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SEROPREVALENCE OF ENZOOTIC ABORTION AND BORDER DISEASE IN SMALL RUMINANTS IN AL-BASRA PROVINCE, IRAQ

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Abstract

There is need to establish effective tools to control abortion in Iraqi sheep and goats; therefore, this study aims to detect the prevalence of enzootic abortion (*Chlamydophila abortus*) and border disease (*Pestivirus*) in sheep and goats using specific an indirect enzyme-linked immunosorbent assay (ELISA) kits. An overall 180 animals (100 ewes and 80 does) were selected randomly form different areas in Al-Basra province /Iraq, during December 2018 to January 2020, and subjected to venous blood collection and to documentation the history data of reproductive disorders. Totally, the results were revealed on 29.44% and 21.11% seropositive animals to *C. abortus* and border disease virus respectively. Significant increases in seropositive does (33.75% and 28.75%) in comparison to ewes (26% and 15%) were reported in both, *C. abortus* and border disease virus, infections respectively. Concerning to ODs as well as to IRPC and %IN of both diseases respectively, there are insignificant differences were detected between seropositive ewes and does. Regarding to reproductive disorders, no significant variation between seropositive and seronegative study ewes and does in both infections was reported. Abortion, dystocia and stillbirth for *C. abortus*, and abortion and dystocia for border diseases were the most significant reproductive disorders found among seropositive ewes. Abortions, dystocia, metritis, and stillbirth are the most significant reproductive disorders found among seropositive does for *C. abortus* but not for border disease virus. In conclusion, enzootic abortion and border disease are common infection among Iraqi flocks of sheep and goats, and further investigation using molecular diagnostic assays is needed.

Keywords**:** Sheep, Goats, Abortion, Iraq, *Chlamydophila abortus*, *Border disease virus*.

Introduction

Several infectious agents have been implicated in the etiology of ovine and caprine abortion which is an important cause of loss to the Iraqi sheep and goats industry. Enzootic abortion (EA) a bacterial disease caused by *Chlamydophila abortus* as well as the border disease (BD) caused by a *Pestivirus*, are two of the commonest causes of abortion in Iraq which both are reported in sheep, but only enzootic abortion detected in goats (Al-Rubayie and Hasso, 2014; Fahad and Salman, 2017). *Chlamydophila abortus* is a highly contagious pathogen which infected small ruminants particularly sheep resulting in a great economic losses due to annual abortions that rated 1-5% in diseased immune flocks and can be reach to 30% in recently infected flocks (Milne *et al.*, 2009). The infection sometimes causes placentitis with necrotic changes in the cotyledons and accumulation of reddish brown exudates in intercotyledonary areas; however, most infections in sheep and goats are asymptomatic apart from late term abortion or stillbirth (Hadley *et al.*, 1992; Buxton *et al.*, 2002). EA is usually transmitted through inhalation of infected barn dust or ingestion of contaminated food and water, and although infected animals develop immunity after abortion, they might remain carriers of the organism in their reproductive tract for up to 3 years (Rodolakis and Mohamad, 2010; Rodolakis, 2014). Also, rams/bucks may also acquire *C. abortus* from infected ewes/does and may spread the disease to other ewes at the time of breeding (Papp and Shewen, 1996; Aitken and Longbottom, 2007).

Border disease virus is a helical, enveloped, and non-cytopathic RNA virus which has been reported mostly as a sporadic cases a across many parts of the world, and rarely as outbreaks in association with persistent infection with bovine viral diarrhea virus (Kaiser *et al.*, 2016; Feknous *et al.*, 2018). The virus transmission occurs through contact with secretions and excretions of body fluids and tissues from infected animals, and spread rapidly through a flock (Kessell *et al.*, 2011). Persistently infected sheep and goats, usually asymptomatic seronegatives, are the major active reservoir which shed large quantities of virus in the urine, feces, and saliva (Pugh and Baird, 2012). However, if a pregnant ewe or doe is infected, the virus may be transmitted vertically to the embryo or fetus, and outcomes ranging from embryonic re-absorption to normal birth depending on the stage of gestation (Nettleton *et al.*, 1998; Pugh and Baird, 2012).

