First report on molecular detection of *Chlamydia abortus* infection in sheep flocks in Mosul, Iraq

Y.A. Rhawy[1] and O.M. Al-Iraqi[2]

[1]Department of Animal Resource, Directorate of Agriculture Nineveh, [2]Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

Chlamydia abortus is considered one of the significant causes of abortion in the sheep population across the country and leads to heavy economic losses. The current study aimed to detect *Chlamydia abortus* infection by microscopic examination using a Modified Ziehl-Neelsen stain and conventional polymerase chain reaction in sheep flocks in Mosul, Iraq. A total of 157 different samples (49 aborted fetuses, 52 placentas, and 56 vaginal swabs) were collected from 60 flocks from August 2022 to January 2023 in different areas of Mosul. A smear was made for microscopic examination, and Biopsies were taken from the placenta and the organs of aborted fetuses for PCR use 16S rRNA gene and OMP2 gene primers. Results indicate that the proportion of positive samples in the microscopic examination was 18.47%, showed in a large number of red dot-shaped bodies in the intercellular space, while the percentage of positive samples was 11.46% for the S16rRNA gene, while it was 4.45% for the OMP2 gene in molecular diagnostics as PCR. The Sensitivity, Accuracy, and Specificity were 78, 89, and 89%, respectively, for microscopic examination by comparison with PCR as a gold standard, and it was found that there was a moderate strength association between microscopic examination and PCR. We concluded that Chlamydia could be diagnosed using a microscopic field examination when a quick diagnosis is needed. While in Molecular Diagnostics, the 16S rRNA gene was the best for confirming the detection of Chlamydia.

Introduction

Chlamydial abortion results in significant reproductive disorders in numerous sheep-farming regions worldwide, with New Zealand and Australia as notable exceptions (1). The disease is particularly prevalent in flocks that are intensively maintained throughout the lambing season, and it remains the most frequent reason for abortion in many countries worldwide. The primary host of the disease is sheep; goats are also impacted, as are cattle and deer (2). Greig first identified chlamydia abortion in 1936 under the name enzootic abortion of ewes (EAE) (3), and the disease was for the first time detected in Iraq (4). Chlamydia is an obligate intracellular, Gram-negative organism that has a unique biphasic life cycle; they have two morphological structures called the Elementary Body (EB), which is the infective form, and the Reticulate Body (RB), a form of the metabolically active bacterium (5). In scientific reports, more than 60 Chlamydial diseases are known in mammals, in addition to infecting 465 species of domestic and wild birds, as well as causing 30 diseases in humans; this is because they closely resemble in their biological characteristics that they are only represented by a single genus Chlamydia, which includes all currently described species (6). Chlamydia is classified as a biological paradox. One paradoxical trait is the group's unified antigenic

structure; this antigenic unit includes a wide range of pathogenicity, which is why the importance of these organisms should not be overlooked. Chlamydia has been listed by the OIE as a class B notifiable disease. Among the most important of these types is *Chlamydia abortus*, which causes Chlamydial abortion, a disease of high importance that is also referred to as Ovine enzootic abortion (OEA) and Enzootic abortion of ewes (EAE), which is endemic in sheep. *Chlamydia abortus* can be detected by microscopic examination by using special stains like modified Ziehl-Neelsen MZN, which will show EB of *Chlamydia abortus* in a large number of red dot-shaped bodies on a blue background that colors the epithelial cells and the rest of the bacteria (7). Clinically, Abortions in late pregnancy typically do not have any warning signs before they happen. Ewes do not appear to experience systemic effects, but goats can develop metritis and retain the placenta. Following an abortion, vaginal discharge that lasts up to 3 weeks is standard. Stillbirths, weak-born lambs, and kids that die soon after birth cause additional losses (5), as necropsy findings Aborted fetuses do not have any apparent abnormalities. The inter-cotyledonary areas are thickened, edematous, and leathery, and the placental cotyledons are necrotic and hemorrhagic. Chlamydial organisms can be observed in the cytoplasm of swollen trophoblasts in direct placental smears using modified Ziehl-Neelsen MZN or other suitable stains (5); aborted fetuses do not have any apparent abnormalities. The inter-cotyledonary areas are thickened, edematous, and leathery, and the placental cotyledons are necrotic and hemorrhagic. Chlamydial organisms can be observed in the cytoplasm of swollen trophoblasts in direct placental smears using modified Ziehl-Neelsen MZN or other suitable stains (5). Chlamydia can be diagnosed by many techniques and confirmed by a Polymerase chain reaction that allows direct identification of the causal agent and species differentiation based on DNA from clinical samples. Many different PCR protocols have been used and adopted by various researchers. It has been established that PCR is highly durable for regular use and for further confirmation assays and has proven to be the most sensitive among several protocols, but the fact is that primers have been created. Based on the traditional classification of species according to the latter classification, some tests that could be pertinent to veterinary diagnostic laboratories often rely on published conventional PCR techniques on specific genetic targets such as the S16rRNA gene, OMP2, and the MOMP gene, each specific to an antigen region, between a specific genus and species, ideal targets for PCR diagnostics, additionally for assays of intra-species differentiation and need to use more than one gene to confirm infection (8). Middle Eastern countries have reported Chlamydia. Therefore, numerous studies were accomplished in Turkey; Chlamydia was identified by PCR technique in 6% of the vaginal swab samples of ewes and goats (9), and in Iran (10) found that 24.1% of sheep have *Chlamydia abortus* by real-time PCR technique, furthermore, in Saudi Arabia, Chlamydia was isolated from tissue samples taken from goat and sheep, like aborted fetuses and vaginal swabs (11). In Jordan, 21.8% of sheep and 11.4% of a goat was positive for Chlamydia by complement fixation test (CFT) (12), and in Syria, a positive titer for Chlamydia in sheep and goats was 11.7%, and 10.8%, respectively were detected by ELISA (13). Abortion is caused by many factors such as toxoplasmosis (14), although there are many studies on the detection of abortion caused by Chlamydia in sheep in Iraq (15-21) and in Nineveh (22,23), also Alameen and Dahl (24) mentioned that *Chlamydia abortus* is one of the primary causes of abortion in Nineveh.

