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Original Article

Serological Investigation of Bovine *Chlamydia abortus* in Wasit Province, Iraq

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Abstract

Chlamydia abortus is one of the most common infectious and zoonotic bacteria, which causes abortion in different ruminants as well as other animals and humans. For the first time in Iraq, the current study aimed to identify the prevalence of *C. abortus* in cattle using the enzyme-linked immunosorbent assay (ELISA). A total of 276 venous blood samples were collected from November (2021) to January (2022), subjected to obtaining sera that ELISA tested. Infection severity and estimation of the relationship between serum positivity and risk factors (including age, sex and region) were evaluated. An overall 17.03% of samples were being positive for *C. abortus*, which classified as mild (63.83%), moderate (25.53%) and severe (10.64%) infections with OD values of 0.3271±0.0085, 0.527±0.0139 and 0.7084±0.0256, respectively. The association of positivity to risk factors revealed that there was significant variation in their values as follows: for age, significant increases in prevalence and risk factors were detected in cattle aged < 3 years when compared to 3-6 and > 6 years; whereas, for sex, significant increases were found in females more than males. For the region, significant higher and lower prevalence and risk exposure to were reported in Al-Hai and Al-Kut, respectively. The association of mild, moderate and severe infections to risk factors showed that moderate infection was elevated significantly in cattle of <3 years, while the mild infection increased significantly in cattle aged 3-6 and >6 years with the absence of severe infection in both last age groups. In females and males and all study regions, mild infection appeared more significantly than moderate and severe infections (P<0.05). The present study was the first serological detection of *C. abortus* in cattle in Wasit province (Iraq); furthermore, studies are necessary to estimate the prevalence of *C. abortus* in cattle and other field animals.

Keywords: *Chlamydophila abortus*, Enzootic abortion, ELISA, Border disease, Cattle

1. Introduction

Chlamydia abortus is a non-motile, coccoid, pleomorphic, gram-negative, obligatory intracellular bacterium that belongs to the Chlamydiaceae family of the Chlamydiales Order classified under the phylum of Chlamydiota (1). *Chlamydia* spp. has a unique life cycle, including the non-infectious reticulate form found only inside the cell and the smaller and relatively inert infectious elementary form taken by a host cell and remains inside a membrane-bound inclusion body in the cytoplasm of a cell (2). Direct and indirect infection transmissions have been reported as a result of the excretion of organisms in large amounts by the expelled placenta, uterine discharges, and feces of infected and aborted animals (3). In many countries around the world, *C. abortus* is a cause of abortion and fetal losses, mainly in mammals, in particular ruminants (cattle, sheep, and goats) and non-mammalian hosts, causing tremendous economic losses (4-6).

In Iraq, the incidence of reproductive diseases in cattle appears to be increasing over the years. Chlamydia diseases are frequently asymptomatic, and clinical signs

are seen individually in cattle, often noticed as a non-specific loss in reproduction, sub-clinical mastitis, pneumonia, and weight loss (7). Also, most adult animals that have been infected before pregnancy show no clinical signs of infection, with the organism arriving in a dormant phase (8). In pregnant cows, *C. abortus* infection occurs during the 6th to 8th months of gestation, particularly among heifers in their first pregnancy, sometimes resulting in weak and premature calves (9, 10). In aborted cases, a presumptive diagnosis can be made based on gross pathological lesions in cotyledons and intercotyledonary membranes and confirmed by either detection of elementary bodies in stained smears (11) or isolation of organisms by culture (12). However, both methods cannot be applied in epidemiological studies due to difficulties in isolation, requiring high expertise and special laboratories (13). Other methods include antigen detection using immunochemical and molecular assays and antibody detection using serological techniques, characterized by their high sensitivity and specificity (14). The Enzyme-linked immunosorbent assay (ELISA) is one of the most specific available tests and is usually applied in epidemiological surveys (15).