The basis for positive diagnosis of infection with *C. abortus* or border disease virus depends on a history of abortion in sheep and goats, evidence of placentitis, demonstration of organism or its antigens in affected placenta for *Chlamydophila*, or from abomasums, pancreas, kidney, thyroid, and testicular tissues of aborted fetus for border disease virus (Quinn *et al.*, 2011; Cebra and Cebra, 2012). Serological tests such as fluorescent antibody test (FAT), complement fixation test (CFT), agar gel immunodiffusion (AGID) test, and enzyme-linked immunosorben assay (ELISA) are useful to detect exposure in unvaccinated animals. The demonstration of specific *C. abortus* or border disease virus is specific evidence of infection particularly in tested samples that show a significant rise in antibody titers (Hovers *et al.*, 2014; OIE, 2018; O'Neill *et al.*, 2018).

In Basrah province / Iraq, there are no available data associated with EA and BD in sheep and goats. Therefore, the current study aims to detect the prevalence of *C. abortus* and border disease virus in ewes and does, serologically, using an indirect ELISA, and estimate the relation of antibody titers to the history of reproductive disorders.

Materials and Methods

Ethical approval

The present study is approved and performed under the Council of College of Veterinary Medicine, and the authority of the Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Study animals

A total of 180 animals involving 100 ewes and 80 does from many areas in Basra province / Iraq, were selected randomly for this study during December 2018 to January 2020. Under aseptic conditions, approximately 5ml of venous blood was drained by a disposable syringe into a free-anticoagulant glass gel tube. At laboratory, all blood samples were centrifuged at 4000rpm for 5minutes, and sera were saved into 1.5ml labeled Eppendorf tubes that kept frozen at -4°C until be tested serologically. Case history data concerning to reproductive disorder of study animals were documented based on their owner's.

Serological testing

***Chlamydophila abortus* :** 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 serum samples of study ewes and does. The solutions of kit and serum samples were prepared, diluted, and reacted. After adding of Stop Solution, optical density (OD) of tested samples was read at a wavelength of 450nm using the Microplate ELISA reader (BioTek, USA). For interpretation of OD's results, IRPC (Relative Index ×100) was measured applying this formula: IRPC= [(OD_{450} Sample – Mean $OD_{450}^{\text{Negative Control}}$) / (Mean $OD_{450}^{\text{Positive Control}}$ – Mean $OD_{450}^{\text{Negative Control}}$)] × 100. The results of sample testing were considered as negative when the OD value of a sample was ≤ 40.0, and positive when the OD value of a sample was >40.0.

***Border disease virus* :** An indirect-blocking ELISA kit (HIPRA, Spain) coated with the specific *Pestivirus* proteins (P80) as the antigen, which designed to detect of monoclonal IgG antibodies was used in this study. All kit reagents and serum samples were prepared, diluted, and reacted in according to manufacturer's instruction. After adding of Stop Solution, optical density (OD) of tested samples was read at a wavelength of 450nm using the Microplate ELISA reader (BioTek, USA). For interpretation of OD's results, %IN value was detected using this formula: %IN = [Mean $OD_{450}^{\text{Negative Control}}$ – OD_{450}^{Sample} / Mean $OD_{450}^{\text{Negative Control}}$] × 100. The results of sample testing were considered as negative when the %IN value <50, low positive when the %IN ≥ 50 and <80, and high positive when the %IN ≥80.

Statistical analysis

All obtained data were documented using the Microsoft Office Excel (*2016*) program, and analysed statistically using the IBM/SPSS (*version 23*) program. Chi-square (x^2) test was applied to detect significant differences between the positive findings of sheep and goats at a value of P<0.05 (Petrie and Watson, 2013).

Results

Chlamydophila abortus

Of 180 serum samples tested by an indirect ELISA for detection of specific IgG antibodies, the overall results were revealed on 53 (29.44%) seropositive animals for *C. abortus*. Among seropositives, there were 26 (26%) sheep and 27 (33.75%) goats. Statistically, there insignificant differences (P<0.048) were showed between positive sheep and goats (Table 1).

