Assiut University web-site: www.aun.edu.eg

SEROPREVALENCE RATE OF *COXIELLA BURNETII* IN COWS' SERUM IN ASSIUT GOVERNORATE, EGYPT

REEM M. ALSAADAWY [1]; SYLVIA O. AHMED [1] AND HELAL F. HETTA [2]

[1] Department of Zoonoses, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.
[2] Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Received: 24 January 2023; **Accepted:** 28 February 2023

ABSTRACT

This study was conducted to determine the seroprevalence of *Coxiella burnetii* in cows from Assiut Governorate, Egypt. A total of 268 blood samples were collected from cows (176 females and 92 males). The age of these animals ranged between 2 months and more than 3 years. Screened for C. burnetii using indirect enzyme-linked immunosorbent assay. A total of 15.67% (42/268) was serologically positive. The seropositivity was high in females (19.56%) than males (13.63%). The native breed was more infected (17.44%) than Fresian (13.33%) and mixed ones (11.76%). In cows, a higher seroprevalence was observed in the age group more than 1 to 2 years (22.38%). Dairut city recorded the highest seroprevalence rate (40.74%) of *C. burnetii*. *C. burnetii* seroprevalence was higher in hot season (20.10%) than cold season (5.95%). Cows free from ticks recorded higher seroprevalence (22.58%) than those infested with ticks (12%). Cows with fever and pneumonia recorded higher seroprevalence (14.29%) compared to animals with other signs. In conclusion, findings of this study revealed the wide spread of *C. burnetii* infection among cows at Assiut Governorate, Egypt.

Keywords: *C. burnetii*, ELISA, Pneumonia, Q Fever, Ticks.

INTRODUCTION

Globally contagious zoonosis known as Q fever is carried on by the gram-negative, strictly intracellular bacteria *Coxiella burnetiid* (*C. burnetii*) (Woldehiwet, 2004). The absence of distinct clinical indications among infected livestock makes diagnosis difficult, and *C. burnetii* infection in livestock frequently goes undetected (Cutler *et al.,* 2007).

Corresponding author: Reem M. Alsaadawy
E-mail address: reemalsaadawy@vet.aun.edu.eg
Present address: Department of Zoonoses, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

Q fever illness can occasionally lead to late-stage abortions, stillbirths, or the delivery of fragile infants. However, there is no much efforts made to control this pathogen in animals, since it is not thought to cause economically significant animal disease (Maurin and Raoult, 1999).

Since the impact of Q fever has been recognized, serologic surveys have been carried out to determine the disease's prevalence in both humans and animals. Because the disease is difficult to isolate and doesn't grow on standard laboratory bacteriological media, culture of the disease is rarely performed (Kilic *et al.,* 2005).

Numerous clinical and seroepidemiological studies were carried out in our area, suggesting that Q fever is a common illness in Egypt (Hussein, 1993; Mazyad and Hafez, 2007). Recently, there are a few data about this disease in cows in our community. Therefore, the current investigation was conducted to estimate the seroprevalence rate of *C. burnetii* in cows' serum in Assiut Governorate.

MATERIALS AND METHODS

Sampling

The sample in this study was collected between March 2021 and February 2022.A sum of 268 blood samples were collected from cows (176 females and 92 males), which apparently appear healthy and diseased at Assiut Governorate. The age of these animals ranged between 2 months and more than 3 years in cows.

Five ml of blood was drawn from each animal via jugular vein puncture and collected on plain serum tubes for serological examination. Samples were transported in coolers to the Zoonosis laboratory, Faculty of Veterinary Medicine, Assiut University. The serum was separated within 24 hours after sampling. All sera were stored at – 20°C for further investigation.

ELISA test

The animal sera were tested for the presence of specific anti- *C. burnetii* IgG antibodies using ELISA CHECKIT Q-fever test with a mixture of *C. burnetii* phases I and II, according to manufacture procedure (IDEXX Switzerland GmbH, Stations Strasse 12,CH-3097, liebefeld-Bern) (REF, QFT1135T).

