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PREVALENCE OF CHLAMYDOPHILA ABORTUS IN ABORTED SMALL RUMINANTS IN SLEMANI AND DUHOK GOVERNORATES

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ABSTRACT

This study was conducted to estimate the prevalence of chlamydiosis (*Chlamydophila abortus*) in aborted ewes and does from 66 flocks in 8 different districts of two provinces, Slemani and Duhok in Kurdistan Region-Iraq from October 2014 to the end of June 2015. Indirect enzyme-linked immunosorbent assay (i-ELISA) was used to analyze the serum samples that were collected from 271 sheep and 97 goats. Giemsa and modified Ziehl-Neelsen (MZN) stains were used for direct staining examination of the liver of aborted fetuses, vaginal swabs, and placentae of aborted sheep and goats. A total of 368 aborted sheep and goats serum samples were screened for the presence of *Cp. abortus* antibodies. The results show 53 (14.40 %) serum samples were positive for *Cp. abortus* antibodies using i-ELISA; including 36 (13.04 %) in Slemani and 17 (18.47 %) in Duhok province. The overall prevalence rate of chlamydiosis was 15.50 % in ewes and 11.34 % in goats. The infection rate of *Cp. abortus* 18.24 % found in ewes, aged ≤two years was higher than in the ewes aged >two years. Conversely, does aged >two years showed a higher infection rate than does aged ≤two years. However, the difference in the rate of infection between the two age groups wasn't statistically significant (P>0.05). The abortion rate was higher in the late stage of pregnancy, compared to the early and mid stages of pregnancy in both animal species. However, the difference was statistically significant (P< 0.05) in ewes only. In all animals, *Cp. abortus* infection occurred only once and caused abortion in 9.13 % and 6.97 % of sheep and goats, respectively. The intracytoplasmic inclusion bodies of Chlamydia in the placentae of sheep appeared as small, purple-colored bodies when stained by Giemsa stain and as red bodies using MZN staining inside the cytoplasm of the cells.

KEYWORDS: Aborted Sheep and goats , *Chlamydophila Abortus*, i-ELISA, Slemani And Duhok Governorates.

INTRODUCTION

Ovine chlamydiosis, enzootic abortion of ewes (EAE), or ovine enzootic abortion (OEA), all refer to the same disease, which is caused by a gram-negative intracellular bacterium named *Chlamydophila abortus* (*Cp. abortus*) (OIE, 2023). This disease causes various reproductive failures in sheep and goats, such as abortion mainly late in the final 2-3 weeks of pregnancy, premature labor, stillbirths, the delivery of weak lambs with low birth weight that generally fail to survive beyond 2 days and grossly inflamed placentas (OIE, 2023). Chlamydiae are obligate intracellular organisms and unlike other bacteria, the chlamydial development life cycle is characterized by two distinct developmental forms elementary bodies (EBs) and reticulate bodies (RBs) (Witkin et al., 2017)(Jury et al., 2023). The EBs are infectious, with rigid cell wall, which contains the major outer membrane protein (MOMP) and other proteins. The RBs are non-infectious, metabolically active forms of Chlamydiae capable of synthesizing RNA, DNA and proteins (Abad et al., 2011). *Cp. abortus* is also a zoonotic pathogen that is transmitted from animals to humans (Burgener et al., 2022) via the oral route from contaminated hands as well as clothes or food after direct handling of infected sheep or goats (Radostits et al., 2006; Vlahović et al., 2006; Selim et al., 2021; Burgener et al., 2022). Although *Cp. abortus* is not very contagious in people, the consequences of the disease for pregnant women in close contact with infected sheep and goats are

disastrous (Rodolakis and Mohamad, 2010). Worldwide, the disease caused by this organism is responsible for large economic losses through the wastage and loss of kids and lambs and, also through lost milk production (Longbottom et al., 2002; Alderton, 2013; Al-Tabbaa et al., 2020). There is a clear need for improved control strategies for OEA that include more effective diagnosis, prophylaxis and farm management systems (Rocchi et al., 2009). The disease spreads easily, particularly at lambing time when large numbers of bacteria are excreted with placentas and vaginal discharges (Aitken, 2000). Direct microscopic examination, pathogen isolation, serological tests (complement fixation test (CFT), Enzyme-linked immunosorbent assay (ELISA) and immunofluorescence), immunohistochemistry and DNA-based methods (polymerase chain reaction and DNA microarray) are used for diagnosis (Anonymous, 2004). Serological examination is more frequently used in routine diagnosis because organism isolation is difficult and time-consuming (Anonymous, 2004; Sachse *et al*., 2009). This study was conducted to achieve the following aims: To determine the prevalence of ovine enzootic abortion (OEA), in flocks of aborted sheep and goats. To determine some risk factors (age, gestation period, and frequency of abortion) which are associated with *Cp. abortus* infection in the animals.

MATERIALS AND METHODS

Sampling

This present study was carried out at the College of Veterinary Medicine/ Duhok University, College of Veterinary Medicine/ Sulaimani University and Kalar Veterinary Directorate, Kurdistan region-Iraq. The samples were collected from different flocks (66 flocks) in 8 districts of Sulaymaniyah and Duhok provinces during the kidding and lambing seasons from October 2014 to the end of June 2015 (Table, 2). A total of 368 serum samples and 24 aborted fetuses were collected, in addition to 33 vaginal swabs and 10 placenta specimens for the diagnosis of *Cp.abortus* infection in aborted does and ewes using direct staining method and serological tests (Table, 1).

Table (1): Details of specimens collected from aborted sheep and goats according to location

Location	Sheep specimens				Goats specimens			
	Serum	Placenta	Vaginal Swabs	Liver	Serum	Placenta	Vaginal swabs	Liver
Kalar	127	5	12	11	32	0	6	2
Kifri	52	1	1	1	13	1	2	1
Saydsadq	14	1	2	3	1	0	1	1
Chamchamal	36	1	2	2	1	0	1	0
Sumel	27	1	3	3	20	0	1	0
Akrei	4	0	0	-	18	0	0	0
Zakho	5	0	0	-	8	0	0	0
Ameidi	6	0	1	-	4	0	1	0
Total	271	9	21	20	97	1	12	4

Blood

Ten ml of blood was drawn aseptically by vacutainer tubes without anticoagulant from the jugular vein of aborted dams, those who have history of abortion since 1.5- 2 months after abortion. The blood was submitted to a laboratory and then the serum was separated by centrifugation of blood at 3000 RPM for 5 minutes, then serum was put in a clean plastic screw cap vial, and kept in a deep freeze at - 20 ^{o}C until performed the tests. The collected serum was used for detection of antibodies against *Cp. abortus* using ELISA.