Table 1 : Total results of *C. abortus* infections by indirect ELISA

Type of animal	Total No.	Seropositive	Statistical analysis
Sheep	100	26 (26 %) B	*P < 0.048*
Goats	80	27 (33.75 %) A	
Total No.	180	53 (29.44%)	127

Variation in large vertical letters refers to significant differences (P<0.05)

Concerning to values (M ± SD) of ODs of seropositive animals, the study showed that there no significant differences (P<0.093) in levels of ODs between sheep (0.484 ± 0.52) and goats (0.467 ± 0.33), (Figure 1).

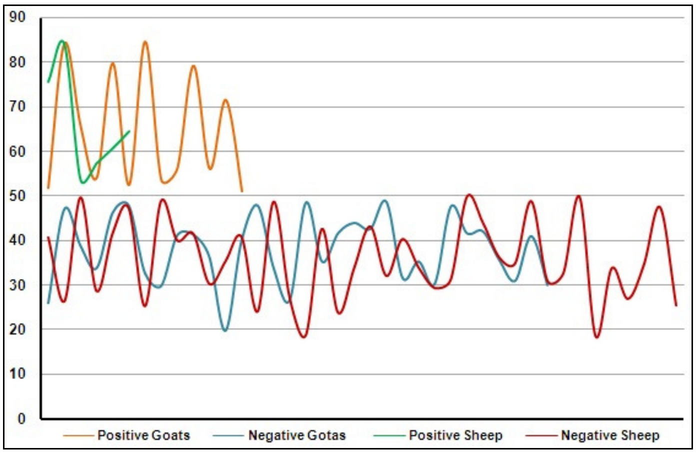

Fig. 1 : ODs level of IgG antibodies against *C. abortus* among study animals

For IRPC, no significant variation (P>0.097) was detected between seropositive sheep (54.28 ± 8.19) and goats (57.33 ± 12.34), (Figure 2).

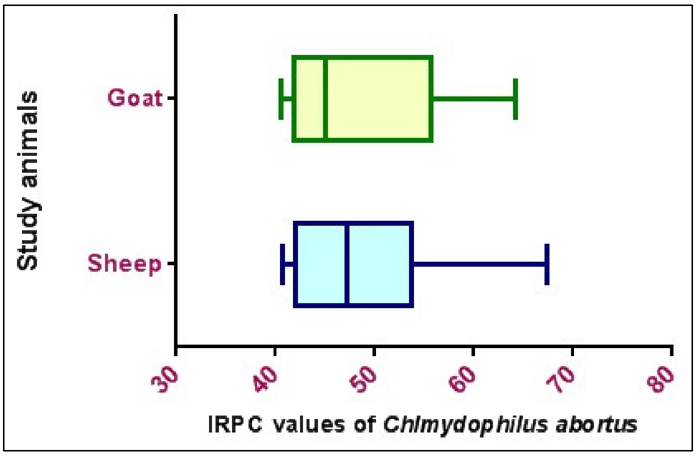

Fig. 2 : IRPC level among study animals tested by an indirect ELISA

Border disease virus

The positive findings for detection of specific IgG targeting P80 of border disease virus in a totally of 180 tested sera were 38 (21.11%); of which, 15 (15%) sheep and 23 (28.75%) goats (Table 2).

Table 2 : Total results of border disease virus infection by indirect ELISA

Type of animal	Total No.	Seropositive	Statistical analysis
Sheep	100	15 (15 %) [B]	*P < 0.039*
Goats	80	23 (28.75 %) [A]	
Total No.	180	38 (21.11%)	142

Variation in large vertical letters refers to significant differences (P<0.05)

In association to ODs of seropositive animals, the study showed that there were insignificant differences (P<0.084) between the values (M±SD) of sheep (0.239±10.15) and goats (258±9.76), (Figure 3).

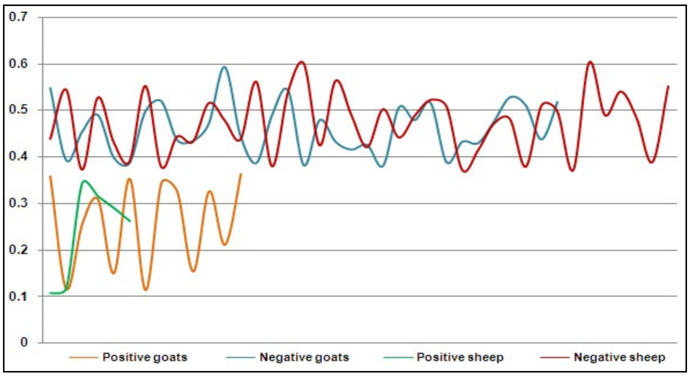

Fig. 3 : ODs level of IgG antibodies against Pestivirus among study animals

Also, significant differences (P<0.05) in values of %IN were not observed (P<0.079) between seropositive sheep (66.04±4.16) and seropositive goats (62.83±3.25), (Figure 4).

