



Original Article

Serological and Histopathological Investigation of *Chlamydia abortus* in Aborted Ewes in Wasit, Iraq

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Abstract

Chlamydia abortus is one of the most important pathogens, which causes a marked economic loss in small ruminants, in particular sheep, worldwide. This study aimed to detect the prevalence of *C. abortus* in the sera of aborted ewes in Wasit province, Iraq, using the enzyme-linked immunosorbent assay (ELISA), followed by the investigation of the main histopathological alterations that occurred in some organs of the dead newborns. Out of 180 tested samples by ELISA, 32.22% of the evaluated animals showed positive reactions to IgG antibodies toward *C. abortus*. Concerning the titers of the infection of seropositive ewes, there were significant increases in values of moderate level of antibody titer (55.17%), compared to mild (32.76%) and severe (12.07%) levels of infection. History data showed a significant variation ($P<0.05$) in the existence of seropositive ewes with other field animals. However, significant increases ($P<0.05$) were reported in the seropositive ewes existed with goat (91.38%) and cattle (84.48%), while significant decreases ($P<0.05$) were observed in positive ewes found in donkeys (8.62%), horses (3.45%), and camels (0%). Regarding the histopathology results, the findings of the small intestine showed marked necrosis of intestinal villous, hyperplastic tissues, and necrosis in some mucosal glands; however, in the liver, there was small ductal proliferation with mild portal fibrosis, necrotic debris, and focal areas of hemorrhage in the parenchyma. In conclusion, the findings of this study represent the first Iraqi data concerning *Chlamydial* detection in aborted ewes; however, further studies in other regions and animal species are necessary to know the actual prevalence of organisms and initiate active measures for control and prevention.

Keywords: Abortion, ELISA, IgG, Iraq, Small ruminant

1. Introduction

Chlamydia abortus is one of the most important pathogens, which causes a marked economic loss in small ruminants, in particular sheep, worldwide (1). *C. abortus* (previously *Chlamydia psittaci* biotype1/serotype1) is a bacterium having a tendency to the placentas of ruminants causing a disease generally known as ovine enzootic abortion (2). The bacterium can cause an identical infection in goat, cattle, horses, and pigs (2, 3). There is no doubt that abortion products play a significant role in the

contamination of environments and transmission of infection to naïve ruminants due to the existence of a large number of bacteria in discarded placenta, discharges of vagina of aborted sheep, and remnants of dead newborns (4). Clinical expressions of disease are, in general, asymptomatic without apparent clinical symptoms of any impending abortions, except for probable alteration in behaviors or vaginal discharges prior to or a day from the occurrence of abortion (5). However, barren ewes, stillbirths, delivery of small weakly newborns, or lamb with hairy fleeces,

abnormal bodily conformations, and tremors were also reported (6).

The costs of chlamyphilosis to the industries of small ruminants are greatly difficult for determining due to a great decrease in the number of surviving newborns and abortions throughout the final stage of pregnancy (7). If there is a rapid and reliable diagnosis, controlling schemes can decrease the influence of disease in recent and future and prevent viable economic losses to the flock (8). Although definitive diagnosis of both infections is the isolation of the organism from the infected organs, it is continuously not practical in a diagnostic situation because it is labor-intensive, expensive, time-consuming, and cannot be applied for epidemiological studies (9). Several serological diagnostic methods have been developed to detect *C. abortus* in different samples and animal species with variable degrees of sensitivity and specificity (10). Enzyme-linked immunosorbent assay (ELISA) is one of the most widely used techniques for serological diagnosis of *C. abortus*, which detected specific antibodies at wide ranges of chlamydia's antigen involving polymorphic outer membrane proteins and major outer membrane proteins (11). In Wasit, an eastern Iraqi province, no epidemiological studies have been performed so far to detect the prevalence of *C. abortus* in sheep, and no Iraqi studies aimed to detect the prevalence of *Chlamydia* infection in aborted ewes. Therefore, this study aimed to detect the prevalence of IgG antibodies against *C. abortus* in the recently aborted cases of sheep in Wasit province using ELISA; moreover, it was attempted to investigate the histopathological effects of organisms on some fresh organs of dead newborns or aborted fetuses.