The serological prevalence of *C. abortus* varies considerably between regions of a country as well as between different countries (7, 16). Due to the zoonotic potential, *C. abortus* has attracted increasing interest. However, available data about the prevalence of the organism in cattle in Iraq remains low and needs to be supported using advanced diagnostic assays. Therefore, for the first time, this study was designed to investigate the seroprevalence of *C. abortus* in cattle in Wasit province (Iraq) and to identify the severity of infection among seropositive animals. Additionally, our study aimed to estimate the association of positive findings with risk factors, age, sex, and region.

2. Materials and Methods

2.1. Study Animals

Two hundred seventy-six cattle of different ages and sexes were selected randomly from different areas (four regions, Al-Kut, Al-Hai, Al-Numaniyah, Sheikh Saad) in Wasit province (Iraq) from November (2021) to January (2022). Each study animal was subjected to draining jugular venous blood under aseptic conditions into vacuum-free-anticoagulant glass tubes centrifuged at 5000 rpm (5 minutes). The sera were transferred into labelled Eppendorf tubes and frozen in a refrigerator at 4 ºC until examined serologically by ELISA.

2.2. Serological Examination by *ELISA*

Following the manufacturers' instructions for the Bovine Chlamydia abortus ELISA Kit (SunLong Biotech, China), the serum samples, solutions, and micro ELISA strip plate were prepared. For each ELISA kit, the procedure involved adding 50µl of positive and negative control solutions, in duplicate, to the first wells (1-4) with adding 40µl of Sample Dilution Buffer and 10µl of sera to all other wells (5-95) except the blank well (96). The microplate was sealed, incubated (37ºC / 30 min), and washed with the Diluted Washing Buffer 5 times. A total oϕ 50 µl of HRP-Conjugate was added to all wells except the blank, and the microplate was sealed, incubated (37ºC / 30 min), and washed with the Diluted Washing Buffer 5 times. A total oϕ 50 µl of each Chromogen A and B was added to each well (except the blank), and the microplate was incubated (37ºC / 15 min). A total oϕ 50 µl of Stop Solution was added to all wells, and the microplate was placed in the Microtiter Plate Reader (BioTek, USA) to measure absorbance at an optical density (OD) of 450nm. For determining results, effectiveness identification, critical value (CUT OFF) calculation, and positive/negative judgments were determined at <253 for negative OD and ≥253 for positive OD. Based on their values, the positive ODs were classified into 3 categories; mild (253-450), moderate (451-600) and severe (>600) infections.

2.3. Statistical Analysis

All obtained data were documented using the Microsoft Office Excel version 2016 (Microsoft, USA) software and analyzed statistically using GraphPad Prism version 9.0.2 (GraphPad Inc, USA). One-Way ANOVA and Odds-Ratio were applied to detect

significant differences between groups in the severity of infections and to estimate the association between seropositivity and risk factors (including age, sex and region), respectively. Each value was represented as either a percentage (%) or Mean±Standard Error (M±SE). Variation was considered significant at $P<0.05$.

3. Results

The findings of ELISA results revealed 17.03% (47/276) positive samples that involved 63.83% (30/47) mild, 25.53% (12/47) moderate and 10.64% (5/47) severe infections (Figures 1 and 2). In addition, values of mild, moderate and severe infections were 0.3271±0.0085, 0.527±0.0139 and 0.7084±0.0256, respectively (Figure 3).

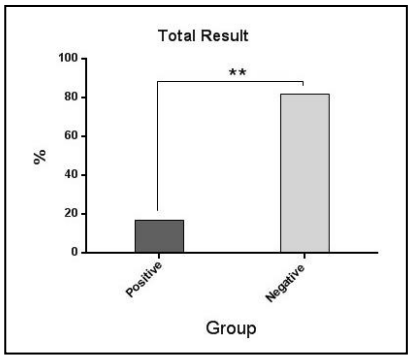

Figure 1. Total results of testing of *Chlamydia abortus* infection in 276 cattle by ELISA
($P\leq0.0017$) **