This study aimed to detect *Chlamydia abortus* using microscopic examination by Modified Ziehl-Neelsen stain (MZN) and conventional polymerase chain reaction (c-PCR) and to compare them and to find the appropriate test, furthermore to explore the definitive sample for diagnosis.

Materials and methods

Ethical approval

This study was ethically permitted by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Mosul (UM.VET.2022.069) on 15 Sep 2022.

Animals of study

Our study was conducted on 157 samples from 75 ewes representing 60 flocks (10903 ewes). We took all three types of samples from the same ewe as possible. Samples were attended from ewes who suffered from abortion 1-3 years of age of local breed (Awassi, Hamdani, local crossbreed) from various areas of Mosul, Iraq; the study was conducted from August 2022 to January 2023.

Sample collection

Samples included taking swabs from the vagina of aborted ewes, the placenta, and the stomach contents of aborted fetuses. Cotton swabs were used to smear the glass slides for the Microscopic Examination. Biopsies were taken from the placenta and from the organs of aborted fetuses (liver, abomasum), which were transported in special containers and under refrigerated conditions 4-8 °C and a buffer solution (Sucrose-Phosphate-Glutamate Buffer, SPG BUffer) was added to them (25), It was kept at a temperature of -20°C until PCR made the diagnosis, samples included 49 aborted fetuses, 52 placentas, and 56 vaginal swabs.

Microscopic examination

Swabs were spread on glass slides to make smears, left to completely dry, and then fixed with absolute methanol, and a Ziehl-Neelsen stain prepared by the SYRBIO (Syria) was used. A carbol fuchsin stain was used for 15-20 minutes. The slide was washed with water, and diluted acid alcohol was

used for color reduction for 15-20 seconds. Then, the slide was washed with water, and the contrast methylene blue stain was used for 1-2 minutes. The slide was left to dry and then examined under a light microscope using a 40x magnification lens first and then a 100x magnification lens to detect the Elementary Body (EB), which appeared in red on a blue background that colors the epithelial cells and the rest of the bacteria. The acid alcohol was diluted by 1 ml of concentrated acid alcohol to 200 ml of distilled water (26).

Extraction of DNA

A DNA extraction kit (ADDBIO, South Korea) was used to extract the DNA from the samples. The procedure was carried out by the manufacturer's instructions.

Amplification of DNA

Primers prepared from (Macrogen, South Korea) were used to amplify specific parts of DNA during the polymerase chain reaction, and their sequences and sizes are shown in table 1.

Table 1: Primer sequence of 16-S rRNA gene and OMP2 to confirm *Chlamydia abortus* in infected sheep

Gene	Primers	A sequence of the primers (5- to 3-)	Size (pb)	Reference
16S rRNA	16SIGF	GATGAGGCATGCAAGTCGAACG	278	(27)
	16SIGR	CCAGTGTTGGCGGTCAATCTCTC		
OMP2	F	ATG TCC AAA CTC ATC AGA GGA G	587	(28)
	R	CCT TCT TTA AGA GGT TTTACC CA		

Master mix preparation

The master mix for all polymerase chain reactions was prepared using the master mix kit (GeNetBio, South Korea), and for each reaction, a pair of specialized primers representing the gene to be detected was used. After the additives had been properly mixed, they were distributed in a volume of 15 microliters on Eppendorf tubes with a capacity of 200 microliters. After that, the DNA was added in a volume of 3 microliters in the particular tube for each sample so that each tube's overall volume was 20 microliters.

Programming the thermocycler

After preparing the tubes of the PCR mixture, they were placed in a thermocycler to amplify the gene to be detected. Denaturation at 94°C for 10 minutes for one cycle was the first step in the thermocycler program. Then, 40 cycles of denaturation were performed. (94°C for 45 seconds), Forty cycles of annealing for 45 seconds at (60°C for 16 S IGF, 16 S IGR, and 55°C for OMP-F, OMP-R), and 40 cycles of extension (72°C for 1 minute) a final extension were run for seven minutes at 72 °C, according to the manufacturer's instructions (Macrogen, South Korea).