Aborted fetuses

Twenty-four aborted fetuses were collected and submitted to the laboratory under a sterile condition for direct microscopic examination. After the aborted fetuses were transferred to the laboratory for anatomical process, they were cleaned and disinfected with 70 % alcohol, then the carcasses were opened under sterile conditions. The direct impression smear was prepared from the liver by cutting the surface of hepatic tissue and then touching it with filter paper to remove excess blood. Later the liver piece was placed and pressed gently onto a microscopic slide to impression smear preparation and stained with both Giemsa and modified Ziehl-Neelsen'stains for direct examination. They were examined microscopically for demonstration of Chlamydophila organisms.

Vaginal swabs

A total of 33 sterile vaginal swabs were collected directly from the vagina of recently aborted dams within a maximum time after of 72 hours after cleaning the external vaginal orifice with 70% alcohol. They were examined microscopically for the demonstration of the causative agent using direct microscopic examination after being stained with both Giemsa and modified Ziehl-Neelsen'stains.

Placenta

The 10 placental samples were obtained from aborted dams which were submitted to a laboratory for examination by direct impression smears which were stained with both Giemsa and modified Ziehl-Neelsen'stains for detection of the causative agent.

Staining method (presumptive method)

All samples were collected from the liver of aborted fetuses, placenta and vaginal swabs were stained with both modified Ziehl–Neelsen and Giemsa stain (Timoney *et al.*, 1988a; (Markey, B.; Leonard, F.; Archambault, M.; Cullinane, A. and Maguire, 2013)

Serological tests

All sera obtained from aborted dams were tested using ELISA for detection of *Cp.abortus*, infection.

Statistical analysis

The data analysis was performed by chi-square test using the Statistical Analysis Software (SAS) program**.** the differences were considered to be statistically significant when the P value less than (0.05) while the P value more than (0.05) was regarded as non-significant (Bowers, 2008).

RESULTS

This study was carried out to determine the prevalence of Chlamydophilosis (*Cp. abortus*) using monoclonal antibody (IgG) type ELISA from the serum of aborted ewes and does, and direct microscopic examination of staining specimens (Giemsa and MZN stains) taken from the liver of aborted fetuses, vaginal swab and placenta of aborted dams. According to the results, the overall prevalence rate was 42/271 (15.50 %) in ewes and 11/97 (11.34 %) in does. The results also revealed a significant increase of infection in goats in Duhok province only when compared to Sulaymaniyah. The present study showed no significant difference in the infection between Duhok and Sulaymaniyah provinces (Table, 2).

Table (2): The prevalence of *Cp. abortus* in ewes and does by ELISA test in both Sulaymaniyah and Duhok provinces.

Province	Sheep			Goat		
	No. of examined animals	Positive No. (%)	Negative No. (%)	No. of examined animals	Positive No. (%)	Negative No. (%)
Sulaymaniyah	229	35 (15.28)	194 (84.72)	47	1 (2.13)A	46 (97.87)
Duhok	42	7 (16.67)	35 (83.33)	50	10(20.00)B	40 (80.00)
Total	271	42 (15.50)	229 (84.50)	97	11 (11.34)	86 (88.66)

- Different letters indicate a statistically significant difference (P<0.05).
- The rate of *Cp. abortus* among sheep in both provinces shows no significant difference (P>0.05).
- The rate of *Cp. abortus* among sheep and goats in both provinces shows no significant difference (P>0.05).

The different prevalence rates between aborted sheep and goats were recorded among different districts in Sulaimanyah and Duhok provinces, as shown in (Fig.1). In the districts of Sulaymaniyah, the highest rate was found in sheep in Saydsadq district 8/14 (57.14 %); whereas the lowest rate was found in Chamchamal 2/36 (5.56 %). The rates in other

districts were 20/127 (15.75 %) in Kalar, and 5/52 (9.62 %) in Kifri. However, the infection recorded for goats only in one district, namely Kalar was 1/32 (3.12 %); whereas the other areas were found infection-free. In the districts of Duhok different rates also were recorded. The highest rate was recorded in Akrei 2 /4 (50 %) in sheep, and the lowest rate was in Sumel 2 /27 (7.41 %). The rates in other districts were 1 /5 (20 %) in Zakho, and 2 /6 (33.33 %) in Amedi. Also, the highest rate of prevalence in goats was 6 /20 (30 %) in Sumel; whereas the lowest rate was 2 /4 (11.11 %) in Akrei. The prevalence in goats rate was 2 /8 (25 %) in Zakho and no case was recorded in Amedi.

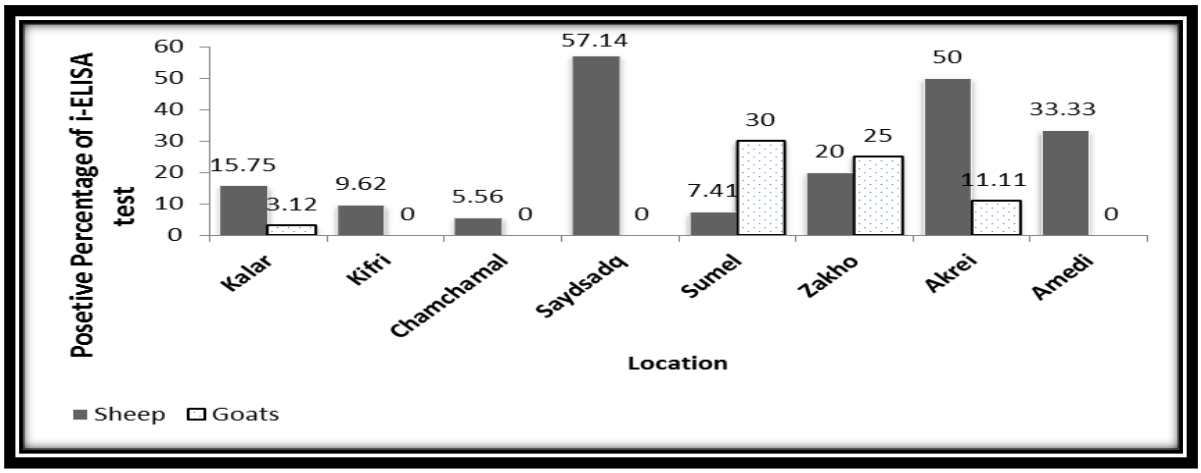

Fig. (1): The percentage of positive *Cp. abortus* using ELISA in different districts in both Sulaymaniyah and Duhok provinces.

In sheep, the infection rate was higher in the age group ≤ 2 years than in the age group > 2 years: 29 (18.24 %) compared to 13 (11.61 %). In goats, on the other hand, converse rates were noticed for the two age groups: > 2 years, 6 (16.22 %), compared to 5 (8.33 %) in ≤ 2 years. The results are depicted in Table (3).