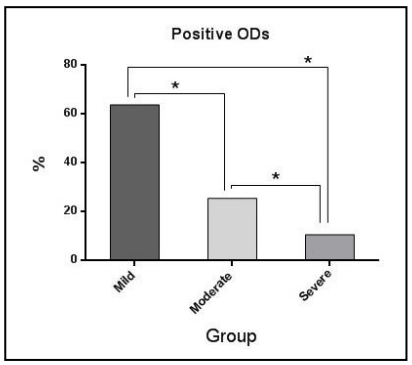

Figure 2. Distribution of percentage of positive ODsaccording to the severity of *Chlamydia abortus* infection. ($P<0.05$) *

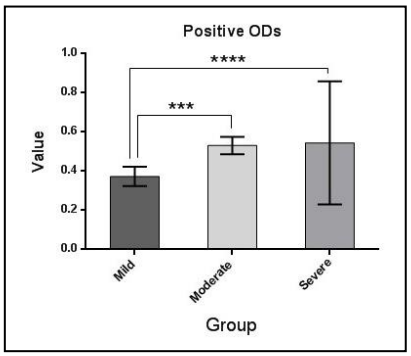

Figure 3. Distribution of values of positive ODs according to severity *Chlamydia abortus* infection
($P\leq0.0009$) ***, ($P\leq0.0001$) ****

Concerning risk factors, age, sex and region, the prevalence of positive findings was significantly variable (Table 1). For age, significant increases ($P<0.043$) in the prevalence of *C. abortus* were detected in cattle aged < 3 years (31.71%) in comparison with 3-6 (15.46%) and >6 (13.77%) groups that both showed insignificant differences ($P>0.05$) in their values. Also, cattle of < 3 years appeared at a significantly higher risk of infection (2.19) than 3-6 (0.86) and >6 (0.68). Regarding sex, there were significant increases ($P<0.034$) in prevalence and risk exposure of females (19.56% and 3.33, respectively) to *C. abortus* when compared to males (5.88% and 0.3, respectively). For the region, significantly higher prevalence and risk exposure to *C. abortus* were reported in Al-Hai (33.33% and 2.87, respectively), while lowered in Al-Kut (4.35% and 0.2, respectively) when compared to Al-Numaniyah (13.04% and 0.71, respectively) and Sheikh Saad (17.39% and 1.03, respectively).

Regarding the association between risk factors and the level of severity of infection, results reported a significant variation ($P<0.05$) in their values (Table 2). In this study, we found that cattle of <3 years showed a significant elevation ($P<0.04$) in moderate (46.15%) and severe (38.46%) *C. abortus* infection when compared to mild (7.69%) infection; however, cattle aged 3-6 and >6 years showed a significant increase of

mild infection (73.33% and 94.74%, respectively) when compared to moderate (36.36% and 5.62%, respectively) infection. Additionally, no severe positive infections were seen in the 3-6 and >6 age groups. In females and males, mild infection (63.64% and 66.67%, respectively) demonstrated a significant (P<0.05) higher prevalence than moderate (27.27% and 0%, respectively) and severe (9.09% and 33.33%, respectively) infections. Among all study regions, mild seropositive *C. abortus* infection findings were significantly more significant than moderate and severe infections (P<0.05).

Table 1. Association of positivity to risk factor in *Chlamydia abortus* infection (Total positives: 47 / 276)

Factor (Group)	Positive / tested	Prevalence (%)	Odds ratio	Risk	*P*-value
Age (Year)					
< 3	13 / 41	31.71 *	2.75	2.19	0.043
3-6	15 / 97	15.46	0.84	0.86	
>6	19 / 138	13.77	0.63	0.68	
Sex					
Female	44 / 225	19.56 *	3.89	3.33	0.034
Male	3 / 51	5.88	0.257	0.3	
Region					
Al-Kut	3/69	4.35	0.17	0.2	0.022
Al-Hai	23/69	33.33 *	3.817	2.87	
Al-Numaniyah	9/69	13.04	0.667	0.71	
Sheikh Saad	12/69	17.39	1.034	1.03	

Significance * (P<0.05)

Table 2. Association between severity of infection to risk factor *Chlamydia abortus* infection (Total positives: 47)