Electrophoresis in an agarose gel:

A 1.5% agarose gel concentration was prepared by dissolving 1.5% agarose in 100 ml of 1Tris-borate-EDTA X buffer, adding three microliters of (Gel red safe) dye. After that, seven microliters of the amplification products resulting from the polymerase chain reaction were placed in the specified pits (DNA Invitrogen, USA), ladder 100bp, and four microliters of it were placed in the first hole in the agarose gel. The power supply was connected to 80 volts and (300) milliamps for 60 minutes. The gel was placed in a particular imaging device (Gel Documentation System BioRad, USA) that sheds ultraviolet light to detect amplification products, and then the images of the results were compared with a 100 bp DNA ladder.

Statistical analysis

Data from our study were analyzed by IBM-SPSS (IBM Corp., Armonk, NY, USA) v21. Using the Chi-square test to test the association between the two tests (29). Cramer's Value was used to determine the strength of the association between the two tests. Cramer's Value is a number between zero and 1, indicating the strength of association between the two tests (30,31). Additionally, the microscopic examination's sensitivity, specificity, and accuracy were assessed compared to the PCR as a gold standard (32). Accuracy, Sensitivity, and Specificity were calculated according to the following Mathematical formulas: Sensitivity= TP/ TP+FN, Specificity=TN/TN+FP, Accuracy=TP+TN/TP+TN+FP+FN. Using Spearman's rank coefficient of correlation to measure the correlation between test results according to the type of sample (5), where the data of the animals from which the samples were collected were identified in their three types (placenta, fetus, vaginal smear), which there were 26 ewes to determine the association between the types of samples (29).

Results

The results showed that out of total 157 samples comprise of (52 placenta, 49 fetuses, and 56 vaginal swabs), 18.47% were identified positive using microscopic examination stained with Modified Ziehl-Neelsen/MZN and 11.46% were confirmed for Chlamydia abortus by Conventional Polymerase chain reaction (c-PCR) for (16S rRNA) (Table 2).

Table 2: The number and percentage of samples with *Chlamydia abortus* using microscopic examination (MZN) and PCR

Tests	N. of samples	N. of positive samples	Percentage (%)
MZN	157	29	18.47%
PCR		18	11.46%

Laboratory diagnosis of *Chlamydia abortus*

Chlamydia was detected, according to the results in the samples examined under Microscopic examination using the Modified Ziehl-Neelsen stain in different proportions, as they were 16.3, 26.9, and 12.5% for each of the samples of the placenta, aborted fetus, and vaginal swab, respectively, smears stained with Modified Ziehl- Neelsen -MZN show Elementary Body (EB) of *Chlamydia abortus* in a large number of red dot-shaped bodies in the intercellular space and also in the cytoplasm of the trophoblasts of the placenta (Figures 1 and 2).
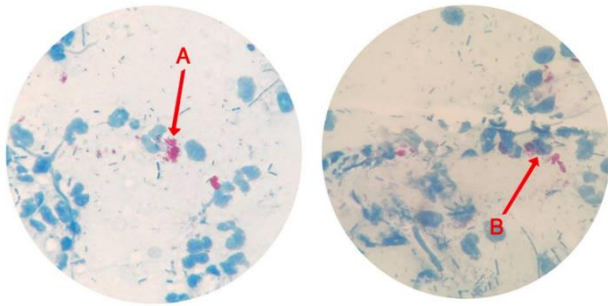

Figure 1: Vaginal smear stained with Modified Ziehl-Neelsen- MZN stain, A-Extracellular EB, B-Intracellular EB (X1000).
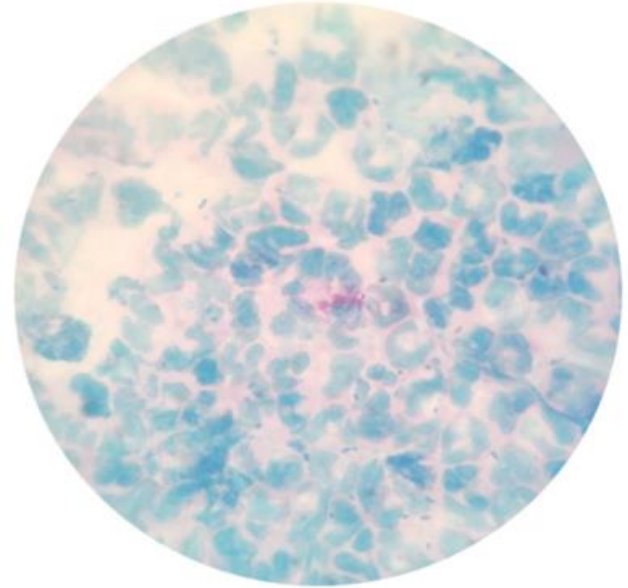

Figure 2: Placental smear showed EB of *Chlamydia abortus* stained with Modified Ziehl-Nielsen (MZN) stain (X1000 Light Microscope).