2. Materials and Methods

2.1. Animal and Sample Collection

A total of 180 aborted ewes found in different areas in Wasit province, Iraq, were included in the current study from September to October (2021). Each study animal was subjected to drain 5 ml of venous blood samples into a free-anticoagulant tube to obtain sera by centrifugation at 4000 rpm for 5 min. Additionally, the tissue specimens of some bodily organs were collected

from freshly aborted fetuses into plastic containers that contain 10% neutral buffered formalin.

2.2. Serology

Following the manufacturer's instruction (HIPRA, Spain), an indirect-ELISA kit coated with the specific antigen of *C. abortus* was used for detection and quantification of specific IgG in the serum samples of the study ewes and does. The solutions of the kit and serum samples were prepared, diluted, and reacted. After adding the Stop Solution, the optical density (OD) of the tested samples was read at a wavelength of 450 nm by an ELISA reader (BioTek, USA). To interpret the OD's results, IRPC (Relative Index $\times 100$) was measured applying this formula: $IRPC = [(OD_{450}^{Sample} - Mean\ OD_{450}^{Negative\ Control}) / (Mean\ OD_{450}^{Positive\ Control} - Mean\ OD_{450}^{Negative\ Control})] \times 100$. The results of sample testing were considered negative when the OD value of a sample was ≤ 40.0 , and positive when the OD value of a sample was >40.0 .

2.3. Statistical analysis

All study data were analyzed statistically using the IBM/SPSS (version 23). The Chi-square (χ^2) and t-test were also used to detect significant differences between values at $P < 0.05$.

3. Results

Out of 180 tested samples by ELISA, 58 (32.22%) animals showed a positive reaction to IgG antibodies toward *C. abortus*, whereas 122 (67.78%) cases were seronegative (Figure 1). Concerning the titers of infection among the seropositive ewes, the findings of this study reported a significant increase in the values of the moderate level of antibody titer (32/58; 55.17%), compared to mild (19/58; 32.76%), and severe (7/58; 12.07%) levels of infection (Figure 2). History data showed a significant variation ($P < 0.05$) in the existence of seropositive ewes with other field animals (Figure 3). However, significant increases ($P < 0.05$) were reported in the seropositive ewes existed in goats (53/58; 91.38%) and cattle (49/58; 84.48%); however, significant decreases ($P < 0.05$) were observed in positive ewes found in donkeys (5/58; 8.62%), horses (2/58; 3.45%), and camels (0/58; 0%).

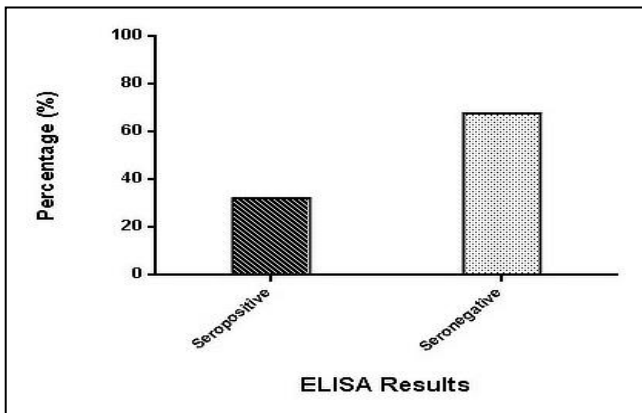


Figure 1. Total results for serological testing of 180 aborted ewes by ELISA
Significance * ($P < 0.05$)

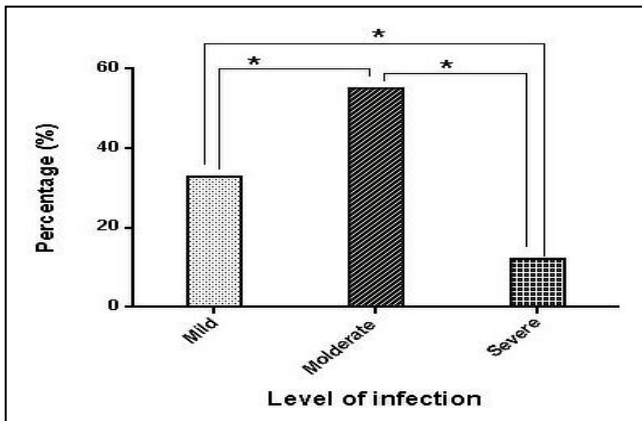


Figure 2. Level of infection among 58 seropositive ewes according to their ODs
Significance * ($P < 0.05$)