Table (3): The infection rate of *Cp. abortus* according to age of the aborted animals.

Age/ year	Sheep			Goats		
	Total	Positive No. (%)	Negative No. (%)	Total	Positive No. (%)	Negative No. (%)
≤2	159	29 (18.24)	130 (81.76)	60	5 (8.33)	55 (91.67)
>2	112	13 (11.61)	99 (88.39)	37	6 (16.22)	31 (83.78)
Total	271	42 (15.50)	229 (84.50)	97	11 (11.34)	86 (88.66)

▪ The rate of *Cp. abortus* related to the age in sheep and goats shows no significant differences (P>0.05).

Table (4) shows a high rate of abortion due to *Cp.abortus*, 35/119 (29.41 %) which was only detected in the last stage of pregnancy (> 3 months), and 7/ 94 (7.45 %) in the mid-gestation period, but not recorded in the early stages of aborted sheep. In the goats, on the other hand, a higher rate was seen in the last stage of gestation period 8 (19.05 %), but for the early and mid-gestation periods the rate was 1/29 (3.45 %) and 2/26 (7.69 %) respectively.

Table (4): The infection rate of *Cp. abortus* according to the time of abortion.

Time of abortion	Sheep			Goat		
	Total	Positive No. (%)	Negative No. (%)	Total	Positive No. (%)	Negative No (%)
Early gestation (1-1.5 month)	58	0 (0.00)	58 (100)	29	1 (3.45)	28 (96.55)
Mid gestation (2-3 month)	94	7 (7.45)	87 (92.55)	26	2 (7.69)	24 (92.31)
Late gestation (>3month)	119	35 (29.41)	84 70.59	42	8 (19.05)	34 (80.95)
Total	271	42 (15.50)	229 (84.50)	97	11 (11.34)	86 (88.66)

▪ The rate of *Cp. abortus* in aborted sheep about time of abortion was significant (P< 0.05). However, in aborted goat there was no significant differences (P> 0.05).

Out of 271 aborted ewes, 252 had aborted one time only; out of the latter number of ewes, only 23 (9.13 %) showed positive results to *Cp. abortus* by the ELISA test. But out of 97 aborted does, only 86 had aborted one time, and only 6 out of 86 (6.97 %) yielded positive results to the test. It should be noted that two-time or more than two-time abortions were not recorded in the present study (Table, 5).

Table (5): Relationship between *Cp. abortus* infection and frequency of abortion.

Number of abortion	Sheep			Goat		
	Total	Positive No. (%)	Negative No. (%)	Total	Positive No. (%)	Negative No (%)
Single	252	23 (9.13)	229 (90.87)	86	6 (6.97)	80 (93.02)
2 times	18	0 (0)	18 (100)	10	0 (0)	10 (100)
>2 times	1	0 (0)	1 (100)	1	0 (0)	1 (100)
Total	271	23 (8.49)	248 (91.51)	97	6 (6.19)	91 (93.81)

▪ Only cases of *Cp. abortus* were excluded from other causes.

▪ The rate of *Cp. abortus* in aborted sheep and goats with the frequency of abortion was not significantly different between single, two times and more than two times of abortion (P> 0.05).

Direct microscopic examination Examination was made of direct smears taken from the liver of the aborted fetuses, and placenta and vaginal swabs of dams using modified Ziehl-Neelsen (MZN) and Giemsa stains. By the latter method, the identification of the agent was done depending based on demonstration of elementary bodies of *Chlamydia*. The inclusions appeared as small, and purple in color with packed elementary bodies (Fig. 2); whereas, by using MZN staining, they appeared as red bodies inside the cytoplasm of the cells (Fig. 3).

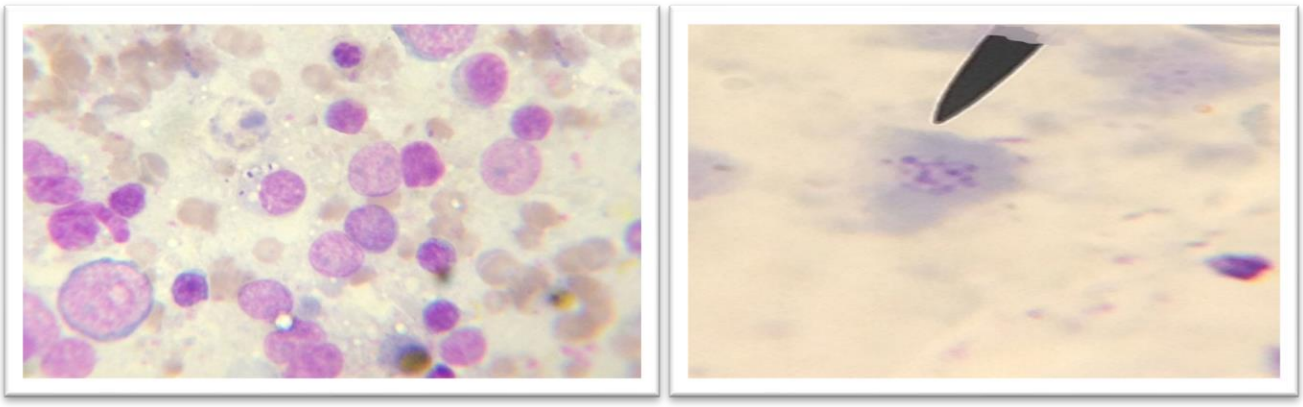

Fig. (2): Direct smear, Giemsa stain, elementary bodies of *Chlamydia* appearing purple in color in different sizes and numbers in the cytoplasm of the infected placenta of aborted sheep (X-100).

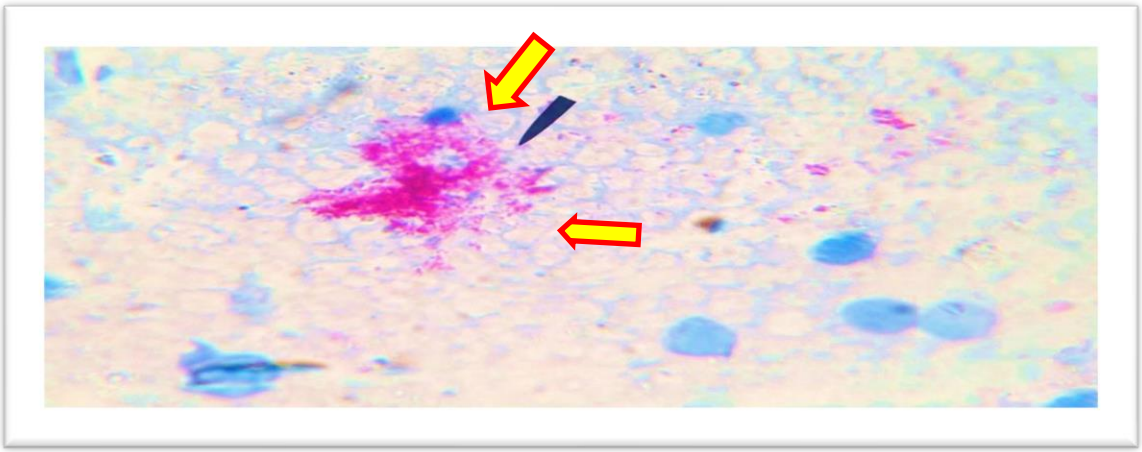

Fig. (3): Direct smear, MZN stain. Elementary bodies of Chlamydia appear as intracellular red, coccobacillus rods usually occur in clamps (X-100).