Molecular detection of *Chlamydia abortus* bacteria

The results showed that the molecular detection using (c-PCR) 18 samples 11.46% were confirmed by the 16S rRNA gene with an amplicon size of 278pb, while seven samples 4.45% for the OMP2 gene. These results are presented in (Table 3). The amplification products of the gene S16rRNA of the positive samples showed a size of 278 pb and 578 pb for OMP2 compared to the negative control after electrophoresing in a 1.5% agarose gel (Figure 3 and 4).

Table 3: Percentages of primers for the two genes (S16rRNA, OMP2) using conventional PCR

Gene name	Primers	Sequences 5'-3'	Size (bp)	Positive samples	Percentage (%)
S16rRNA	16SIGF 16SIGR	GATGAGGCATGCAAGTCGAACG CCAGTGTTGGCGGTCAATCTCTC	278	18	11.46%
OMP2	F R	ATG TCC AAA CTC ATC AGA GGA G CCT TCT TTA AGA GGT TTTACC CA	587	7	4.45%

In the comparison of the two methods' results, the microscopic examination (MZN) with the polymerase chain reaction (PCR) as a gold standard, it was found that there were 14 valid positive samples for both tests, 124 valid negative samples for both tests, 15 false positives, and four false negative samples and sensitivity was 78%, specificity was 89%, accuracy was 89%. Positive predictive Value (PPV) was 48%, and Negative predictive value (NPV) was 97%. According to the result of statistics, there is a moderate strength association between microscopic examination (MZN) and the PCR, as the chi value was 47.48 and the

Cramar Value 0.55 at a significant level of P<0.01 in diagnosing *Chlamydia abortus* in sheep herds in Mosul (Table 4).

From the results of different samples (placenta, fetus, vaginal swab) examined with a microscopic examination by Modified Ziehl-Neelsen stain and PCR, they were positive placenta 14.2%, aborted fetus 15.3%, and vaginal swab 5.3% samples, furthermore, by Spearman's rank coefficient there is a positive correlation between samples of fetuses and vaginal swabs with samples of the placenta, as the correlation value was 0.7654 and 0.7706 for samples of

fetuses and vaginal smears, respectively, at a significant level of P<0.01 (Table 5).
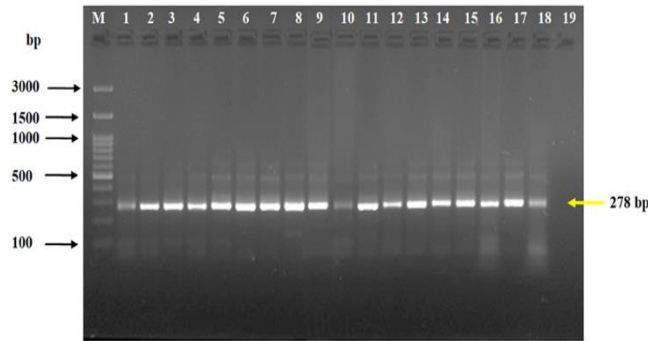

Figure 3: Agarose gel electrophoresis of PCR products for detecting *Chlamydia abortus* using primer 16SIGF-F and 16SIG-R of the gene (16S rRNA). Path M: Marker is 100 bp. Lane 1 - 18 represents positive samples with an amplicon size of 278 bp; lane 19 represents the negative control.
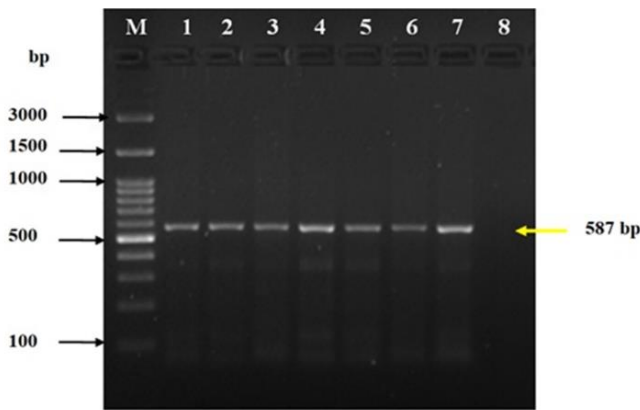

Figure 4: Agarose gel electrophoresis of PCR products for detecting *Chlamydia abortus* using primer OMP-F and OMP-R of the (OMP2) gene. Lane M: Marker represents 100 bp size. Lane 1 - 7 represents positive samples with amplicon size 587 bp; Lane 8 represents negative control.

Table 4: Comparison of microscopic examination (ME) and conventional PCR technique (c-PCR)

	Conventional PCR technique		
	Positive	Negative	Total
Positive	14*	15	29
Negative	4	124**	128
Total	18	139	157

* = true positive, **= true negative.