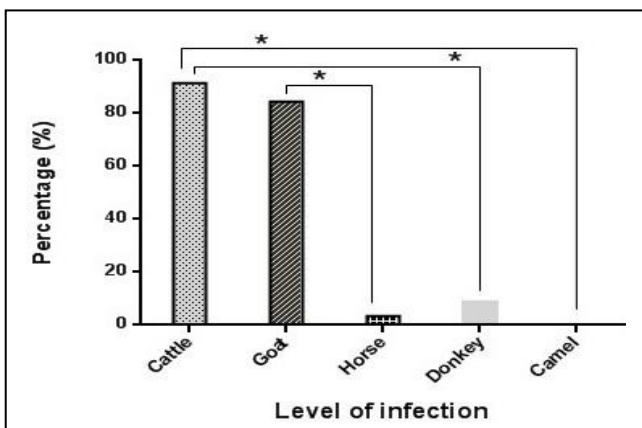


Figure 3. Existence of seropositive ewes with other field animals
Significance * ($P < 0.05$)

Based on the histopathology results, the findings of microscopic examination for intestinal specimens of aborted fetuses revealed lymphoid depletion, the irregular appearance of intestinal villi, diffuse infiltration of mononuclear cells in lamina propria, focal aggregation of cellular inflammatory cells, and necrotic debris in adjacent glandular tissue (Figures 4,5). In the liver, there were marked dilation and congestion in the portal and central veins, mild periportal fibrosis and hyperplasia of bile duct epithelia and thrombus, dilation of sinusoid with focal heterophil aggregation, and necrosis of ductal tissues (Figures 6, 7). In the lung, edema and emphysema were recognized with the presence of marked hemorrhage in peribronchial tissues and thrombus in a branch of the pulmonary artery. Focal electatic lesions were scattered in some cases (Figure 8). In the kidney, karyopyknosis in the epithelial cells of renal tubules, focal necrosis in cortex with the appearance of hyaline changes in cytoplasm, and detection of pyknosis in the nucleus were observed in more severely affected cases. Furthermore, there were degenerative changes in small arteries and hemorrhages in other affected aborted fetal cases (Figure 9).

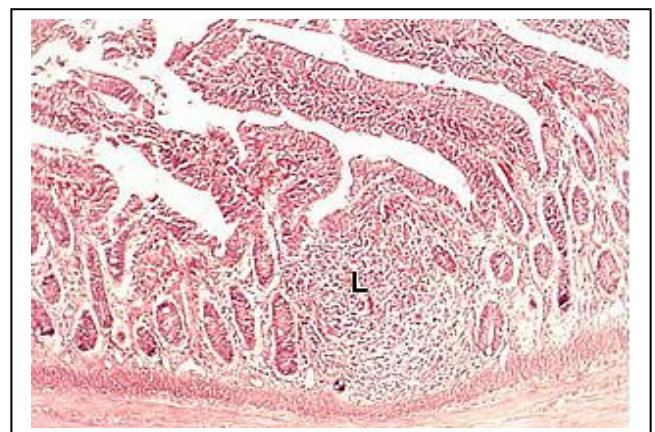


Figure 4. Histopathology of small intestinal specimens showing lymphoid depletion and irregular appearance of intestinal villi (H&E, 100x)

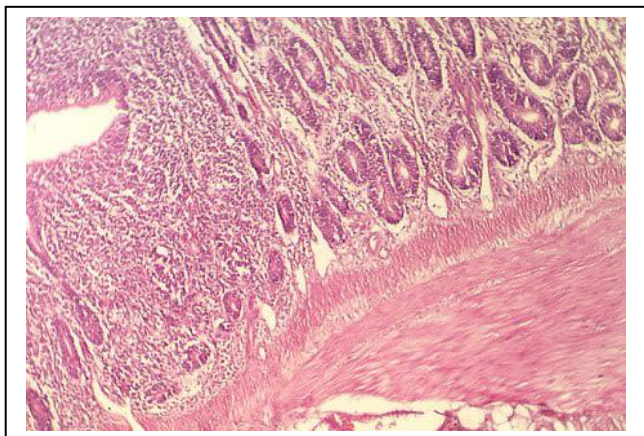


Figure 5. Histopathology of small intestinal specimens showing hyperplasia of submucosal glands with the prominence of lymphoid associated tissue (H&E, 100×)

