49 seropositive goats with *C. burnetii* by ELISA

Factor	Positive		P-value
	No.	Prevalence (%)	
Reproductive status			
Abortion	19	38.78 *	
Stillbirth	8	16.33	
Congenital birth	4	8.16	0.035
Low fertility	6	12.24	
Normal	12	24.49	
Milking status			
Mastitis	6	12.25	0.041
Low milk production	28	57.14 *	
Normal	15	30.61	
Physical status of feces			
Diarrhea	7	14.29	
Constipation	4	8.16	0.022
Normal	38	77.55 *	
Presence /absence of other domestic animals			
Only sheep	11	22.45	
Only cattle	2	4.08	0.017
Sheep and cattle	31	63.27 *	
Absence of domestic animals	5	10.2	

Significance * (P<0.05)

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Table (3): Results of demographic risk factors among 49 seropositive goats

Factor	Total No.	Positives		P-value
		No.	%	
Age				
< 2 years	52	2	3.85	0.026
≥ 2 - < 4 years	40	9	22.5	
≥ 4	94	38	40.43 *	
Region				
Al-Numaniyah	31	6	19.36	0.053
Al-Hai	87	23	26.44	
Al-Kut	68	19	27.94	

Significance * (P<0.05)

Discussion

Milk of infected livestock is the usual route for shedding *C. burnetii*; however, contamination by feces or through direct and indirect contacts with infection site of the abortion or periparturient can occurs (18, 19, 20). In domestic animals, *C. burnetii* diagnosis is greatly important not only for identification of diseased flocks but also for determining the pathogen source and risks for transmission to human (15). In Iraq, Q fever is interestingly neglected among animals as well as humans. In this study, the findings showed that seroprevalence of Q fever among lactating does was 26.34% that higher (8.6%) than detected previously in Iraq (16). These results might be reflected by increasing the existence of organism in goats as well as in cattle and sheep. Therefore, we suggested that shedding via milk in does might act as most commonly route for spread *Coxiella* in environments. In other countries, the prevalence of disease is 6.5% in Greece (21), 7.5% in India (22), 8.7% in Spain (23), 9.3% in Northern Ireland (24), 13% in Chad (25), 13% in Italia (26), 16.8% in Egypt (27), 18.7% in Albania (28), 35% in Mexico (29), 56% in Oman (30), 65.8% in Iran (31), and 73% in France (32). This variation in prevalence of *C. burnetii* could be due to differences in sensitivity and specificity of applied diagnostic tools, types of tested samples, sampling method (individuals or herd), virulence of agent, animal factors (type, age, and sex), system management, and climate. Although, many studies demonstrated that ELISAs have high efficacy in detection of *C. burnetii* infections, not all animals can be identified because either some animals shed *Coxiella* prior to development of specific antibodies, or others does not seroconvert (33, 34). Since 1980, ELISA becomes the most reliable and frequently used test for detection of infection in small ruminants due to it high sensitivity (82-100%) and specificity (93-96%), (9). Most commercially available kits can detect anti-phase II antibodies or both anti-phase I and II antibodies; therefore, the test is preferred for large-scale screening of the infection status livestock (13). However, the type of antigen coated ELISA's kit might play an active role in obtained results of each study.

Our findings showed that the moderately infected animals were elevated significantly in comparison to weakly and moderately infected goats. These findings may reflect the frequent exposure of study animals to moderately dose of organism or that *Coxiella* has a moderate pathogenicity. Roest et al. (2012) demonstrated that the immune response to *Coxiella*

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infection could be diagnosed post 14 days and remains for up to 13 weeks after-infection, suggesting that immune responses may last for months or even years (32). Roest et al. (2020) demonstrated that immune response resulted due to inoculation of non-pregnant and pregnant goats is idetintical suggesting that both indicating an incidence of disease in both status (35). Gharban and Yousif (2020a) found that the seropositive animals to *Coxiella* have high differences in peak titers as well as in peak of antibodies suggesting the fast changes throughout a relative short period during an increasing of antibody concentrations after exposure and body response; whereas, decreases in antibody concentration being more slowly and take longer period (1).

Absence of significant differences in values of body temperature, pulse and respiratory rates of both seropositive and seronegative study goats were observed in the present study. Regarding case history data, we showed that abortion, low milk production and presence of other farm animals (cattle and sheep) were contributed greatly in increasing the seroprevalence of infection significantly. In large goats herds, abortion rates can reach up to 80% of the infected pregnant animals although healthy kids can also born (32, 33). The existence of abortion waves due to *C. burnetii* infection was reported for sheep and goats by many researchers (37-39). Different reports have detected that *Coxiella* can result in reproductive disorders in cattle such as infertility, premature delivery, endometritis, metritis, and mastitis (40, 41). It showed that the residency of *Coxiella* in mammary gland tissue has a direct relation with the level of *C. burnetii* antibodies in milk, clinical or subclinical mastitis, reduced milk production, decreased fat content, and increased protein content in milk (42, 43). Other systemic disorders were seen in present study required further analysis. Experimental evidences of the abortifacient potential of *C. burnetii* in cattle reinforces the view that natural infection can sometimes lead to disease, either as sporadic cases of bovine abortion or as more serious outbreak in sheep and goats (44).

The presence of other animals at pasture in particularly cattle and sheep implicated with significant increasing a prevalence of *C. burnetii* (45, 46). However, the level and nature of interaction between livestock and other animals might vary by management type that can affect on seropositivity (47). As detected previously, the increasing of herd size and high density of animals are contributed factors in increase transmission of infection between animals (48). Many reports detected that there is a strong spatial correlation in seropositivity between the flocks of small ruminants and cattle population, suggesting that *C. burnetii* circulates in farms of small ruminants during lambing with increasing the chance of spreading infection to other animals (49-51).

Epidemiological studies in animals suggested that *C. burnetii* infection is highly prevalent in regions, and that prevalence varied by host species and diagnostic techniques (5). According to our findings, prevalence of Q fever was differed insignificantly meaning that Q fever is endemic in Iraq. We showed also that there was significant proportional relationship between positivity and age factor. This finding was not surprised since adult animals appeared more sensitivity to infection than younger as reported previously (52, 53), and that the risk of infection may be correlated with the time that a host reaches adulthood as adults exposed to *C. burnetii* and tick bites for extended periods (54). Epidemiological data clearly show an

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age-related increase in the incidence and severity infection (55). In goats, the increased risk, at this age, seems not to be explained by differences in specific work activities or frequency of animal contact.

Conclusion

Very few well-designed studies dealing with the prevalence of *C. burnetii* infection in domestic ruminants, particularly goats, were available. Our results showed a higher seroprevalence among lactating goats suggesting that Q fever is of considerable importance in this animal in Iraq, and that, humans consumed milk and/or their non-pasteurized derivatives for these animals are at high risk to gain the infection. Shedding prevalence is crucial in estimating the risk of transmission of infection between ruminants or herds and from ruminants to humans. Therefore, published and well-designed prevalence studies for sampling and testing procedure are needed to help researchers, agricultural organizations, veterinarians and farmers.

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