Factor	Total Positive	Mild	Moderate	Severe	*P*-value
Age (Year)					
< 3	13	1 (7.69%)	7 (46.15%) *	5 (38.46%)	0.04
3-6	15	11 (73.33%) *	4 (36.36%)	0 (0%)	0.024
>6	19	18 (94.74%) *	1 (5.26%)	0 (0%)	0.013
Sex					
Female	44	28 (63.64%) *	12 (27.27%)	4 (9.09%)	0.023
Male	3	2 (66.67%) *	0 (0%)	1 (33.33%)	0.018
Region					
Al-Kut	3	2 (66.67%) *	1 (33.33%)	0 (0%)	0.015
Al-Hai	23	14 (60.87%) *	6 (26.09%)	3 (13.04%)	0.024
Al-Numaniyah	9	8 (88.89%) *	0 (0%)	1 (11.11%)	0.013
Sheikh Saad	12	6 (50%) *	5 (41.67%)	1 (8.33%)	0.037

Significance * (P<0.05)

4. Discussion

The cattle industry had several challenges that affected its further development. Reproductive disorders represent one of these challenges worldwide and may be due to intrinsic and extrinsic factors imposed on the herd and individual animals, such as genotyping traits, feeding, contaminants and toxins in feeds or other environmental factors (17). *Chlamydia abortus* is one of the most common infectious bacteria with serious effects for infected pregnant animals and humans as well as for their fetuses. In this study, 17.03% of study cattle were positive serologically to *C. abortus*. In Iraq, only one recent study in Ninevah province detected that the seroprevalence of *C. abortus*

in cattle was 0.82% (18).Worldwide, the seroprevalence of *C. abortus* in cattle was 1.69% in Belgium (19), 4.44% in Ireland (20), 4.65% in India (21), 17.83% in China (22), 26.4% in Poland (23), 26.92% in Turkey (24), 45% in Australia (25), 48.4% in Iran (26) and 51.3% in Taiwan (27). This variation in the prevalence of *C. abortus* between our study and others might be attributed to different factors such as the virulence of chlamydial strains and possibly innate immunity amongst animals, frequent exposure of study animals to infected or carrier animals, uncontrolled restriction for movement of diseased cattle from the contaminated region, nutritional deficiency, bad management, grazing strategies, breed of cattle, size of examined samples, type of serological test and its efficacy (sensitivity and specificity), and geographical location of the study. Significant prevalence of mild infection among seropositive cattle may be indicated that cattle are either less sensitive to being infected with *C. abortus*, increasing resistance to infection with advancing age as most study animals were larger than 3 years, or intracellular pathway of the organism which allows to limited exposure and then less developed antibody against infection.

Statistical analysis of risk factors (age, sex and region) revealed a significant difference in the distribution of seropositivity among these factors. For age, our findings demonstrated that cattle aged <3 Years were having a higher positivity rate of *C. abortus* infection than other age groups (3-6 and > 6 years) significantly, suggesting that *C. abortus* is either transferred vertically during pregnancy (inutero infection), their origin is due to subclinical mastitis of Chlamydia or due to reduced maternal immunity, especially after one year of age. (28). In this study, the significant prevalence of positivity in females may be caused by frequent infection by organisms due to its reproductive role and lowered immunity resulting from high stress due to gestation, milk production, and nutritional deficiency. Also, in this study, a low number of tested males and the fact that in Iraq, male cattle received more quantitatively and qualitatively nutritional and managemental attention since male calves were used for slaughter and for insemination purposes. Regarding study regions, cattle of the Al-Hai district showed a higher prevalence of *C. abortus* than in other regions. This might be due to the fact that animals in this region are mainly exposed to organisms, reduced therapeutic and controlled measures or direct contact with the carriers/reservoirs. The association of risk factors to the level of severity of infection confirmed that moderate and severe infections were more prevalent among cattle aged <3 Years; while mild infection appeared significantly in cattle aged 3-6 and >6 years, both females and males, and among all positive cattle throughout all study regions as reported by different studies as the calves are highly susceptible to infectious pathogenic agents, particularly newborn calves that not received adequate amounts of colostrums (18, 29). This study showed that *C. abortus* is widespread in cattle, particularly adult lactating cows, which might represent a potential source of transmission of infection to humans. The classification of positive infections according to their ODs might provide a valuable framework for subsequent investigations of potential farm-level influences causing these larger-than-expected differences in herd antibody seroprevalence to *C. abortus*. While eradication of *C. abortus* in Iraq is unlikely, the most substantial and cost-effective method for reducing its impact could be achieved by targeting control measures such as vaccination, particularly in areas with high seropositivity or cattle with a history of high abortion rates. Cattle might be susceptible to *C. abortus* even if they do not, in general, exhibit disease, and the causes of chronic sub-clinical reproductive problems linked with infertility and mastitis should be studied precisely.