Table 5: Infection percentages of *Chlamydia abortus* according to the type of sample in ewes using MZN and PCR

Sample type	Tested samples	Test type	Positive samples	Percentage (%)
Placenta	52	MZN	14	26.9%
		PCR	8	15.3%
Aborted fetus	49	MZN	8	16.3%
		PCR	7	14.2%
Vaginal swab	56	MZN	7	12.5%
		PCR	3	5.3%

Discussion

Our study aimed to detect Chlamydia in ewes' flocks in Mosul by two laboratory tests to compare between microscopic examination using the Modified Ziehl-Neelsen stain and the c-PCR. This study was also focused on finding the most desirable and representative sample for early diagnosis of diagnosis. The current findings revealed that out of 157 samples collected from different tissues, 29/18.47% were using microscopic examination with the MZN, which is considered a high number of favorable results compared with other researchers (33,34). Chlamydial abortion is an endemic disease in Iraq due to the close movement of sheep between the Iraqi governorates, while our result disagrees with Cati *et al*. (19), Majed *et al*. (23) and Al-Magsoosi *et al*. (35) because positive samples increase if the disease occurs for the first time in the region. It may also be due to several factors, including the number of animals, the type and size of the sample examined within the herd, husbandry practices, the breeding procedures, the health status of the animals, improper breeding, and failure to dispose of aborted fetuses and infected discharge properly. Some other associated factors comprising virulency of the pathogen, susceptibility to infection, and the advanced stage of pregnancy can also play vital roles in the propagation and transmission of this lethal disease (5).

The result of microscopic examination using MZN, shows EB of *Chlamydia abortus*. These results agree with Paolo *et al*. (36) Tsakos (37) and Borel *et al*. (27), as microscopic examination represents an easy and inexpensive method. The preference for this method increases with the availability of a representative sample, such as the placenta taken close to the time of the abortion to ensure the presence of EB and considered a rapid initial test for the early detection of Chlamydia (2), our results disagree with Sargison and Truyers (38) may be due to the absence of EB in the examined microscopic smear or the presence of a small number of EB, which increases the difficulty of diagnosis, in addition to the difference in the eye of the examiner (39). The reason may be that the contrast stain (carbol fuchsin) needs careful discolorations using dilute acetic acid. Excessive decolorization may lead to difficulty detecting EB (40). In molecular detection using a c-PCR assay, *Chlamydia*

abortus was confirmed in 18/11 (46%) and 7/4 (45%) using the 16S rRNA gene and OMP2 gene, respectively. Our results are reflected in similar findings with other researchers in Turkey and Iran (9,25).

The results disagreed with Esmaeili *et al.* (10) because of geographical location, animal breed, grazing and management techniques, inadequate dietary intake, and uncontrolled restrictions on the movement of animals from infected areas. Are factors that may affect the rate of *Chlamydia abortus* infection, geographical areas, environmental elements, soil types (arid, semi-arid), climate, temperature, sunlight, pasture cultivation levels, feeding types, and irrigation sources all play a role in the variation in disease incidence in other countries as well Gillespie and Timoney (41).

Our results disagree with Arif *et al.* (21), which used only one gene ompA. The reason for preferring the 16S rRNA gene in diagnosis by polymerase chain reaction is that the gene is characterized by the fact that it maintains its sequences for one species and changes slowly over millions of years, and its size is appropriate when conducting the process of determining genetic species and the phylogenetic tree (sequencing), These reasons give strength for the compatibility of the 16S rRNA gene with this study and other studies. It was based on this gene in 1999 and proposed in the reclassification of the Chlamydiales family, which expresses 90% of its identity and distinguishes it as a bacterium from other chlamydia-like organisms (42). In addition, *Chlamydia abortus* has only one copy of the 16S rRNA gene, unlike chlamydia species that have two copies, and this makes this gene an advantage in recognizing Chlamydia over other genes used in diagnosis (43). From the statistical results, it is reflected that there was a moderate association strength between microscopic examination and PCR, which agrees with Hanger *et al.* (44) and Madkhali *et al.* (45) that both techniques depend on the presence of bacteria, and the microscopic examination was sensitive enough to be used in the rapid diagnosis of chlamydia infection.

Our results indicate that 26.9% of pathogens were confirmed from the placental, making it essential for diagnosing EAE. These results agreed with Moore *et al.* (29), Paolo *et al.* (36), Tsakos (37), and Creelan *et al.* (46). The placenta is the target tissue for *Chlamydia* that produces lesions. The pathogenesis and primary abnormality occurred due to the ability of *Chlamydia abortus* to colonize in the trophoblasts of the placenta, and the placenta was the most representative for detecting Chlamydia. It also reported that *Chlamydia abortus* can be confirmed in the placenta because shedding occurs on the 85th day of pregnancy and then gradually increases (47).

Results indicate a correlation between all types of samples (placenta, aborted fetus, vaginal swab) because all these samples have the origin of the urogenital tract. These results agree with Livingstone *et al.* (48) that diagnosis should include more than one type of sample and the necessity of collecting it immediately or soon after the abortion (36). Vaginal swabs are less representative than other swabs, which may be due to the continuous vaginal secretions, which dilute the causative agent.

