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Seroprevalence and assessment of risk factors associated with Coxiellosis in cattle in Toukh, Qalubyia

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ABSTRACT

Q fever or Coxiellosis is a worldwide zoonotic bacterial disease caused by *Coxiella burnetii* organism. Coxiellosis incriminated a pronounced economic impact on the veterinary industry, along with presenting serious public health hazards among humans. This current study was conducted during the period from October 2022 to December 2023 to assess the seroprevalence and the possible risk factors associated with *C. burnetii* infection in cattle in Toukh, Qalubyia governorate. The historical background of cattle under investigation indicated contact with small ruminants, and occurrences of reproductive disorders such as abortions and stillbirths. Moreover, clinical investigation of animals revealed the presence of tick infestation. A commercial ELISA kit (IDEXX) was utilized in the current study and the recorded overall seroprevalence rate of *C. burnetii* infection was 47%. Moreover, the statistical analysis also revealed that Coxiellosis is significantly associated with numerous risk factors including the breed of animal, age, contact with small ruminants, and infestation with ticks. These findings indicate that *C. burnetii* is widely spread in cattle in Toukh, Qalubyia governorate. In conclusion, we recommended the establishment of sanitary measures and an appropriate preventive strategy to reduce the spreading of the *C. burnetii* infection among farm animals and humans in Qalubyia governorate.

1. INTRODUCTION

Q or 'query' fever, febris Q or Coxiellosis is a crucial worldwide zoonotic disease that affects both human and animal species. The World Organization for Animal Health (OIE) classifies Q fever as a disease affecting several species(OIE, 2018). Coxiellosis is caused by *Coxiella burnetii*, which is a Gram-negative obligate intracellular bacterium characterized by its antigenic shift (phase variation). *C. burnetii* is divided into 2 forms the first one is highly infectious (phase I) in the host, while the second one a non-infectious (phase II) in culturing (Bielawska-Drózd et al., 2013, Rathish et al., 2022). The primary reservoirs of *C. burnetii* infection are cattle, sheep, and goats (Mohabbati Mobarez et al., 2017). Among animals *C. burnetii* can be transmitted via inhalation of contaminated aerosols and dust, ingesting the contaminated food (hay or straw) or by ticks' bites. While humans become infected via contaminated aerosols or drinking raw milk (Pexara et al., 2018). *C. burnetii* infections may be exhibited clinically or as an asymptomatic infection and pass unnoticed (Mori et al., 2017). Predominantly, it causes reproductive disorders in ruminants, such as weak calves, stillbirths, and abortion which mostly occur at the end of gestation. Moreover, *C. burnetii* might be associated with metritis and infertility in cattle (Robi et al., 2023).

The incidence of Coxiellosis in cattle is influenced by various host factors (animal-related factors), such as age, sex and breed. Older animals are more susceptible, and females are more commonly affected. Moreover, certain breeds display increased susceptibility (Alsaadawy et al., 2023). Small ruminants can harbor and transmit the bacteria so contact with these animals represents another significant risk (Selim et al., 2023). Moreover, the transmission of *C. burnetii* by ticks amplifies this risk, highlighting the need for tick control measures. Rather implementing acaricide strategies is crucial for reducing transmission risk. Herd size serves as a significant determinant, as large populations can elevate the risk due to higher animal density and enhanced disease propagation opportunities (Sadiki et al., 2023). Additionally, a history of abortion or stillbirth within animals acts as a concerning indicator of potential *C. burnetii* presence (Tukur et al., 2014). The Enzyme-linked immunosorbent assay (ELISA) is a commonly used method for detecting antibodies in serum samples of infected animals. This assay has demonstrated the ability to detect even low titer of antibodies exhibiting a high level of specificity and sensitivity. As a result, ELISA is recommended over other serological tests such as IFAT and CFT for serological diagnosis of Coxiellosis because ELISA is more practical in large-scale screening and ELISA is more sensitive than CFT (OIE, 2018). There are several studies have been carried out to clarify the epidemiological situation of *C. burnetii* infection in Egypt (Nahed and Khaled, 2012,

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Gwida et al., 2014, Klemmer et al., 2018, Abbass et al., 2020, Alsaadawy et al., 2023). Herein, the objective of the present study is to ascertain the seroprevalence of Coxiellosis in cattle in the Qalubyia governorate, Egypt, as well as identification of the potential risk factors associated with the disease.