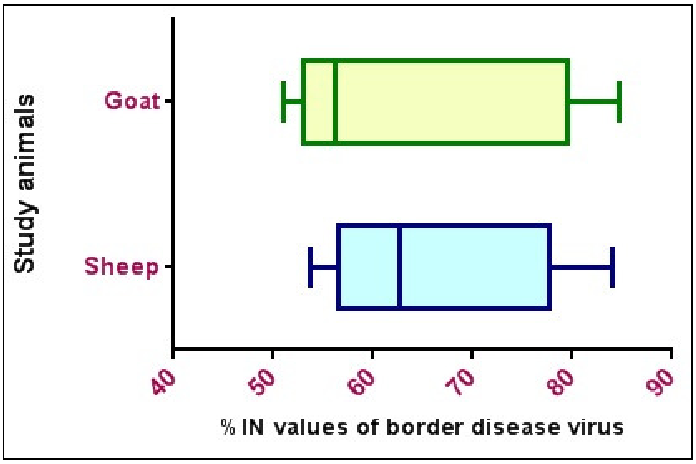

Fig. 4 : %IN level among study animals tested by an indirect ELISA

Clinical examination

History data of reproductive disorders showed that there were insignificant differences (P>0.05) between seropositive and seronegative study ewes for *C. abortus*; however, significant increases (P<0.05) among seropositive ewes were detected in abortion (34.62%), dystocia (15.38%), and stillbirth (11.54%). Regarding to border disease virus, there no significant increases were detected in reproductive disorders of seropositive ewes when compared to seronegatives. Abortion (26.67%) and dystocia (6.67%) were the most significant reproductive disorders found among seropositive ewes (Table 3).

Table 3 : Total results of history data of reproductive disorders among study sheep (No: 100)

Reproductive disorder	Total No.	Disease			
		C. abortus		Border disease virus	
		Seropositive (No: 26)	Seronegative (No: 74)	Seropositive (No: 15)	Seronegative (No: 85)
Abortion	32	9 (34.62%) [Aa]	23 (31.08%) [a]	4 (26.67%) [Ab]	28 (32.94%) [a]
Congenital birth	5	1 (3.85%) [Ca]	4 (5.41%) [a]	0 (0%) [Cb]	5 (5.88%) [a]
Dystocia	13	4 (15.38%) [Ba]	9 (12.16%) [a]	1 (6.67%) [Bb]	12 (14.12%) [a]
Infertility	2	0 (0%) [Da]	2 (2.70%) [a]	0 (0%) [Ca]	2 (2.35%) [a]
Metritis	11	2 (7.69%) [Ca]	9 (12.16%) [a]	0 (0%) [Cb]	11 (12.94%) [a]
Stillbirth	9	3 (11.54%) [Ba]	6 (8.11%) [a]	0 (0%) [Cb]	9 (10.59%) [a]

Variation in large vertical and small horizontal letters refers to significant differences (P<0.05)

History data of reproductive disorders were revealed on insignificant variation (P>0.05) between values of seropositives and seronegatives. However, there significant increases (P<0.05) were reported in abortions (7.41%), dystocia (7.41%), metritis (3.70%), and stillbirth (3.41%) of seropositive animals to *C. abortus*, but not for border disease (P>0.05), (Table 4).