The positive and negative results of the direct examination methods for demonstration of *Chlamydia* species from different samples are shown in Table (6). From the results the *Chlamydia* was demonstrated only from one placenta 1/ 10 (10 %) of aborted sheep, while from the different samples, it had given a negative result.

Table (6): Detection of Chlamydia in different specimens using direct microscopic examination**.**

Samples	Total no. of examined specimen	Sheep			Goats		
		No. of examined specimen in sheep	Giemsa stain	(MZN) stain	No. of examined specimen in goat	Giemsa stain	MZN stain
			Positive (%)	Positive (%)		Positive (%)	Positive (%)
Aborted fetus (liver)	24	20	0 (0)	0 (0)	4	0 (0)	0 (0)
Placenta	10	9	1 (10)	1 (10)	1	0 (0)	0 (0)
Vaginal swab	33	21	0 (0)	0 (0)	12	0 (0)	0 (0)

DISCUSSION

There are many researches available on brucellosis and toxoplasmosis, which are two the main causes of abortion in sheep and goats and are widely spread in e both provinces (Nassrulla, 2007; Mikail, 2011; (Al-Naqshabendy, 2012; Ahmad, 2013; Ameen et al., 2023).Yet, while very little information is available on the prevalence of Chlamydiosis in Iraq. Therefore, by the findings of the current study, it has been confirmed for the first time that *Cp. abortus* is also one of the main causes of abortion in sheep and goats and their prevalence was different according to locations, temperature and time of year. This has been documented by conducting both the direct staining (MZN and Giemsa stains), and the seroprevalence survey using i-ELISA for a limited number of aborted ewes and does in both provinces. In general, a diagnostic method of chlamydiosis by clinical signs is difficult *(*Longbottom *et al*., 2004; Essig and Longbottom, 2015). Many studies have been carried out in different countries throughout the world aiming for the detection of the prevalence of *Cp.abortus* in animals using serological assays including; ELISA (Al-Dabagh et al., 2014) CFT (Osman, 2013), and indirect immunofluorescent-antibody test (IIFAT) (Kennedy *et al*., 2001); by isolation (Kalender *et al*., 2013) as well as molecular technique like PCR (Abad *et al*., 2011)(Dhahir, 2020)(Aras *et al.*, 2021). Thus the ELISA test was applied in the present study because of its accuracy in sensitivity (89-95%) and specificity (100%) as well as the fact that it is considered a potent reliability test for survey of chlamydiosis in sheep and goats (IDEXX Animal Health Updates, 2011), and it's a rapid screening test for infection detection (El-Berbawy and El-Khabaz, 2014). In general, according to the present results, there is no significant difference in infection rate between Sulaymaniyah and Duhok provinces, except for the finding that the infection rate in goats was significantly higher in Duhok (20%) than in Sulaymaniyah province (2.13%). This difference may be due to the uncontrolled cross movement of goats between the two neighboring countries, with Duhok being closer to the Turkish borders than Sulaymaniyah, and also taking into consideration that *Cp. abortus* is considered the main cause of abortion in goats in Turkey (Kalender *et al*., 2013). The ELISA overall seropositivity of 53/368 (14.40%) has been found 36/276 (13.04%) in Sulaymaniyah and 17/92 (18.47%) in Duhok. On the other hand, according to (Mikaeel *et al*., 2016) study the infection rate was 11.9 %. The infection rate of the disease in the north of Iraq (Sulaymaniyah and Duhok provinces) was higher than in some provinces in the South of Iraq. This has been confirmed by comparing the findings of the current study with the findings of (A Cati *et al*., 2008), who recorded that the infection rate of chlamydiosis in different regions of the South of Iraq, including Thiqar, Misan, Al-Muthanna and Al-Basra was 2.15%, 4.08%, 2.66% and 3.91%, respectively. Similarly, (Al-Neaemy, 1999) also recorded an infection rate in Baghdad of 48.86 % and 73.25 %, using CFT and micro-immunofluorescence tests respectively. The prevalence of the disease is higher in the Kurdistan Region of Iraq (15.50 % in sheep and 11.34 % in goats) when compared to other countries the using ELISA technique. For instance, in Al-Qaseem of Saudi Arabia, the prevalence of the disease was 4.55 % in sheep and 5.66 % in goats (Aljumaah and Hussein, 2012). In Iran, the rate of the disease was 8.9 % in sheep fields (Ghorbanpoor *et al*., 2007). In Kars of Turkey and in the west of India, the rates of the infection were 5.38 % and 6%, respectively (Otlu *et al*., 2007; Stone et al., 2012). Some similarities can be seen in the rate of infection among the current study's results and the findings of some researches. For instance, the prevalence rate of the disease in Nineveh governorate using ELISA was 11.2 % in sheep and in Jordan by the same procedure was 21.8 % and 11.4 % in sheep and goats, respectively (Al-Qudah *et al.*, 2004; Al-Dabagh *et al.*, 2014). Besides, in Syria similar rate 15.53 % was recorded (Al-Tabbaa *et al.*, 2020). (Huang *et al*., 2013) postulated that the prevalence of Chlamydosis varied among the studies conducted in the same region and demonstrated that the methods of the disease determination (assay type) was the main reason for this variation. (Al-Dabagh *et al*., 2014) further revealed that the geographic location, animal population, size and type of the samples, use of maternity pens, reproductive and health status of the animal have considerable effect on the obtained results. In addition, (Al-Tabbaa *et al*., 2020) found that ovine chlamydiosis higher in the winter season. In the current study the overall prevalence rate of chlamydiosis was 14.40%, the rate of infection in infected ewes was higher (15.50%) when compared with infected does but the difference was statistically non-significant ($P>0.05$). These results are in agreement with (Al-Qudah *et al.*, 2004) who noted that there was no significant difference in infection rate of chlamydiosis between the two species. On the other hand, the present study's