The results were read using a photometer (Stat Fax-2100.Awareness, technology INC) at a wavelength of 450 nm.

Statistical analysis

The collected data were analyzed using computer program SPSS (Statistical Package for the Social Science) version 25. The data was subjected to analysis of variance using the Chi-square procedure of SPSS software. A probability value (*P*-Value) $P < 0.05$ was considered statistically significant.

RESULTS

ELISA results in animals revealed a high seroprevalence rate in cows, where the seroprevalence of *C. burnetii* in cows was 15.67% (42/268). The seroprevalence rate was higher in female animals 19.56% (18/92) than males13.63% (24/176). The difference was statistically non-significant ($P = 0.205$) (Table 1).

Higher seroprevalence in cows 15/67 (22.38%) was observed in the age group more than 1 to 2Years, followed by 7/34 (20.58%) in the age group more than 3 years; while lower seroprevalences 3/ 25 (12%) were detected in the age groups more than 2 to 3Years, followed by 17/142 (11.97%) in the age group between 2M and 1Year. The differences between age groups were statistically non-significant ($P = 0.201$). (Table2).

According to locality, Dairut city recorded the highest seroprevalence rate of *C. burnetii* in cows 22/54 (40.74%) compared to other cities in Assiut governorate, with significant differences between cities ($P = 0.000$) (Table3). Cows free from ticks recorded higher seroprevalence rate of *C. burnetii* 21/93 (22.58%) than those infested with ticks 21/175(12%). The difference was statistically significant ($P = 0.023$) (Table 4).

The seroprevalence of *C. burnetii* was higher in the hot season 37/184 (20.10%) than in the cold season 5/84 (5.95%) The difference was statistically significant ($P = 0.003$) (Table 5). Higher seroprevalence rate of *C. burnetii* was detected in cows among the native breed 30/172 (17.44%), followed by Fresian 6/45 (13.33%) and mixed 6/51 (11.76%) breeds (Table 6).

The seroprevalence rate of *C. burnetii* was slightly higher in cows with clinical signs 5/31 (16.13%) than apparently healthy cows 37/237 (15.61%) (Table 7). Cows with fever (3/21) and pneumonia (2/14) recorded a higher seroprevalence rate (14.29% for both signs), compared to cows with other clinical signs (0%) (Table 8).

Table 1: Seroprevalence of *C. burnetii* in cows according to the sex.

Sex	Total number of samples	ELISA	
		No. of positive samples	%
Female	92	18	19.56
Male	176	24	13.63
P-value	Non-significant (*P* = 0. 205)		

Table 2: Seroprevalence of *C. burnetii* in cows according to the age.

Age	Total number of samples	ELISA	
		No. of positive samples	%
2M-1 Y	142	17	11.97
More than 1 Y - 2 Y	67	15	22.38
More than 2Y -3Y	25	3	12
More than 3 Y	34	7	20.58
P-value	Non-significant (*P* = 0. 201)		

Table 3: Seroprevalence of *C. burnetii* in cows according to the region.

Regions	Total number of samples	ELISA	
		No. of positive samples	%
Dairut	54	22	40.74
Manfalut	33	6	18.18
Drounica	29	5	17.24
Elzaweaia	115	6	5.21
Dawina	7	0	0
Refa	10	1	10
Abu-Tig	20	2	10
P-value	Significant (*P* = 0.000)		

Table 4: Seroprevalence of *C. burnetii* in cows according to the association with ticks.

Associated with ticks	Total number of samples	ELISA	
		No. of positive samples	%
With ticks	175	21	12
Without ticks	93	21	22.58
P-value	Significant (*P* = 0.023)		

Table 5: Seroprevalence of *C. burnetii* in cows according to the season.

Season	Total number of samples	ELISA	
		No. of positive samples	%
Hot season	184	37	20.10
Cold season	84	5	5.95
P-value	Significant (*P* = 0.003)		

Table 6: Seroprevalence of *C. burnetii* in cows according to the breed.