2. MATERIAL AND MET HODS

2.1. Ethical approval

The ethics committee of the faculty of veterinary medicine at Benha University declared that the study was performed consistent with its guidelines and regulations and it was approved by ethical number (BVFVTM 24-02-23)

2.2. Study areas

This study was conducted on cattle situated in Toukh, Qalubyia governorate of northern Egypt. The climatic factors in this region are characterized by hot and dry summers, occasionally windy springs, and cold, sometimes rainy winters. These weather conditions foster the proliferation of vectors, particularly ticks, contributing to the transmission of *C. burnetii*.

2.3. Animals and collected samples

Cattle were employed as subjects in the present study, with sera samples collected for analysis. The calculation of the sample size was based on previous report indicating a 4.7% seroprevalence of bovine Coxiellosis (Farag et al., 2023) employing the Thrusfield formula (n=((Z)^2 P(1-P))/d^2) (Thrusfield, 2007), where n= sample size, Z = 1.96 for 95%confidence level, P = expected prevalence (4.7% seroprevalence from earlier mentioned report) and d = 5% required precision. Accordingly; the initially determined sample size was 69; however, to enhance precision, a total of 100 cattle were sampled for the investigation. This study was carried out over the period from October 2022 to December 2023 on cattle raised by individual breeders from Toukh, Qalubyia governorate. Clinical investigation of cattle revealed the presence of ticks on the examined animals. Further, the history of these animals declared contact with small ruminants, and the presence of abortions and stillbirth's problems. The blood samples were centrifuged at a speed of 3000 revolutions per minute for a duration of 10 minutes. The sera were stored at a temperature of -20°C until their analysis. Data on risk factors linked to *C. burnetii* infection was taken during sample collection. The questionnaire was taken including various factors such sex (female or male), age (<2, 2-4 and >4 years old), breed (Baladi, Holstein and Mixed), herd size (≤ 5 cows or > 5 cows), application of acaricides (yes or no), infestation of ticks (yes or no), history of abortion and stillbirths (yes or no) and contact with small ruminants (yes or no).

2.4. Serological test and results interpretation

The serum samples were analyzed to detect the presence of antibodies against *C. burnetii* using direct ELISA kits for Q fever from IDEXX Laboratories, USA, following the manufacturer's instructions. The kit is reported to have a sensitivity and specificity of approximately 100%. In addition, this kit was previously validated by(Klemmer et al., 2018). The percentage of the sample to positive control was calculated using the formula (S/P %) = 100 x (sample450 - negative mean450)/ (positive mean450 - negative mean450). Samples with S/P% ≥40% or <30% were considered seropositive or seronegative, respectively. Samples with S/P% between ≥30% and <40% were considered suspect (doubtful) and doubtful samples were considered to be negative samples.

2.5. Statistical analysis

The statistical analysis was carried out using Chi-square using SPSS, ver. 27 (IBM Corp. Released 2013). Data were treated as a complete randomization design according to (Steel et al., 1997). The significance level was set at < 0.05.

3. RESULTS

The result of a serological investigation using an ELISA test revealed that 47 cattle were positive for *C. burnetii* antibodies from the 100 tested serum samples, yielding an overall seroprevalence rate of 47%(47/100). The univariable analysis of animal-related risk factors identified age and breed were greatly significant factors (P = 0.000***, 0.000***respectively). However, sex did not reveal any significant associations with *C. burnetii* seroprevalence (P = 0.136 NS). The seropositivity rate was higher in females (50.68%) than males (37.04%), while older cattle, particularly those older than 4 years, had (67.44%) the highest seropositivity rate. In addition, mixed-breed cattle had a higher rate (61.82%) compared to Holstein (40%) and Baladi (25.71%) breeds (Table 1).