results disagree with several findings by (Tsakos et al., 2001) and (Cislakova *et al.*, 2007), who found the infection rate of the disease to be high in sheep compared to goats. In addition, the results of the current study disagree with (Aljumaah and Hussein, 2012), (Travnicek *et al*., 2002) , (Pinheiro Junior *et al.*, 2010), who revealed that the prevalence of the disease was lower in sheep compared to goats. This difference in the rate of infection between sheep and goats among researchers might be due to the difference in the number of samples taken, the quality and purpose of the study and the geographic regions of the farms; in addition, in our country, most of the farmers prefer to rear a large number of sheep and a small number of goats in their flocks, most often with both species in the same flock under the same management program such as feeding, grazing and watering, and same environmental conditions. All these factors contribute to the occurrence of the disease in both species and give them an equal chance of exposure to the infection. In the present study, the different prevalence rates between aborted sheep and goats were recorded among different districts in Slemani and Duhok provinces, as shown in Figure (1). The current study demonstrated that the age of the animal does not influence in contracting of infection. In sheep, the rate of the infection was higher in age ≤ 2 years 18.24% compared to sheep > 2 years 11.61%. Meanwhile in goats, the rate of the disease was higher > 2 years 16.22% compared to goats in age ≤ 2 years 8.33%. However, the differences were not statistically significant (P>0.05), which means that age cannot be a significant factor in changing the rate of *Cp. abortus* infection in both species. The results revealed that all ages under the same circumstance had an equal chance of acquiring the infection with *Cp.abortus*. In terms of the age variable, the present study's results agree with the findings of (Al-Qudah *et al*., 2004), (Ghorbanpoor *et al*., 2007) and (Huang *et al*., 2013), who conducted their study in Jordan, Iran and China, respectively. Our result was in disagreement with El-berbawy and El-khabaz (2014). In the present study a high rate of abortion due to *Cp. abortus* among sheep and goats was 29.41%, and 19.05 % respectively, which was detected mainly in the last stage of pregnancy. A significant difference was seen between the early, mid and late stages of pregnancy (P<0.05) in sheep; whereas, in goats, there was not any difference between the stages of pregnancy (P>0.05). The current study's findings support the results of Kahn *et al*. (2005), who reported that abortion in sheep mostly happened during the last 14 to 21 days of gestation, while in does, abortion is not common at a specific time or stage of gestation; however, to some extent, it happens around the last 14 to 21 days of gestation. *Cp. abortus* alters the pattern of secretion of the hormones which are responsible for maintaining pregnancy, especially during the late stage of gestation. In the gestation period, chorionic epithelial cells constitute the main source of progesterone; thereafter, progesterone interacts with locally synthesized oestradiol and prostaglandin in regulating of parturition. *Cp. abortus* can alter this mechanism, which causes premature delivery (Aitken, I. D. and Longbottom, 2007). In the findings of the current study, *Cp. abortus* was seen in those sheep and goats which suffered from one abortion only, but neither a two times nor more than two times of abortion was recorded to be due to this infection. (Papp *et al.*, 1994) stated that one of the most characteristic of chlamydiosis in ewes is the long-term immunity, in which the animals do not suffer from the infection (chlamydiosis) anymore. This immunity may prevent future chlamydaemias and stop placental colonisation; also, the cases have been described in those ewes carrying and excreting the microorganism in their reproductive tract during the periovulation period. In addition, (Aitken, I. D. and Longbottom, 2007) who studied the immune status of the animal which suffered from abortion due to *Cp. abortus* and stated that this immunity (long-term immunity) is likely to be a consequence of the maternal acquired immune response. The direct smear was the traditional method used by some workers for detecting chlamydial inclusion bodies (Dagnall and Wilsmore, 1990). In the present study, the samples of liver of aborted fetus, placenta, and vaginal swabs were taken and stained with both Giemsa and MZN stains. Direct smears from the placenta of aborted sheep was performed to reveal the presence of chlamydial inclusion bodies using both MZN and Giemsa stains. The inclusion bodies appeared red color inside the cytoplasm of the cells after using MZN stain (Coles, 1986), while with the Giemsa stain, the bodies appeared in a purple colour (Timoney *et al.*, 1988b). Similar bodies was found by (Barhoom, 2015) in smear taken from organs of aborted fetuses, in North Palestine, while (Ghorbanpoor *et al*., 2007) found similar bodies in the smears which were taken from the liver of infected rabbits with *Cp.abortus*. Because of its simplicity, the direct microscopic examination was chosen; however, the test is not commonly

used due to low sensitivity and specificity compared with other methods (Black, 1997). Since staining artifacts can be considered as chlamydial inclusions, the reading of the stained slides needs to be performed by expert staff (Schoenwald *et al*., 1988). However, for the purpose of diagnosing it, Giemsa staining was unreliable (El-Berbawy and El-Khabaz, 2014). The percentage of positive cases which was determined by placenta of the animals was 10 %. This result agrees with the result of (Pospíšil *et al*., 2000), who experimentally inoculated *Cp. abortus* in testicles of rabbits, after distribution of the organism to other organs, the researcher found the percentage of the infection in the liver, spleen and lungs which to be 40%, 12.5%, and 10%, respectively. In addition, (Ghorbanpoor *et al*., 2007) reported that the percentage of the infection in all organs was 77.1% using Giemsa stain. In the current study, difficulty was seen in obtaining of chlamydial inclusion bodies in some prepared slides, this might be due to some problem of direct smear such as an error in the staining process or as (Rodolakis *et al*., 1998) explained, the time of destaining in acetic acid, which may vary according to the thickness of the smear (Rodolakis *et al.*, 1998). In addition, (Dagnall and Wilsmore, 1990) mentioned that the carbol fuchsin-stained smears require careful decolorisation with diluted acetic acid. Excessive decolorisation or counterstaining can make the demonstration of chlamydial EBs difficult.

CONCLUSIONS

For the first time the *Cp. abortus* was found as a causative agent of abortion among sheep and goats in the Kurdistan region by using the i-ELISA test, which no one has so far mentioned about this agent. ELISA test was appeared as confinement test, and easily used for the detection anti-*Cp. abortus* antibodies in the serum of aborted ewes and does. This test was necessary for assessing the economic impact of the problem and for developing effective control strategies. The single, double and mixed infections were reported in this survey in both provinces. Age and species were not significant factors in the appearance of infection, since no statistically significant difference in infection rate has been observed among species and age group of sheep and goats. The most prevalent chlamydiosis was statistically significant concerning the time of pregnancy in sheep mainly in the last stage of gestation, but it was not significant in goats. Several seronegative results were obtained in this survey, which can be attributed to other causes rather than, toxoplasmosis, brucellosis and chlamydiosis, such as Campylobacteriosis, listeriosis, salmonellosis, leptospirosis, and Q fever.