Conclusion

Chlamydia abortus can be diagnosed by MZN as an initial screening test, and further conformation should be done by using the PCR assay for the 16S rRNA gene.

Acknowledgment

Conflict of interest

There is no conflict of interest.

References

1. Rodolakis A, Laroucau K. Chlamydiaceae and chlamydial infections in sheep or goats. Vet Microbiol. 2015;181:107-118. DOI: 10.1016/j.vetmic.2015.07.010
2. Aitken ID, Longbottom D. Chlamydial abortion. In: Aitken ID, editor. Diseases of sheep. UK: Blackwell Publishing; 2007. 105-111 p.
3. Greig JR. Enzootic abortion in ewes. Vet Rec. 1936;48:1226-1227. [available at]
4. Dhahir SH. Bacteriological and serological study on some infectious causes of ovine and caprine abortion [Ph.D. dissertation]. Baghdad: College of Veterinary Medicine, University of Baghdad; 1990. 23 p.
5. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 11th ed. USA: Elsevier Health Sciences; 2016. 1786-1790 p.
6. Borel N, Polkinghorne A, and Pospischil1A. Review on chlamydial diseases in animals: Still a challenge for pathologists. Vet Pathol. 2018;55(3):374-390. DOI: 10.1177/0300985817751218
7. Svetoslav PM. Chlamydiae and chlamydial infections. 1st ed. Bulgaria: River Publishers; 2018. 191-193 p.
8. Sachse K, Vretou E, Livingstone M. Recent developments in the laboratory diagnosis of chlamydial infections. Vet Microbiol. 2008;135(1-2):2-21. DOI: 10.1016/j.vetmic.2008.09.040
9. Kalender H, Kiliç AH, Eröksüz A, Muz U, Kılınç B. Identification of *Chlamydophila abortus* infection in aborting ewes and goats in eastern Turkey. Rev Med Vet. 2013;164(6):295-301. [available at]
10. 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. DOI: 10.1016/j.smallrumres.2021.106339
11. Fayez M, Elmoslemany A, Alorabi M, Alkafafy M, Qasim I, Al-Marri T, Elsohaby I. Seroprevalence and risk factors associated with *Chlamydia abortus* infection in sheep and goats in eastern Saudi Arabia. Pathogens. 2021;10:489. DOI: 10.3390/pathogens10040489
12. Alqudah KA, Sharif LA, Rauf RY, Hailat NQ, Aldomy FM. Seroprevalence of antibodies to *Chlamydophila abortus* shown in Awassi sheep and local goats in Jordan. Vet Med. 2004;49(12):460-466. DOI: 10.17221/5740-vetmed
13. Roukbi M, Al-Omar AN, Al-Najjar K, Salam Z, Al-Suleiman H, Mourii M and Jourié S. Seroprevalence of antibodies to *Chlamydophila*

abortus in small ruminants in some provinces in Syria. Net J Agric Sci. 2016;4(2):29-34. [available at]

14. Elaadli H, Abo El-Makarem H, Abd Elrahma A H, Shaapan R, Bessat, M. Prevalence and associated risk factors of *Toxoplasma gondii* infection in sheep and aborted women in Egypt. Iraqi J Vet Sci. 2023;37(2):437-445. DOI: 10.33899/ijvs.2022.135777.2518

15. Taha FY, Alhankawe OK. Molecular evidence of schmallenberg virus associated by ovine abortion with fetal anomalies in Nineveh province, Iraq. Iraqi J Vet Sci. 2023;37(1):115-120. DOI: 10.33899/ijvs.2022.133665.2276

16. Naoman UT, Aliraqi OM, Hayawy EL, Albaqqal SM. Clinical and ultrasonographic findings in rams with scrotal enlargement. Iraqi J Vet Sci. 2022;36(I):47-53. DOI: 10.33899/ijvs.2022.135079.2443

17. Noomi BS, Ahmed SS, Khalaf HY, Jafar NA. Immune response strategies of *Brucella melitensis* and their antigens in rats. Iraqi J Vet Sci. 2022;36:27-30. DOI: 10.33899/ijvs.2022.134990.2431

18. Nori W, Ali AI. Toxoplasmosis, a zoonotic infection; A critical and updated analysis: A review article. Iraqi J Vet Sci. 2021;35:95–99. DOI: 10.33899/ijvs.2021.131607.1980

19. Cati JA, Hasso SA, Abbod BC. Detection of (*Chlamydophila abortus*) antibodies in cattle and sheep in the south of Iraq by using iELISA and passive haemagglutination tests. AL-Qadisiya J Vet Med Sci. 2008;7(2):66-72. [available at]

20. Farhad BM, Zanan MAT, Lokman TO. Seroprevalence of *Chlamydophila abortus* in goat farms in Duhok province-Iraq. AL-Qadisiya J Vet Med Sci. 2016;15(1). [available at]

21. Arif ED, Saeed NM, Rachid Sk. Isolation and identification of *Chlamydia abortus* from aborted ewes in Sulaimani province, northern Iraq. Polish J Micobiol. 2020;69(1):65–71. DOI: 10.33073/pjm-2020-009