Table 4 : Total results of history data of reproductive disorders among study goats (No: 80)

Reproductive disorder	Total No.	Disease			
		C. abortus		Border disease virus	
		Seropositive (No: 27)	Seronegative (No: 53)	Seropositive (No: 23)	Seronegative (No: 57)
Abortion	9	2 (7.41%) [Ab]	7 (13.21%) [a]	1 (4.35%) [Ab]	8 (14.04%) [a]
Congenital birth	2	0 (0%) [Ba]	2 (3.77%) [a]	0 (0%) [Aa]	2 (3.51%) [a]
Dystocia	6	2 (7.41%) [Aa]	4 (7.55%) [a]	1 (4.35%) [Aa]	5 (8.77%) [a]
Infertility	0	0 (0%) [Ba]	0 (0%) [a]	0 (0%) [Aa]	0 (0%) [a]
Metritis	4	1 (3.70%) [Aa]	3 (5.66%) [a]	1 (4.35%) [Aa]	3 (5.26%) [a]
Stillbirth	4	1 (3.70%) [Aa]	3 (5.66%) [a]	0 (0%) [Ab]	4 (7.02%) [a]

Variation in large vertical and small horizontal letters refers to significant differences (P<0.05)

Discussion

Abortions by infectious agents in ewes and goats consider as an important cause of great economic loss. In Iraq, many pathogenic etiologies were implicated with abortion in domestic animals such as brucellosis (Al-Tae and Al-Samarrae, 2013; Salman *et al*., 2018), campylobacteriosis (Hasso and Aldraji, 2018), toxoplasmosis (Al-Dabagh *et al*., 2014), enzootic abortion (Fahad and Salman, 2017; Salman *et al*., 2019), and border disease (Dahhir *et al*., 2019). In Al-Basra province, there are no recent available data about prevalence of enzootic abortion and border disease in small ruminants, sheep and goats.

In relation to *C. abortus*, this study reported that the total seropositive result was 29.44%. Also, this study indicated considerably higher serological prevalence of enzootic abortion in goats (33.75%) than sheep (26). In comparison to other Iraqi studies, the total positive findings of current study were higher than 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 (Cati *et al*., 2008), 11.41% (8.44% sheep and 26.67% goats) in Baghdad (Fahad and Salman, 2017), and 4.34% (7.33% sheep and 1.35% goats) in Nineveh (Majed *et al*., 2018). 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 as well as quarantine measures, and absence an active scheme of vaccination), and environmental factors (geographical zone and climatic changes). The higher prevalence of infection in goats which similar to that recorded by other studies (Aljumaah and Hussein, 2012; Fahad and Salman, 2017), is not necessarily an indication of higher susceptibility of goats to *Chlamydophila* as compared to sheep, since some investigations detected no variation between these two species in prevalence of enzootic abortion (Al-Qudah *et al*., 2004), while still other reported even higher prevalence in sheep than in goats (Cislakova *et al*., 2007; Majed *et al*., 2018). In other countries, there are 24.82% in Jordan (Al-Qudah *et al*., 2004), 13.98% in Turkey (Gokce *et al*., 2007), 21.5 in Brazil (Pinheiro *et al*., 2010), 15.2% in Germany (Runge *et al*., 2012), 15.61% in Saudi Arabia (Aljumaah and Hussein, 2012), 20.9% in China (Huang *et al*., 2013), and 25.6% in Iran (Esmaeili *et al*., 2015). Sensitivity and specificity of applied serological diagnostic assay, in addition to availability of predisposing (management and environment) factors could have a role to that variation between our results and worldwide studies. Concerning to levels of ODs and IRPC, no significant differences (P>0.05) were detected between seropositive sheep and goats suggesting endemic stability of infection in areas of tested animals.

For border disease, our study reported that the total seropositive result was 21.11%; involving 15% sheep and 28.75% goats. In Iraq, only one study performed recently in Mosul city to detect of border disease virus infection in sheep and goats, and is revealed on 38.46% total seropositives (Dahhir *et al*., 2019). In comparison to other worldwide studies, prevalence of border disease in small ruminants was 4.3% in Canada (Heckert *et al*., 1994), 29.21% in Egypt (Zaghawa, 1998), 0.5% in Denmark (Tegtmeier *et al*., 2000), 36.3% in Spain (García-Pérez *et al*., 2010), 17% in Iran (Mohammadi *et al*., 2011), 75.9% in Turkey (Tutuncu *et al*., 2011), and 73.1% in Algeria (Feknous *et al*., 2018). As in *C. abortus* infection, significant increases were reported also among seropositive goats. Our findings were in contrast to that detected previously by Zaghawa (1998) who reported that there is no significant differences between both sheep (27.5%) and goats (31.4%), and to (Dahhir *et al*., 2019) who recorded a higher significance in sheep (46.9%) than goats (16%). We thought that this variation in prevalence of border disease virus infection between sheep and goats is correlated to physiological and pathological factors. Macaldwie *et al*. (2003) showed experimentally that goats carried higher worm burdens than lambs at a different stage of development which cause a significant depletion in immunity against other infection. Compared to sheep, which develop a strong immunity around 12 month of age, goats acquire a lower level of immunity to infection (Gorski *et al*., 2004). Furthermore, Mortensen *et al*. (2003) reported that there is a significant reduces in effectiveness of treatment in goats than in sheep. Lack of statistical significance in values of ODs and %IN might be explained as both either endemic stability or equal sensitivity of both animal species to infection.