REFERENCES

A Cati, J., Abbod, B. C., and A Hasso, S. (2008). Detection of (Chlamydophila abortus) antibodies in cattle and sheep in south of Iraq by using of iELISA and passive Heamagglutination tests. *AL-Qadisiyah Journal of Veterinary Medicine Sciences*, *7*(2), 66–72.

Abad, R., Elhafi, G. E., Vijayan, R., and Kumar, S. (2011). Studies on Carrier State of Chlamydophila abortus in Naturally Infected Sheep. *Current Trends in Biotechnology and Pharmacy*, *5*(2), 1123–1129.

Ahmad, R. B. (2013). *Seroprevalence and Detection of Toxoplasma gondii in aborted ewes and does in Sulaimanyhia province. M.Sc. thesis.* College of Veterinary Medicine. University of Duhok.

Aitken, I. D. and Longbottom, D. (2007). Chlamydial abortion. In: In *Diseases of sheep.* (4th ed., pp. 105-112.). Aitken I.D. Blackwell Scientific Ltd, Oxford, UK.

Aitken, I. D. (2000). Chlamydial abortion. In W. . Martin (Ed.), *Diseases of sheep.* (3rd ed. Ed, pp. 81–86). Blackwell Science, Oxford.

Al-Dabagh, I. I., Jasim, B. M., and Jarjees, M. T. (2014). Seroprevalence of antibodies to toxoplasmosis, brucellosis and chlamydiosis in abortive sheep in Nineveh governorate, Iraq. *Iraqi Journal of Veterinary Sciences*, *28*(1).

Al-Naqshabendy, A. A. (2012). *Diagnosis of brucellosis in aborted small ruminants using conventional methods and PCR technique. Ph.D. Thesis.* College of Veterinary Medicine. University of Duhok.

Al-Neaemy, H. (1999). *Isolation and serological diagnosis of Chlamydia psittaci in aborted ewes and their owners. M.Sc thesis.* College of Veterinary Medicine . University of Baghdad.

Al-Qudah, K. M., Sharif, L. A., Raouf, R. Y., Hailat, N. Q., and Al-Domy, F. M. (2004). Seroprevalence of antibodies to Chlamydophila abortus shown in Awassi sheep and local goats in Jordan. *Veterinární Medicína*, *49*(12), 460.

AL-TABBAA, M. F., ALLOUZ, A. K. K., and ALOMAR, Y. (2020). SEROPREVALENCE AND RISK FACTORS OF CHLAMYDIA ABORTUS INFECTION IN AWASI SHEEP IN HAMA GOVERNORATE IN THE MIDDLE REGION OF SYRIA. *Assiut Veterinary Medical Journal*, *66*(167).

Alderton, S. (2013). *Analyse lamb losses now.*

Farmers Weekly Interactive. http://www.fwi.co.uk/livestock/analyse-lamb-losses-now.htm

Aljumaah, R. S., and Hussein, M. F. (2012). Serological prevalence of ovine and caprine chlamydophilosis in Riyadh region, Saudi Arabia. *African Journal of Microbiology Research*, *6*(11), 2654–2658.

Ameen, A. M., Abdulaziz, N. S., and Ghaffar, N. M. (2023). Molecular Detection and Associated Risk Factors of Brucella melitensis in Aborted Sheep and Goats in Duhok Province, Iraq. *Pathogens*, *12*(4), 544.

Anonymous. (2004). *OIE manuel of diagnostic tests and vaccines for terreestrial animals* (5th ed). Chap.

Aras, Z., Gölen, G. S., Sayın, Z., and Haydardedeoğlu, A. E. (2021). *Identification of chlamydophila abortus in abomasum content of aborted sheep and goats by PCR*.

Black, C. M. (1997). Current methods of laboratory diagnosis of Chlamydia trachomatis infections. *Clinical Microbiology Reviews*, *10*(1), 160–184.

Burgener, A.-V., Seth-Smith, H. M. B., Kern-Baumann, S., Durovic, A., Blaich, A., Menter, T., Bruder, E., Roloff, T., Martinez, A., and Borel, N. (2022). A case study of zoonotic chlamydia abortus infection: diagnostic challenges from clinical and microbiological perspectives. *Open Forum Infectious Diseases*, *9*(10), ofac524.

Cislakova, L., Halánová, M., Kovacova, D., and Stefancikova, A. (2007). Occurrence of antibodies against Chlamydophila abortus in sheep and goats in the Slovak Republic. *Annals of Agricultural and Environmental Medicine*, *14*(2).

Coles, E. H. (1986). *Veterinary clinical pathology* . (4th ed.). W.B. Saunders Company, London. pp .

Dagnall, G. J., and Wilsmore, A. J. (1990). A simple staining method for the identification of chlamydial elementary bodies in the fetal membranes of sheep affected by ovine enzootic abortion. *Veterinary Microbiology*, *21*(3), 233–239.

Dhahir E A. (2020). *Molecular Investigation of Some Virulence Factors of Chlamydia abortus Isolated from Aborted Ewes in Slemani Province*.

EL BERBAWY, S. M., and AS EL-KHABAZ, K. (2014). CHLAMYDIA INFECTION IN ABORTED AND CLINICALLY HEALTHY EWES IN ASSIUT GOVERNORATE USING MICROSCOPICAL EXAMINATION AND ELISA. *Assiut Veterinary Medical Journal*, *60*(142), 23–28.

Essig, A., and Longbottom, D. (2015). Chlamydia abortus: new aspects of infectious abortion in sheep and potential risk for pregnant women. *Current Clinical Microbiology Reports*, *2*, 22–34.

Ghorbanpoor, M., Goraninejad, S., and Heydari, R. (2007). Serological study on enzootic abortion of ewes in Ahvaz, Iran. *Journal of Animal and Veterinary Advances*, *6*(10), 1194–1196.

Huang, S. Y., Wu, S. M., Xu, M. J., Zhou, D. H., Danba, C., Gong, G., and Zhu, X. Q. (2013). First record of Chlamydia abortus seroprevalence in Tibetan sheep in Tibet, China. *Small Ruminant Research*, *112*(1–3), 243–245.

IDEXX Animal Health Updates. (2011). *Available at:* https://www.idexx.com/pdf/en_us/livestock-poultry/abortive-disease-information-brochure.pdf

Jury, B., Fleming, C., Huston, W. M., and Luu, L. D. W. (2023). Molecular pathogenesis of Chlamydia trachomatis. *Frontiers in Cellular and Infection Microbiology*, *13*.