Breed	Total number of samples	ELISA	
		No of positive samples	%
Native	172	30	17.44
Freisan	45	6	13.33
Mixed	51	6	11.76
P-value	Non-significant (*P* = 0. 553)		

Table 7: Seroprevalence of *C. burnetii* in cows according to clinical symptoms.

Signs	Total number of samples	ELISA	
		No. of positive samples	%
Apparently healthy	237	37	15.61
With clinical signs	31	5	16.13
P-value	Non-significant (*P* = 0. 948)		

Table 8: Seroprevalence of *C. burnetii* in cows according to type of the signs.

Signs	Total number of samples	ELISA	
		No. of positive samples	%
Fever	21	3	14.29
Pneumonia	14	2	14.29
Anemia	4	0	0
Bloody urine	1	0	0
Delaying in pregnancy	2	0	0
Stuntinggrowth	1	0	0
Blood parasites	3	0	0

DISCUSSION

Because of fever is now widely recognized for how important, and because it is rarely performed on culture due to the organism's inability to grow on standard laboratory bacteriological media, which necessitates a prolonged isolation period and biosafety level III conditions, serological surveys have been carried out to determine the disease's prevalence in both humans and animals (Kilic *et al.*, 2005). The diagnosis of *C. burnetii* infection in both humans and animals can be made using a variety of techniques, however, previous studies reported that ELISA for serological inquiry and PCR for molecular detection of Coxiella DNA are the most

effective methods (Hadush *et al.,* 2016; Ullah *et al.,* 2019).

In this study, the seroprevalence of *C. burnetii* in cows was 15.67% (42/268), nearly similar to the results of a previously conducted study in Bangladesh, where the overall prevalence of Q fever in bulk cow milk was 15.6% (Rahman *et al.,* 2016). In cows, higher *C. burnetii* seroprevalence rates were previously reported as 31% in the Adamawa region of Cameroon (Scolamacchia *et al.,* 2010), 32% in southeastern Ethiopia (Gumi *et al.,* 2013), 20.5% (95 of 463) in Guina (Troupin *et al.,* 2022), 34% in 4 upper Egypt governorates (Gerges *et al.,* 2018) and 36% in Beni Suief followed by 34% in Giza and 32% in Fayoum (Salem *et al.,* 2020). However, lower *C. burnetii* seroprevalence rates in cows were detected in Kenya11% (Wardrop *et al.,* 2016), India 1% (Pradeep *et al.,* 2017), Laikipia 4% (DePuy *et al.,* 2014), and Somali region, Ethiopia 9.6% (Ibrahim *et al.,* 2021).

As detected here, the seroprevalence rate is higher in female animals 19.56% (18/92) than male 13.63% (24/176) and the difference was statistically non-significant (P = 0.205) (Table 1). Previous studies supported these findings in cattle, including studies from Laikipia, Kenya (Larson *et al.,* 2019) and Northern Regions of Cameroon (Zangue *et al.,* 2022). These findings are consistent with earlier findings that showed a higher frequency in females than in males. The increased susceptibility of pregnant cows and the constant release of organisms into the environment following normal parturition or abortion through the placenta, amniotic fluid, vaginal discharge, fetal membranes, and milk may be the causes of higher sero-activity in females (Sakhaee and Khalili, 2010).

As reported in the present study, higher seroprevalence of 22.38% was observed in cows in the age group more than 1 to 2 years, followed by 20.58% in the age group more than 3 years; while lower seroprevalences of 12.5% were detected in the age groups more than 2 to 3 years, followed by 11.97% in the age group between 2M and 1Year. Differences between age groups were statistically non-significant (P = 0.201) (Table 2). Recent studies reported that the risk of getting *C. burnetii* infection among old animals (more than 1 year old) was 23 times higher than the risk among young animals (less than 1 year old) (Aljafar *et al.,* 2020). Furthermore, animals aged between 6-9 years were recorded 1.89 times more likely to have been infected to *C. burnetii* (Zangue *et al.,* 2022). The higher seroprevalence rates in the old animals may be attributed to more exposure to *C. burnetii* antibodies (Kiptanui *et al.,* 2022).