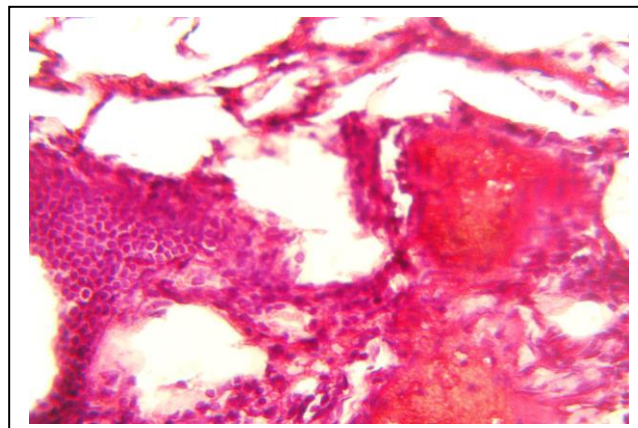


Figure 8. Histopathology of lung specimens showing hemorrhage, thrombus, and emphysema (H&E, 100×)

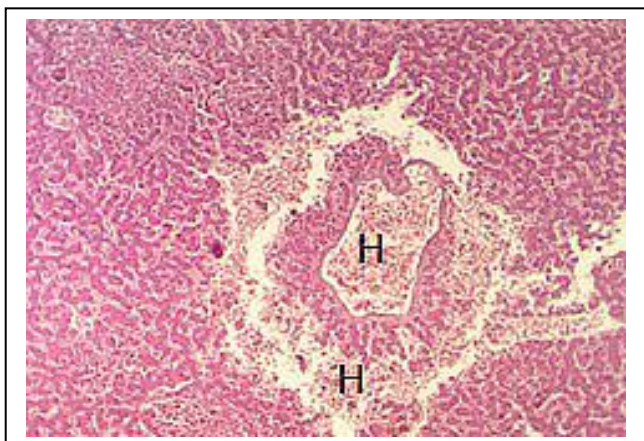


Figure 6. Histopathology of hepatic specimens showing focal hemorrhage and central lobular necrotic lesion in the liver parenchyma with hemorrhage (H&E, 100×)

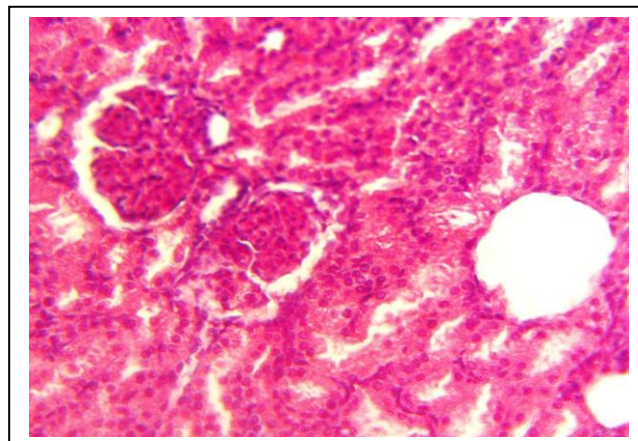


Figure 9. Histopathology of renal specimens showing focal necrosis, hyperemia, and karyopyknosis

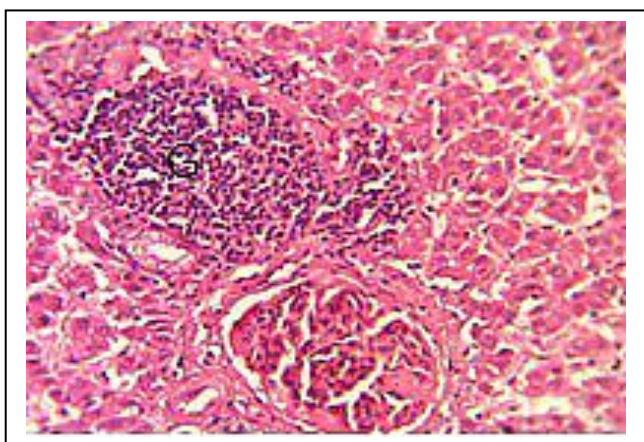


Figure 7. Histopathology of hepatic specimens showing granulomatous lesion in the liver parenchyma mainly adjacent to the portal area (H&E, 100×)