Variables	Categories	No. of animals sampled	No. of positive animals	No. of negative animals	% of positive	Chi-square	Statistic P-value
Sex	Male	27	10	17	37.04	2.227	0.136
	Female	73	37	36	50.68		
		100	47	53			
Age	< 2	25	5	20	20.00	25.984	0.000***
	2-4	32	13	19	40.63		
	> 4	43	29	14	67.44		
		100	47	53			
Breed	Baladi	35	9	26	25.71	15.438	0.000***
	Holstein	10	4	6	40.00		
	Mixed	55	34	21	61.82		
		100	47	53			

Table 1 Univariable analysis of risk factors associated with *Coxiella burnetti* seropositivity among cattle about animal related factors.

NS = Non-significant at (P > 0.05) * = Significant at (P < 0.05) ** = Significant at (P < 0.01) *** = Significant at (P<0.001)

Herd size, acaricide application and a history of abortion and stillbirth did not show a significant relationship (P = 0.918, 0.837, 0.206 NS respectively). Contrary, tick infestation and contact with small ruminants were found to be significantly associated with *C. burnetii* seroprevalence (P = 0.008**, 0.000*** respectively) (Table 2). Cattle with tick infestation had (65.52%) a higher percentage of positive cases compared to those without infestation. Additionally, cattle cohabitating with small ruminants had a significantly higher likelihood (73.17%) of *C. burnetii* infection. Cattle in large herds had slightly higher seropositivity than Cattle in small herds (47.62% and 46.55% respectively). Moreover, cattle without acaricide application (47.69%) had a slightly higher prevalence rate than (45.71%) of those subjected to the acaricide program. Cattle without a history of abortion and stillbirth (50.72%) showed higher seropositivity than (38.71%) cattle that had such a history

Table 2 Univariable analysis of risk factors associated with *Coxiella burnetti* seropositivity among cattle in relation to other risk factors.

Variables	Categories	No. of animals sampled	No. of positive animals	No. of negative animals	% of positive	Chi-square	Statistic P-value
Herd size	≤ 5 cows	58	27	31	46.55	0.011	0.918
	> 5 cows	42	20	22	47.62		
Total		100	47	53			
Acaricide application	Yes	35	16	19	45.71	0.043	0.837
	No	65	31	34	47.69		
		100	47	53			
Infestation of Ticks	Yes	29	19	10	65.52	6.943	0.008**
	No	71	28	43	39.44		
		100	47	53			
Contact with small ruminants	Yes	41	30	11	73.17	18.980	0.000***
	No	59	17	42	28.81		
		100	47	53			
History of abortion and stillbirth	Yes	31	12	19	38.71	1.600	0.206
	No	69	35	34	50.72		
		100	47	53			

NS = Non-significant at (P > 0.05) * = Significant at (P < 0.05) ** = Significant at (P < 0.01) *** = Significant at (P<0.001)

4. DISCUSSION

Q fever infection is proliferating within the cattle population in Egypt (Klemmer et al., 2018, Selim et al., 2023). Various techniques are available for diagnosing *C. burnetii* infection in both humans and animals; however, ELISA for serological investigation and PCR for molecular detection of Coxiella DNA are consistently regarded as the most optimal approaches, according to earlier studies(Khalifa et al., 2016, Saleh et al., 2021).

The current research offers crucial insights into the occurrence of C. burnetti antibodies in cattle in Toukh, Qalubyia Governorate, Egypt, and utilizing the ELISA test as a diagnostic approach. On this study, the overall prevalence rate was 47% in cattle serum samples, which closely resembled the findings of a previous study conducted in the Assuit governorate, where an overall seroprevalence of 50.7% for Q fever was reported in cattle (Abbass et al., 2020). However, *C. burnetii* seropositivity was notably lower in cattle populations across various areas, including Giza, Cairo and El-Fayum governorates it was 13% (Nahed and Khaled, 2012), while in Dakahlia, Port Said and Damietta governorates it was 13.2% (Gwida et al., 2014), 25 Egyptian governorates except Sinai it was 19.3% (Klemmer et al., 2018), in Assuit, Bani-Swif, Dakahlya, Fayoum, Giza, and Minya governorates it was 38.5% (Mo'awad et al., 2022), Assuit was 15.67% (Alsaadawy et al., 2023), and Sohag was at 4.7%(Farag et al., 2023). The differences observed in Coxiella seroprevalence could potentially be explained by variations in sample sizes, animal husbandry practices, and regional climate (Menadi et al., 2020).