As recorded in the current study, cows free from ticks recorded a higher seroprevalence rate (22.58%) than those infested with ticks (12%). The difference was statistically significant (P = 0.023) (Table 4). This result may be due to continuous spraying of animals with insecticides to kill ticks., confirming the results previously reported in the Netherlands' outbreak, where ticks were detected to play no significant role in *C. burnetii* transmission (Sprong *et al.,* 2012).

The present study revealed that *C.burnetii* seroprevalence in cows was higher in the hot season (20.10%) than in the cold season (5.95%). The difference was statistically significant (P = 0.003) (Table 5). Higher detection of *C. burnetiid* in cows in the hot season in the current study may be due to increased stress on animals, which plays arole in decreasing animal immunity and tick spreading.

Higher seroprevalence rate of *C. burnetii* was detected in cows among the native breed (17.44%), followed by Fresian (13.33%) and mixed breed (11.76%), and the difference was statistically non-significant (P = 0.553) (Table 6). This finding may be associated with genetics and exposure to the causative agent (Kiptanui *et al.,* 2022), increasing the number of native breed animal samples in this study and lowering immunity of this breed than others. Moreover, the seroprevalence rate of *C. burnetii* was slightly higher in cows

with clinical signs (16.12%) than those apparently healthy (15.61%) and the difference was statistically non-significant (*P* = 0.948) (Table7). This result may be due to the rarely appearance of Q fever signs in animals. Furthermore, cows with fever and pneumonia recorded a higher seroprevalence rate of *C. burnetii* (14.28% for both signs), compared to cows with other clinical signs (0%) (Table8). This finding confirmed that fever and pneumonia are the principal manifestations of Q fever in diseased animals (Marrie, 1995; Smit *et al.*, 2012).

CONCLUSION

In comparison to other previous studies in Egypt, the findings of this study show a high seroprevalence of Q fever among diseased and apparently healthy cows. However, other animals and people in the study region may contract *C. burnetii* infections from these animals.

REFERENCES

Aljafar, A.; Salem, M.; Housawi, F. Zaghawa, A. and Hegazy, Y. (2020): Seroprevalenceand risk factors of Q-fever (C. burnetii infection) among ruminants reared in the eastern region of the Kingdom of Saudi Arabia.Tropical Animal Health and Production, 52(5): 2631-2638.

Cutler, S.J.; Bouzid, M. and Cutler, R.R. (2007): Q fever. Journal of Infection, 54(4): 313-318.

DePuy, W.; Benka, V.; Massey, A.; Deem, S.L.; Kinnaird, M.; O'Brien, T.; Wanyoike, S.; Njoka, J.; Butt, B. and Foufopoulos, J. (2014): Q fever risk across a dynamic, heterogeneous landscape in Laikipia County, Kenya. EcoHealth,11(3): 429-433.

Gerges, A.A.; Sobhy, M.; Fathi, A.; Hams, M.A.M.; Abou-Gazia, KH.A. and Mohamed, H.F. (2018): Detection of Neospora caninum and Coxiella burnetii antibodies in milk and serum of infected dairy cattle. Assiut Veterinary Medical Journal, 158(64): 49-57.

Gumi, B.; Firdessa, R.; Yamuah, L.; Sori, T.; Tolosa, T.; Aseffa, A.; Zinsstag, J. and Schelling, E. (2013): Seroprevalence of brucellosis and Q-fever in southeast Ethiopian pastoral livestock. Journal of Veterinary Science & Medical Diagnosis, 2(1): 10.4172/2325-9590.1000109.

Hadush, A.; Kandi, V. and Pal, M. (2016): Epidemiology and public health implications of Q fever. Perspectives in Medical Research,4(3): 42-46.

Hussein, A.A.A. (1993): Incidence of Q fever among infected and inapparently infected animals and man in Upper Egypt. PhD, Assiut University.