Authors' Contribution

Study concept and design: H. H. E. A.
Acquisition of data: H. A. N.
Analysis and interpretation of data: I. M. A.

Drafting of the manuscript: I. M. A.
Critical revision of the manuscript for important intellectual content: H. H. E. A.
Statistical analysis: H. A. N.
Administrative, technical, and material support: H. H. E. A.

Ethics

The current study was accepted by and carried out under the authorization of the Scientific Committee in the College of Alkut University (Wasit, Iraq) and by the College of Veterinary Medicine, University of Basrah (Basrah, Iraq).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Sachse K, Bavoil PM, Kaltenboeck B, Stephens RS, Kuo C-C, Rosselló-Móra R, et al. Emendation of the family Chlamydiaceae: proposal of a single genus, Chlamydia, to include all currently recognized species. Syst Appl Microbiol. 2015;38(2):99-103.
2. Gitsels A, Van Lent S, Sanders N, Vanrompay D. Chlamydia: what is on the outside does matter. Crit Rev Microbiol. 2020;46(1):100-19.
3. Rönn MM, Wolf EE, Chesson H, Menzies NA, Galer K, Gorwitz R, et al. The use of mathematical models of chlamydia transmission to address public health policy questions: a systematic review. Sex Transm Dis. 2017;44(5):278.
4. Blumer S, Greub G, Waldvogel A, Hässig M, Thoma R, Tschuor A, et al. Waddlia, Parachlamydia and Chlamydiaceae in bovine abortion. Vet microbiol. 2011;152(3-4):385-93.
5. Borel N, Thoma R, Spaeni P, Weilenmann R, Teankum K, Brugnera E, et al. Chlamydia-related abortions in cattle from Graubunden, Switzerland. Veterinary Pathology. 2006;43(5):702-8.
6. Vidal S, Kegler K, Greub G, Aeby S, Borel N, Dagleish MP, et al. Neglected zoonotic agents in cattle abortion: tackling the difficult to grow bacteria. BMC veterinary research. 2017;13(1):1-13.
7. Softic A, Asmare K, Granquist EG, Godfroid J, Fejzic N, Skjerve E. The serostatus of Brucella spp., Chlamydia abortus, Coxiella burnetii and Neospora caninum in cattle in three cantons in Bosnia and Herzegovina. BMC Vet Res. 2018;14(1):1-9.
8. Barati S, Moori-Bakhtiari N, Najafabadi MG, Momtaz H, Shokuhizadeh L. The role of zoonotic chlamydial agents in ruminants abortion. Iran J Microbiol. 2017;9(5):288.
9. Macías-Rioseco M, Silveira C, Fraga M, Casaux L, Cabrera A, Francia ME, et al. Causes of abortion in dairy cows in Uruguay. Pesquisa Veterinária Brasileira. 2020;40:325-32.
10. Struthers JD, Lim A, Ferguson S, Lee JK, Chako C, Okwumabua O, et al. Meningoencephalitis, vasculitis, and abortions caused by Chlamydia pecorum in a herd of cattle. Vet Pathol. 2021;58(3):549-57.
11. Kumar P, Khanna G, Batra S, Sharma VK, Rastogi S. Chlamydia trachomatis elementary bodies in synovial fluid of patients with reactive arthritis and undifferentiated spondyloarthropathy in India. Int J Rheum Dis. 2016;19(5):506-11.
12. Esmaeili H, Hamedi M, Madani S. Isolation of Chlamydia spp. from Ewes and Does in Iran. Arch Razi Inst. 2017;72(4):249-53.
13. Arif ED, Saeed NM, Rachid SK. Isolation and identification of Chlamydia abortus from aborted ewes in Sulaimani Province, Northern Iraq. Pol J Microbiol. 2020;69(1):65.
14. Esmaeili H, Bolourchi M, Mokhber-Dezfouli MR, Teimourpour A. Detection of Chlamydia abortus and risk factors for infection in small ruminants in Iran. Small Rumin Res. 2021;197:106339.
15. Al-Ahmed TA, Salman SS. Seroprevalence of enzootic abortion and border disease in small ruminants in al-basra province, Iraq. Plant Arch. 2020;20(2):2722-7.
16. Adesiyun AA, Knobel DL, Thompson PN, Wentzel J, Kolo FB, Kolo AO, et al. Sero-epidemiological study of selected zoonotic and abortifacient pathogens in cattle at a wildlife-livestock interface in South Africa. Vector-Borne Zoonotic Dis. 2020;20(4):258-67.
17. Gharban HA, Yousif AA. Serological and molecular phylogenetic detection of Coxiella burnetii in lactating cows, Iraq. Iraqi J Vet Med. 2020;44(E0)):42-50.
18. Majed R, Maab A, Omer A, Hussein A. Preliminary study of seroprevalence of chlamydophila abortus amongst cattle in ninavah province. Adv Anim Vet Sci.