In relation to the analysis of risk factors, tick infestation and contact with small ruminants were highlighted as prominent factors influencing *C. burnetii* seroprevalence. Ticks serve as important vectors for both mechanical and biological transmission of Coxiellosis (Navaei, 2023). Ticks-infested cattle showed a higher percentage of positive cases (65.52%), while only (39.44%) of the non-infested group tested positive, This result aligns with a previous study recorded by (Asadi et al., 2014, Amin et al., 2022). In contrast (Elelu et al., 2020) reported that the difference between the seroprevalence of *C. burnetii* and tick infestation did not attain statistical significance. Moreover, a significantly elevated seroprevalence rate of (73.17%) was recorded when cattle shared an environment with small ruminants, this finding is in accordance with (Menadi et al., 2020). This might linked to the increased susceptibility of hosts, especially goats and sheep where they act as the most susceptible farm animals and at high risk, serving as potential carriers capable of transmitting the disease to other ruminants (Bwatota et al., 2022).

Significantly, age and breed played crucial roles in impacting *C. burnetii* seroprevalence, particularly older cattle aged above 4 years, which exhibited a higher seroprevalence rate of (67.44%) followed by (40.63%) in the 2-4 years age category; meanwhile, the lowest seroprevalence rate of (20%) was observed in cattle under 2 years. These results were in line with those revealed by (Alvarez et al., 2012, and Barlozzari et al., 2020). This might be due to cumulative exposure to the pathogen over time, coupled with lowered immunity in older animals (Deressa et al., 2020).

Moreover, mixed-breed cattle showed a higher rate of (61.82%), exceeding Holstein (40%) and Baladi (25.71%) breeds. This might be due to Variations in immune system characteristics and genetic diversity, impacting susceptibility to infection (Gharban and Yousif, 2020). A similar finding was documented by (McCaughey). On the other side, The seroprevalence of *Coxiella burnetti* infection was higher in the native breed of cattle than in other breeds (Tukur et al., 2014, Alsaadawy et al., 2023).

The seroprevalence rate is higher in females (50.68%) than in males (37.04%), Previous studies supported these findings in cattle (Sadiki et al., 2023). The increased prevalence in females could potentially result from the strong affinity of *C. burnetii* for the uterus and mammary glands and commonly, females were kept for extended periods for breeding, while males were utilized for meat production purposes (Elelu et al., 2020). Cattle in herds with more than five animals had a slightly higher (47.62%) seroprevalence than cattle in small herds with five or fewer animals (46.55%). This result is in contrast with that obtained by(Obaidat and Kersh, 2017) who significantly reported that herd size is a risk factor for *C. burnetii* seropositivity in cattle large herds. The increased seroprevalence in larger herds could be attributed to higher animal density, leading to a higher risk of transmission through direct contact and inhalation of aerosols (Paul et al., 2012). Cattle without acaricide application had a slightly higher prevalence rate than those subjected to the acaricide program (47.69% and 45.71% respectively) (Tukur et al., 2014), This result also proved that the presence of ticks and other modes of transmission (inhalation and ingestion) are playing an important role in the transmission of *coxiella burnetti* among animals (Pexara et al., 2018). Cattle that had a history of abortion and stillbirth showed a lower seroprevalence of *Coxiella burnetii*, with (38.71%) testing positive compared to (50.72%) of cattle without such history. This may be linked to the nature of *C. burnetii* infection which is symptomatic or asymptomatic and may pass unnoticed (Mori et al., 2017).

5. CONCLUSIONS

In conclusion, this study throws light on the increasing prevalence of *C. burnetii* infection within the cattle population in the Qalubyia Governorate, Egypt. Utilizing ELISA testing as a diagnostic approach, the results revealed an overall seroprevalence rate of 47%. Various epidemiological determinants, including tick infestation, the coexistence of small ruminants, age, and breed of animal, exert an impact on the transmission of the infection. These findings provide valuable insights into the epidemiology of *C. burnetii* infection among cattle in Egypt and highlight the importance of tick control and managing the presence of small ruminants to mitigate the spread of the disease. Additionally, increasing community awareness concerning the zoonotic transmission and economic impact of *C. burnetii* infection is critical in preventing its propagation.

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