22. Al-Dabagh E, Jasim BM, Jarjees MT. Seroprevalence of antibodies to toxoplasmosis, brucellosis, and chlamydiosis in abortive sheep in Nineveh governorate, Iraq. Iraqi J Vet Sci. 2014;28:21-25. DOI: 10.33899/ijvs.2014.89467

23. Majed R, Maab AF, Omer AH, Hussein AK. Preliminary study of seroprevalence of *Chlamydophila abortus* amongst cattle in Nineveh province. Adv Anim Vet Sci. 2018;6(3):135-8. DOI: 10.17582/journal.aavs/2018/6.3.135.138

24. Alameen EK, Dahl MO. Abortion in ewes in Nineveh governorate, Iraq: A systematic review and meta-analysis. Iraqi J Vet Sci. 2022;36:681–688. DOI: 10.33899/ijvs.2021.131343.1942

25. Barati S, Bakhtiari NM, Shokoohizadeh L. Genotyping of *Chlamydia abortus* using multiple loci variable number of tandem repeats analysis technique. BMC Vet Res. 2022;18:54. DOI: 10.1186/s12917-022-03142-6

26. Markey BK, Leonard FC, Cullinane A, Archambault M Maguire M. Clinical veterinary microbiology. 2ⁿᵈ ed. USA: Elsevier; 2013. 407-413 p.

27. Borel N, Thoma R, Spaeni P, Weilenmann R, Teankum K, Brugnera E, Zimmermann DR, Vaughan L, Pospischil A. Chlamydia-related abortions in cattle from Graubunden, Switzerland. Vet Pathol. 2006;43:702–708. 10.1354/vp.43-5-702

28. Ababneh HS, Ababneh MK, Hananeh WM, Alsheyab FM, Jawasreh KI, Al-Gharaibeh MA, Ababneh MM. Molecular identification of chlamydial cause of abortion in small ruminants in Jordan. Trop Anim Health Prod. 2014;46:1407–1412. DOI: 10.1007/s11250-014-0654-x

29. Moore DS, McCabe GP, Craig BA. Introduction to the practice of statistics. 6ᵗʰ edition. USA: WH Freeman; 2009. 190 p.

30. Jeffrey H. Contingency tables, chi-squared and Cramer's V towards Data, Science, 2021. https://towardsdatascience.com/contingency-tables-chi-squared-and-cramers-v-ada4f93ec3fd (accessed 6.13.23).

31. Franco F, Di Napoli A. Reliability assessment of a measure: The kappa statistic. Giornale di Tecniche Nefrologiche e Dialitiche. 2016; 28(4):289-292. DOI: 10.5301/GTND.2016.16402

32. Baratloo A, Hosseini M, Negida A, El Ashal G. Part 1: Simple definition and calculation of accuracy, sensitivity, and specificity. Spring. 2015;3(2):48-49. DOI: 10.22037/EMERGENCY.V3I2.8154

33. 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):2722-2727. [available at]

34. Ali HM, Al-Bayati HL. Serological and histopathological investigation of *Chlamydia abortus* in aborted ewes in Wasit, Iraq. Arch Razi Inst. 2022;77(3):1105-1111. DOI: 10.22092/ari.2022.357270.2009

35. Al-Magsoosi HE, Nasear HA, Majeed HI. Serological investigation of bovine *Chlamydia abortus* in Wasit province, Iraq. Arch Razi Inst. 2022;77(6):2371-2377. DOI: 10.22092/ari.2022.358620.2270

36. Paolo Di, Alvarado LA, Pinedo MF, Origlia J, Fernández G, Uzal FA, Travería, GE. First report of caprine abortions due to *Chlamydia abortus* in Argentina. Vet Med Sci. 2019;5:162–167. DOI: 10.1002/vms3.145

37. Tsakos P. Experimental infection of pregnant ewes with enteric and abortion-source *Chlamydophila abortus*. Vet Micobiol. 2001;82:285-291. DOI: 10.1016/S0378-1135(01)00389-3

38. Sargison ND, Truyers IGR, Howie FE, Thomson JR, Cox AL, Livingstone M, Longbottom D. Identification of the 1B vaccine strain of *Chlamydia abortus* in aborted placentas during the investigation of toxaemic and systemic disease in sheep. N Z Vet J. 2015;63:284-287. DOI: 10.1080/00480169.2015.1018365

39. Häcker G. Biology of chlamydia, current topics in microbiology and immunology. USA: Springer International Publishing; 2018. 134-138 p. DOI: 10.1007/978-3-319-71232-1

40. Dagnall GR, Wilsmore AJ. A simple staining method for identifying chlamydial elementary bodies in the fetal membranes of sheep affected by ovine enzootic abortion. Vet Microbiol. 1990;21:233-239. DOI: 10.1016/0378-1135(90)90034-S

41. Gillespie JH, Timoney JF. Hagan and Bruner's infectious diseases of domestic animals.7ᵗʰ ed. USA: Cornell University Press; 1981. 127-150 p.