Ibrahim, M.; Schelling, E.; Zinsstag, J.; Hattendorf, J.; Andargie, E. and Tschopp, R. (2021): Sero-prevalence of brucellosis, Q-fever and Rift Valley fever in humans and livestock in Somali Region, Ethiopia. PLOS Neglected Tropical Diseases,15(1): e0008100.

Kilic, S.; Pasa, S.; Babur, C. and Ozlem, M. (2005): Investigation of Coxiella burnetii antibodies in sheep in Aydin region, Turkey. Revue de Médecine Vétérinaire, 156(6): 336-340.

Kiptanui, J.; Gathura, P.; Kitala, P. and Bett, B. (2022): Seroprevalence Estimates of Q Fever and the Predictors for the Infection in Cattle, Sheep, and Goats in Nandi County, Kenya. Veterinary Medicine International, 2022: 3741285.

Larson, P.S.; Espira, L.; Grabow, C.; Wang, C.A.; Muloi, D.; Browne, A.S.; Deem, S.L.; Fèvre, E.M.; Foufopoulos, J. and Hardin, R. (2019): The sero-epidemiology of Coxiella burnetii (Q fever) across livestock species and herding contexts in Laikipia County, Kenya.Zoonoses and Public Health,66(3): 316-324.

Marrie, T.J. (1995): Coxiellaburnetii (Q fever) pneumonia.Clinical Infectious Diseases, 21(Supplement 3): S253-S264.

Maurin, M. and Raoult, D.F. (1999): Q fever.Clinical Microbiology Reviews, 12(4): 518-553.

Mazyad, S. and Hafez, A.O. (2007): Q fever (Coxiellaburnetii) among man and farm animals in North Sinai, Egypt.Journal of the Egyptian Society of Parasitology, 37(1): 135-142.

Pradeep, J.; Stephen, S.; Pooja, P.; Akshayavardhini, A.; Sangeetha, B. and Antony, P.X. (2017): Coxiellosis in domestic livestock of Puducherry and Tamil Nadu: Detection of Coxiella burnetii DNA by polymerase chain reaction in slaughtered ruminants. Veterinary World, 10(6): 667.

Rahman, M.; Alam, M.; Islam, M.; Bhuiyan, A. and Rahman, A. (2016): Serological and molecular evidence of Q fever in domestic ruminants in Bangladesh. Veterinary medicine international, 2016: 9098416.

Sakhaee, E. and Khalili, M. (2010): The first serologic study of Q fever in sheep in Iran. Tropical Animal Health and Production, 42(7): 1561-1564.

Salem, R.R.; Elhofy, F.I.; Hassaballa, M.S. and Abd El Tawab, A. (2020): Seroprevalence detection of Coxiella burnetii antibodies in milk and serum of dairy cattle by recent methods. Benha Veterinary Medical Journal, 38(1): 57-60.

Sayed, A. (2000): Epidemiological studies on some rickettsioses in man and dogs in Upper Egypt. PhD, Assiut University.

Scolamacchia, F.; Handel, I.G.; Fevre, E.M.; Morgan, K.L.; Tanya, V.N. and De C. Bronsvoort, B.M. (2010): Serological patterns of brucellosis, leptospirosis and Q fever in Bos indicus cattle in Cameroon. PloS one, 5(1): e8623.

Smit, L.A.; van der Sman-de Beer, F.; Opstal-Van Winden, A.W.; Hooiveld, M.; Beekhuizen, J.; Wouters, I.M.; Yzermans, J. and Heederik, D. (2012): Q fever and pneumonia in an area with a high livestock density: a large population-based study.PloS one,7(6): e38843.

Sprong, H.; Tijsse-Klasen, E.; Langelaar, M.; De Bruin, A.; Fonville, M.; Gassner, F.; Takken, W.; Van Wieren, S.; Nijhof, A. and Jongejan, F. (2012): Prevalence of Coxiellaburnetii in ticks after a large outbreak of Q fever. Zoonoses and Public Health,59(1): 69-75.