History data of reproductive disorders in sheep and goats of present study recorded that there are no significant variation between seropositive and seronegative sheep and goats in both infections. However, abortion, dystocia, and stillbirth were appeared more significantly among seropositive sheep and subsequently goats to *C. abortus*. This meaning that there a high risk of reproductive disorders in seropositive small ruminants, and intensive management is favored to be interrupted the spread of infection among flocks. Previously, it was believed that all the chlamydial diseases in sheep and goats including abortion, polyarthritis, and conjunctivitis were caused by *Chlamydia psittaci* and that the organism could also be found in the intestine of sheep with no clinical signs of disease. *Chlamydophila abortus*, previously *Chlamydia psittaci* immunotype / serovar 1, has been shown to be a different and separate organism to *C. psittaci* and reclassified as a different genus (Karlsen *et al*., 2008; Joseph *et al*., 2015). Therefore, enzootic abortion frequently occurs as a reproductive flock/herd disorder in the absence of other so-called *Chlamydia* syndromes such as conjunctivitis, polyarthritis, gastroenteritis, mastitis, and meningoencephalitis. It should be appreciated that ovine is not a spectrum of disease caused by a single bacterial agent but rather a group of different disease syndromes caused by different chlamydial species (Aljumaah and Hussein, 2012). Gerber *et al*. (2007) reported that the newly infected flocks, the percentage of abortion may reach up to 30% in pregnant ewes in the last trimester or give birth to weak or dead lambs. After abortion, ewes in these flocks may develop a protective immunity; and subsequently, yearly losses in endemically infected flocks may decrease to a lower level with sheep either born into the flock or newly introduced animals likely to suffer abortions during their initial pregnancies (Stuen and Longbottom, 2011). In pregnant ewes and does, border disease virus is of great concern for small ruminant producers because of the problems it causes when infecting susceptible pregnant ewes/does. Abortion, stillbirths, and unviable lambs/kids are the main losses caused by infection with this pestivirus (García-Pérez *et al*., 2010). The most relevant epidemiological feature of pestiviral infections is their ability to generate persistent infection that occurs when pregnant ewes/does infected at first stage of pregnancy (Menzies, 2011). Infection during early embryonic and fetal development can lead to the birth of persistently infected animals that remain immunotolerant and seronegative for life and shed the virus in large quantities (Mohammadi *et al.*, 2011). Consequently, small ruminants in contact with persistently infected animals quickly become infected and develop protective long-standing neutralizing antibodies (Kirkland *et al.*, 2019). Therefore, it suggested that many reproductive disorders observed in seronegatives ewes/does might be caused by presence of underestimate pestivirus activity due to persistently infected individuals.

Conclusion

The prevalence rates of *C. abortus* and border disease virus refer to a widespread of infection among small ruminants. Goats may act as an active reservoir for transmission of abortive causes to other field animals. *C. abortus* as an important potential a public health hazard for human as well as a mysterious cause of abortion, additional monitoring efforts should be aimed. Based on our findings, we suggesting that border disease virus infections are common in Iraq. Further knowledge regarding the prevalence, distribution, and epidemiology of ovine and caprine enzootic abortion and border disease using highly sensitive and specific tests must be carried out periodically and annually to confirm its implication in abortion for developing effective control strategies, and to assess their economic impacts.

Author's Contributions

Tamadhir A. Al-Ahmed was responsible for blood samples collection from study animals, history data documentation, and analysis of results; and Sufian S. Salman was responsible for serological testing. Both authors contributed in writing this manuscript, as well as in reading and approving the final manuscript.

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