42. Everett KD, Andersen AA. Identification of nine species of the Chlamydiaceae using PCR-RFLP. Int J Syst Bacteriol. 1999;49(2):803-813. DOI: 10.1099/00207713-49-2-803

43. Thomson NR, Yeats C, Bell K, Holden MG, Bentley SD, Livingstone M, Cerdeño-Tárraga, AM, Harris B, Doggett J, Ormond D, Mungall K, Clarke K, Feltwell T, Hance Z, Sanders M, Quail MA, Price C, Barrell BG, Parkhill J, Longbottom D. The *Chlamydophila abortus* genome sequence reveals an array of variable proteins that contribute to interspecies variation. Genome Res. 2005;15:629-640. DOI: 10.1101/gr.3684805

44. Hanger J, Loader J, Wan C, Beagley KW, Timms P, Polkinghorne A. Comparison of antigen detection and quantitative PCR in the detection of chlamydial infection in koalas (*Phascolarctos cinereus*). Vet J. 2013;195:391-393. DOI: 10.1016/j.tvjl.2012.07.024

45. Madkhali AM, Ghzwani AH, Al-Mekhlafi HM. Comparison of rapid diagnostic test, microscopy, and polymerase chain reaction for the detection of *Plasmodium falciparum* Malaria in a low-transmission area, Jazan region, southwestern Saudi Arabia. Diagn Basel Switz. 2022;12:1485. DOI: 10.3390/diagnostics12061485

46. Creelan JL, McCullough SJ. Evaluation of strain-specific primer sequences from an abortifacient strain of ovine *Chlamydophila abortus* (*Chlamydia psittaci*) for the detection of EAE by PCR. FEMS Microbiol Lett. 2000;190:103-108. DOI: 10.1111/j.1574-6968.2000.tb09270.x

47. Maley SW, Livingstone M, Rodger SM, Longbottom D, Buxton D. Identification of *Chlamydophila abortus* and the development of lesions in placental tissues of experimentally infected sheep. Vet Microbiol. 2009;135:122-127. DOI: 10.1016/j.vetmic.2008.09.031

48. Livingstone M, Wheelhouse N, Stephen W, Maley L, Longbottom D. Molecular detection of *Chlamydophila abortus* in post-abortion sheep at oestrus and subsequent lambing. Vet Micobiol. 2009;135:134-141. DOI: 10.1016/j.vetmic.2008.09.033

التقرير الأول عن الكشف الجزيئي لعدوى الكلاميديا المجهضة في قطعان الأغنام في الموصل، العراق

يعرب عبد الخالق رحاوي[1] و أسامة موفق العراقي[2]

[1]قسم الثروة الحيوانية، مديرية زراعة نينوى، [2]فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تعد الكلاميديا المجهضة أحد الأسباب الرئيسية للإجهاض في الأغنام في جميع أنحاء العالم وتؤدي إلى خسائر اقتصادية كبيرة. هدفت الدراسة الحالية إلى الكشف عن عدوى الكلاميديا المجهضة بالفحص المجهري باستعمال صبغة زيل نيلسن المعدلة وتفاعل البلمرة المتسلسل التقليدي في قطعان الأغنام في الموصل، العراق. تم جمع ما مجموعه ١٥٧ عينة مختلفة شملت ٤٩ جنينًا مجهضًا و ٥٢ مشيمة و ٥٦ مسحة مهبلية من ٦٠ قطيعًا في الفترة من اب ٢٠٢٢ إلى كانون الثاني ٢٠٢٣ في مناطق مختلفة من الموصل. تم عمل مسحات للفحص المجهري وأخذ خزعات من المشيمة وأعضاء الأجنة المجهضة للتشخيص بتفاعل البلمرة المتسلسل PCR باستعمال بادئات الجين 16S rRNA والجين OMP2. وتشير النتائج إلى أن نسبة العينات الموجبة في الفحص المجهري بلغت ١٨،٤٧٪ والتي أظهرت في عدد كبير من الأجسام الأولية للكلاميديا والتي كانت على شكل نقطة حمراء في الحيز بين الخلايا وأيضًا في سيتوبلازم الأرومة الغــــاذية، في حين بلغت نسبة العينات الموجبة ١١،٤٦٪ للجين S16rRNA، و٤،٤٥٪ للجين OMP2 في التشخيص الجزيئي. وكانت الحساسية والدقة والنوعية ٧٨٪، ٨٩٪، و٨٩٪ على التوالي للفحص المجهري بالمقارنة مع تفاعل البلمرة المتسلسل كمعيار ذهبي، وقد وجد أن هناك قوة علاقة معتدلة بين الفحص المجهري وتفاعل البلمرة المتسلسل. استنتجت هذه الدراسة إلى أنه يمكن تشخيص الكلاميديا باستخدام الفحص المجهري عندما تكون هناك حاجة إلى تشخيص سريع. بينما في التشخيص الجزيئي، كان جين 16S rRNA هو الأفضل لتأكيد تشخيص الكلاميديا.