Kalender, H., Kiliç, A., Eröksüz, H., Muz, A., Kilinç, Ü., and Taşdemir, B. (2013). Identification of Chlamydophila abortus infection in aborting ewes and goats in Eastern Turkey. *Rev Med Vet*, *164*(6), 295–301.

Kennedy, H. E., McCullough, S. J., Graham, D., Cassidy, J., Malone, F. E., and Ellis, W. A. (2001). Detection of chlamydial antibody by fetal serology—an aid to the diagnosis of ovine abortion. *Journal of Veterinary Diagnostic Investigation*, *13*(1), 30–35.

Longbottom, D.; Fairly, S.; Chapman , S.; Psarrou, E.; Vretou, E. and Livingstone, M. (2004). Serological diagnosis of ovine enzootic abortion by enzyme-linked immunosorbent assay with a recombinant protein fragment of thepolymorphic outer membrane protein POMP90 of Chlamydophila abortus. *J. Clin. Microbiol*, *40*, 4235-4243.

Longbottom, D., Fairley, S., Chapman, S., Psarrou, E., Vretou, E., and Livingstone, M. (2002). Serological diagnosis of ovine enzootic abortion by enzyme-linked immunosorbent assay with a recombinant protein fragment of the polymorphic outer membrane protein POMP90 of Chlamydophila abortus. *Journal of Clinical Microbiology*, *40*(11), 4235–4243.

Markey, B.; Leonard, F.; Archambault, M.; Cullinane, A. and Maguire, D. (2013). *Clinical Veterinary Microbiology* (2nd ed). Mosby Elsevier, New York.

Mikaeel FB; Taha ZM; Omer LT. (2016). Sero-prevalence of Chlamydophila abortus in goat farms in Duhok Province-Iraq. *AL-Qadisiya Journal of Vet. Med. Sci.*, *15*(1), 34–37.

Mikail, F. (2011). *Serodiagnosis of anti-toxoplasma antibodies in aborted ewes in some localities of Duhok province.* College of Veterinary Medicine . University of Duhok.

Nassrulla, O. J. (2007). *Serosurvay of brucellosis in sheep and goat in Sulaimanyah governorate. M.Sc. Thesis.* College of Veterinary Medicine. University of Duhok.

OIE Terrestrial Manual . Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organisation for Animal Health; Paris, France: 2023. Office International des Epizooties. Chapter 3.8.5—Enzootic abortion of ewes (ovine chlamydiosis). (n.d.).

Osman, W. A. (2013). Comparative evaluation of indirect ELISA, CF test and PCR for diagnosis of ovine enzootic abortion (Ovine chlamydophilosis). *Global Veterinaria*, *11*(1), 65–70.

Otlu, S., Sahin, M., Unver, A., and Celebi, O. (2007). Detection of Brucella melitensis and Chlamydophila abortus antibodies in aborting sheep in the Kars province of Turkey. *Bulletin of the Veterinary Institute in Puławy*, *51*(4).

Papp, J. R., Shewen, P. E., and Gartley, C. J. (1994). Abortion and subsequent excretion of chlamydiae from the reproductive tract of sheep during estrus. *Infection and Immunity*, *62*(9), 3786–3792.

Pinheiro Junior, J. W.; Mota, R. A.; Piatti, R. M.; Oliveira, A. D.; Silva, A. D. and Abreu, S. D. (2010). Seroprevalence of antibodies to Chlamydophila abortus in ovine in the State of Alagoas, Brazil. *Brazilian J. of Microbiol*, *41*(2), 358–364.

Pospíšil, L., Věžník, Z., Zralý, Z., and Diblikova, I. (2000). Systemic dissemination of Chlamydiae after genital infection in rabbits. *Veterinární Medicína*, *45*(6), 159–161.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W., and Constable, P. D. (2006). *Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier Health Sciences.

Rocchi, M. S., Wattegedera, S., Meridiani, I., and Entrican, G. (2009). Protective adaptive immunity to Chlamydophila abortus infection and control of ovine enzootic abortion (OEA). *Veterinary Microbiology*, *135*(1–2), 112–121.

Rodolakis, A., and Mohamad, K. Y. (2010). Zoonotic potential of Chlamydophila. *Veterinary Microbiology*, *140*(3–4), 382–391.

Rodolakis, A., Nettleton, P., and Benkirane, A. (1998). *Manual for laboratory diagnosis of infectious abortions in small ruminants*.

Sachse, K., Vretou, E., Livingstone, M., Borel, N., Pospischil, A., and Longbottom, D. (2009). Recent developments in the laboratory diagnosis of chlamydial infections. *Veterinary Microbiology*, *135*(1–2), 2–21.

Schoenwald, E., Schmidt, B. L., Steinmetz, G., Hosmann, J., Pohla-Gubo, G., Luger, A., and Gasser, G. (1988). Diagnosis of Chlamydia trachomatis infection—culture versus serology. *European Journal of Epidemiology*, *4*, 75–82.

Selim, A., Manaa, E. A., Waheed, R. M., and Alanazi, A. D. (2021). Seroprevalence, associated risk factors analysis and first molecular characterization of chlamydia abortus among Egyptian sheep. *Comparative Immunology, Microbiology and Infectious Diseases*, *74*, 101600.

Stone, D. M.; Kumthekar, S.; Chikweto, A.; Thomas, D.; Tiwari, K. and Sharma, R. N. (2012). Exposure to zoonotic abortifacients among sheep and goats in Grenada. *Inter. J. of Anim. and Vet. Advan*, *4*(2), 113–118.

Timoney, J. F.; Gillespie, J. H.; Scott, F. W. and Barlough, J. E. (1988a). *Hagan and Bruner's microbiology and infectious diseases of domestic animals.* (8th ed.). Cornell University Press, USA.

Timoney, J. F.; Gillespie, J. H.; Scott, F. W. and Barlough, J. E. (1988b). *Hagan and Bruner's microbiology and infectious diseases of domestic animals* (8th ed.). Cornell University Press, USA.

Travnicek, M., Kovacova, D., Bhide, M. R., Zubricky, P., and Cislakova, L. (2002). Field evaluation of an iELISA and CF test for detection of IgG antibodies against Chlamydophila abortus in goats, sheep and rams. *VETERINARNI MEDICINA-PRAHA-*, *47*(7), 195–198.

Tsakos, P., Siarkou, V., Kastanidou, C., and Papadopoulos, O. (2001). Epidemiological survey in sheep and goats for chlamydia and brucella (In Greek). *Journal of the Hellenic Veterinary Medical Society*, *52*(5), 410–421.