Troupin, C.; Ellis, I.; Doukouré, B.; Camara, A.; Keita, M.; Kagbadouno, M.; Bart, J.M.; Diallo, R.; Lacôte, S. and Marianneau, P. (2022): Seroprevalence of brucellosis, Q fever and Rift Valley fever in domestic ruminants in Guinea in 2017–2019.BMC Veterinary Research,18(1): 1-11.

Ullah, Q.; El-Adawy, H.; Jamil, T.; Jamil, H.; Qureshi, Z.I.; Saqib, M.; Ullah, S.; Shah, M.K.; Khan, A.Z. and Zubair, M. (2019): Serological and molecular investigation of Coxiella burnetii in small ruminants and ticks in Punjab, Pakistan.International Journal of Environmental Research and Public Health,16(21): 4271.

Wardrop, N.A.; Thomas, L.F.; Cook, E.A.; de Glanville, W.A.; Atkinson, P.M.; Wamae, C.N. and Fevre, E.M. (2016): The sero-epidemiology of Coxiella burnetii in humans and cattle, Western Kenya: evidence from a cross-sectional study. PLoS Neglected Tropical Diseases,10(10): e0005032.

Woldehiwet, Z. (2004): Q fever (coxiellosis): epidemiology and pathogenesis. Research in Veterinary Science, 77(2): 93-100.

Zangue, C.T.; Kouamo, J.; Ngoula, F.; Tawali, L.P.; Talla, M.M.; Mbeba, L.Y.K.; Doumtsop, C.L.M. and Tangwa, B.V. (2022): Seroprevalence and Risks Factors Associated with Coxiella burnetii Infection in Slaughterhouse Zebu Cattle (Bos indicus) from Northern Regions of Cameroon. Epidemiologia,3(4): 434-442.

معدل الانتشار المصلى لكوكزيلا بيرنيتي في مصل الأبقار في محافظة أسيوط ، مصر

ريم محمد السعداوي ، سيلفيا اسامه ابراهيم ، هلال فؤاد حته

E-mail: reemalsaadawy@vet.aun.edu.eg Assiut University web-site: www.aun.edu.eg

أجريت هذه الدراسة لتحديد الانتشار المصلي لكوكزيلا بيرنيتي فى الأبقار من محافظة أسيوط ، مصر. تم جمع ٢٦٨عينة دم أبقار (١٧٦ أنثى و ٩٢ ذكر) . تراوح عمر هذه الحيوانات بين ٢ أشهر إلى أكثر من ٣ سنوات في الأبقار. بعد الفحص باستخدام اختبار القياسة المناعية الإنزيمية (ELISA) كانت النتيجة ١٥,٦٧ ٪ (٢٦٨/٤٢) إيجابية مصليا كما كانت الإيجابية المصلية عالية في الإناث (١٩,٥٦%) عن الذكور (١٣,٦٣%) .السلالة المحلية سجلت أعلى نسبة إصابة (١٧,٤٤%) عن الفريزيان (١٣,٣٣%) والسلالة المختلطة (١١,٧٦%). كما لوحظ ارتفاع معدل الانتشار المصلي في الأبقار في الفئة العمرية أكثر من ١ سنه الى ٢ سنين (٢٢,٣٨%). وقد سجلت مدينة ديروط أعلى معدل انتشار مصلي (٤٠,٧٤%) بين الأبقار. معدل الانتشار المصلي لكوكزيلا بيرنيتي كان أعلى في موسم الصيف (٢٠,١٠%) أكثر من موسم الشتاء (٥,٩٥%) . كما سجلت الأبقار الخالية من القراد انتشارا مصليا أعلى (٢٢,٥٨%) من تلك المصابة بالقراد (١٢%) ، أوضحت الأبقار المصابة بالحمى والالتهاب الرئوي انتشارا مصليا أعلى مقارنة بالحيوانات ذات الأعراض الأخرى. كشفت الدراسة عن انتشارا واسعا لعدوى الكوكزيلا بيرنيتي بين الأبقار في محافظة أسيوط ، مصر.