4. Discussion

Abortions by infectious agents in ewes and goats are considered an important cause of great economic loss. In Iraq, many pathogenic etiologies, such as brucellosis (12), campylobacteriosis (13), toxoplasmosis (14), enzootic abortion (7), border disease (15), neosporosis (16), and coxiellosis (17), were implicated with abortion in domestic animals. Our finding reported that the total prevalence of IgG antibodies against *C. abortus* was 32.22%. In comparison with other international studies, the total positive findings of the current study were higher than those detected previously in other provinces (2.15% of sheep in Dhi-

Qar, 2.66% of sheep in Al-Muthanna, 3.91% of sheep in Al-Basra, 4.08% of sheep in Maysan (18), 11.41% [8.44% of sheep and 26.67% of goats] in Baghdad (19), and 4.34% [7.33% of sheep and 1.35% of goats] in Nineveh) (20).

Many factors might play a role in increasing the rate of infection involving pathogen factor (increasing the threat of attacks to pathogen and rising of resistance rate to antibiotic over time), management factor (low-quality feeding or pasturing, lack of endogenous and exogenous prophylactic therapy, quarantine measures, and absence of an active scheme of vaccination), and environmental factors (geographical zone and climatic changes). The higher prevalence of infection in goats was similar to those recorded by other researchers (21).

Regarding other countries, these corresponding values are 24.82% in Jordan (22), 13.98% in Turkey (23), 21.5% in Brazil (24), 15.2% in Germany (25), 15.61% in Saudi Arabia (21), 20.9% in China (26), and 25.6% in Iran (27). The sensitivity and specificity of the applied serological diagnostic assay, in addition to the availability of predisposing (management and environmental) factors, could have a role in the variation between our results and those of studies worldwide. Concerning the levels of infection, moderate infection was appeared significantly suggesting either these animals are exposed frequently to the organism or that the pathogen has moderate-severe pathogenicity. In addition, the clinical diversity of infection might be raised from a complex interplay between host and pathogen, and genetic variation could result in increasing the susceptibility to infection within the host and an increasing capacity for virulence within the pathogen.

Our findings reported significant increases of seropositivity among aborted sheep in the existence of other domestic animals at the same field in particular goats and cattle. Numerous researchers referred to the role of goats in the transmission of infection to other animals and humans, especially in small farms that are unable to provide separate housing for breeding (28,

29). Gonzalez-Astudillo, Henning (30) mentioned that many producers underestimated the seriousness of the biosecurity around reproductive diseases, and *Chlamydia* in goats is a reportable condition, in which diagnosis could be done by additional laboratory testing for aborted ewes and testing of their fetal tissues.

The presence of *C. abortus* was also detected in non-aborted (31) and aborted (32) goats. Low or lack of association among the seropositive study of ewes, as well as the existence of equine and camelid might be attributed to the low number of study samples or low prevalence of organisms in these animals (32). However, placenta and aborted fetuses are the tissues with the highest bacterial load, and therefore, the best source for bacterial isolation (5). The sample flock husbandry conditions made it unfeasible to collect these tissues. In the current study, histopathological testing of fresh available fetal samples revealed variable lesions as described previously by other studies (33).

To the best of our knowledge, this is the first serological report performed in Iraq for the detection of *C. abortus* in the aborted ewes. Based on the obtained data, it can be concluded that both infections are endemic in Wasit province, and the positive sheep to *Chlamydia* represent an important potential public health hazard for humans. Further studies should be performed in other regions and on other animals to detect the actual prevalence rates of infections, as well as the role of other domestic animals in the transmission of infection. The development of effective control strategies and the application of vaccination schedules are of importance to decrease economic losses resulting from these infections.

Authors' Contribution

Study concept and design: H. H. M. A. and L. H. A.

Acquisition of data: H. H. M. A. and L. H. A.

Analysis and interpretation of data: H. H. M. A. and L. H. A.

Drafting of the manuscript: H. H. M. A. and L. H. A.

Critical revision of the manuscript for important intellectual content: H. H. M. A. and L. H. A.

Statistical analysis: H. H. M. A. and L. H. A.

Administrative, technical, and material support: H. H. M. A. and L. H. A.

Ethics

The present study was approved by the Scientific Committee of the College of Medicine, Wasit University, Wasit, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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