2018;6(3):135-8.
19. Yin L, Schautteet K, Kalmar I, Bertels G, Van Driessche E, Czaplicki G, et al. Prevalence of Chlamydia abortus in Belgian ruminants. Vlaams Diergeneeskd Tijdschr. 2014;83(4):164-70.
20. Wilson K, Sammin D, Harmeyer S, Nath M, Livingstone M, Longbottom D. Seroprevalence of chlamydial infection in cattle in Ireland. Vet J. 2012;193(2):583-5.
21. Didugu H, Reddy CEN, Ramanipushpa R, Ramaraju S, Reddy M, Satyanarayana M, et al. Serological investigation of Chlamydial infection among ruminants in Krishna district of Andhra Pradesh, India. J Livest Sci. 2016;7:187-91.
22. Qing-You L, Min-Jun X, Jing-Hua F, Xian-Hui H, De-Shun S, Kui-Qing C, et al. Seroprevalence of Chlamydia infection in dairy cattle in subtropical southern China. Afr J Microbiol Res. 2013;7(19):2010-3.
23. Niemczuk K. Prevalence of antibodies against Chlamydophila psittaci and Chlamydophila abortus in cattle in Poland. A preliminary report. Bull Vet Inst Pulawy. 2005;49(3):293.
24. Gokce H, Kacar C, Genc O, Sozmen M. Seroprevalence of Chlamydophila abortus in aborting ewes and dairy cattle. Bull Vet Inst Pulawy. 2007;51:9-13.
25. Biesenkamp-Uhe C, Li Y, Hehnen H-R, Sachse K, Kaltenboeck B. Therapeutic Chlamydophila abortus and C. pecorum vaccination transiently reduces bovine mastitis associated with Chlamydophila infection. Infect Immun. 2007;75(2):870-7.
26. Esmaeili H, Bolourchi M, Kalbasi S, Hamedi M. Anti Chlamydia abortus antibodies in aborted cattle in some farms around Tehran, Iran. Iranian Journal of Veterinary Clinical Sciences. 2016;10(1).
27. Wang FI, Shieh H, LIAO YK. Prevalence of Chlamydophila abortus infection in domesticated ruminants in Taiwan. J Vet Med Sci. 2001;63(11):1215-20.
28. Al-gharban HA. Serological diagnosis of persistent infection with Anaplasma marginale bacteria in cattle: Hasanain AJ. Al-gharban1 and Salim H. Dhahir. Iraqi J Vet Med. 2015;39(1):33-9.
29. Anderson ML. Infectious causes of bovine abortion during mid-to late-gestation. Theriogenology. 2007;68(3):474-86.