Vlahović, K., Dovč, A., and Lasta, P. (2006). Zoonotic aspects of animal chlamydioses-a review. *Veterinarski Arhiv*, *76*, S259–S274.

Witkin, S. S., Minis, E., Athanasiou, A., Leizer, J., and Linhares, I. M. (2017). Chlamydia trachomatis: the persistent pathogen. *Clinical and Vaccine Immunology*, *24*(10), e00203-17.

پوخـــــته

ئەم توێژینەوە بریتیە لە دەرخستنی ڕێژەی توشبوون بە نەخۆشی کلامیدیا لەمەڕو بزنی بەرهاویشتە لە 66 مێگەل و لە 8 شوێنی جیاجای پارێزگای سلێمانی و دهۆك لە هەرێمی کوردستانی عیراق لە ماوەی نێوان مانگی گەڵارێزانی 2714 تاکو کۆتای مانگی جۆزەردانی 2715.لەم توێژینەوەیەدا 271 نموونەی خوێن لە مەڕو 97 نموونە لە بزن کۆکرایەوە ئەم خوێنانە دوای مەین زەردەوەکەی جیاکرایەوە کەبرتیبوو لەو بەشەی پشکنینی سیرۆلۆجی بۆئەنجام ئەدریت هەموو نموونەکان تاقیکردنەوەی لکانی ئەنزیم لەسەر ڕوویەکی بەرگری (بەئامیری ئەلایزا ناڕاستەوخۆ) پشکنینی بۆ ئەنجامدرا. لایەنێکی تری ئەم لێکولینەوەیە بریتیبوو لە کۆکردنەوەی بەچکە بەرهاویشتەکان,Vaginal swabsو پزدانی مەڕو بزنە بەرهاویشتەکان بە هۆی سلایدی بۆیە کراو بە بویەی زیل–نیلسن (MZN) و گیمزا .لە تێکڕای ئەو 368 نموونەی زەرداوی خوێنەی کەکۆکرابووە لە مەڕو بزنە بەر هاویشتەکان ڕێژەی پۆزەتیڤەکان (بەئامێری ئەلایزا ناڕاستەوخۆ) 53(14,40٪) بوو لە هەریەك سلێمانی 36 (13,04٪) و لە دهۆکیش 17 (18,47٪)بوو. وە هەروەها تێکڕای ڕێژەی نەخۆشی کلامیدیا لەمەڕدا 15,50٪ و لە بزنیشدا 11,34٪ بوو. وە هەروەها لە مەڕدا ڕێژەی هەردوو نەخۆشی (Double infection) کلامیدیا لەگەڵ هەریەکە لە نەخۆشی تای ماڵتا (برۆسیللوسز) یا مشەخۆری چەماوەی گوێدی (تۆکسۆپلاسمۆسز) 3,32٪ و 3,69٪ بوو بە دوای یەکدا. لە بزندا 2,06٪ ی نموونەکان توشبوون بە هەردوو نەخۆشی کلامیدیا و مشەخۆری چەماوەی گوندی (تۆکسۆپلاسمۆسز) وەهەر لەهەمان ئاژەڵ 3,09٪ ی نموونەکان توشبوون بە هەر سێ نەخۆشی یە کە (Triple infection). کاتێك ئەنجامەکان بەگوێرەی تەمەنی ئاژەڵە بەرهاویشتەکان بەراوردکرا, ڕێژەی نەخۆشیەکە لەو مەڕانەی کەتەمەنیان لە دوو ساڵ و کەمتر لە دووساڵە 18,24٪ زیاتربوو لەومەڕانەی کەتەمەنیان لە دوو ساڵ بەرەو سەرەوەیە. بە پێچەوانەوە لە بزنەکاندا ڕێژەی ئەم نەخۆشیە لەتەمەنی دوو ساڵ بەرەو سەرەوە زیاتربوو. لە کاتێکدا بە پێی ئامار هیچ جیاوازی یەکی بایەخدار بە گوێرەی تەمەنی ئاژەڵەکان نە بینرا (P>0.05). لە ئاژەڵەکاندا ڕیژەی بەرهاویشتەکان بە هۆی کلامیدیاوە زیاتر بینرا لە قوناغی کۆتای بەراورد بە سەرەتاو نا وەڕاستی قۆناغەکانی سکپری وە بە پێی ئاماریش تەنها لە مەڕدا جیاوازی بایەخدار بینرا لەنیوان قۆناغەکانی سکپری (P< 0.05). لەم توێژینەوەدا ئەوەمان بۆ دەرکەوت کە کلامیدیا یەکجار ئەبێتە هۆی بەراویتە بوون لە مەڕو بزنەکان و ڕیژەکەش بریتیەلە 9,13٪ و 6,97٪ بە دوای یەکدا. لەژیر ماکروسکۆپدا تەنها لەناو خانەکانی پزدانی مەڕیکدا (EBs) ی کلامیدیا بینران بە شێوەی بچووکی ڕەنگ ئەرخەوانی بە بۆیەی گیمزاو بەشێوەی سور بە بۆیەی زیل نیلسن (MZN).

الخلاصـــة

تهدف الدراسة الحاليه الى تحديد انتشار داء الكلاميديا في النعاج و الماعز المجهضة في 66حقل موزعه على 8 أقضيه تابعه لمحافظتي السليمانية و دهوك في اقليم كوردستان العراق للفترة من تشرين الأول 2014 ولغايه نهايه شهر حزيران 2015. تم اجراء اختبار الاليزا غيرالمباشر على 271 و 97 عينه من امصال النعاج و الماعز المجهضه على التوالي، فضلا عن اجراء الفحص المباشر لأعضاء الأجنه المجهضة (الكبد), المسحات المهبليه والمشيميه بأستخدام صبغه الزيل نيلسن والكيمزا. أظهرت نتائج الدراسة الحالية ان نسبة الاصابة الكلية بداء الكلاميديا 53 (14,40٪) وتضمنت 36 حالة (13,04٪) في السليمانية و 17حالة (18,47٪) في دهوك. في حين بلغت نسبة الاصابة في الاغنام 15,50٪ وفي المعز 11,34٪. عند مقارنة النتائج مع عمرالحيوانات المجهضة، لوحظ ان نسبة الاصابة ازدادت في النعاج التي تراوحت اعمارهم عامين أو أقل. بينما لوحظ العكس تماماً في الماعز فقد ان الاعمار التي تقل عن عامين معرضة للاصابة والاجهاض بنسب اعلى. ومع ذلك, احصائياً لايوجد فرق في نسبة الاصابة بين المجموعتين من ناحية العمر (P>0.05). ولوحظ ايضا ان النسبة الاعلى للأجهاض تحدث في المراحل الاخيرة من الحمل في كلا النوعين وازدادت معنوياً في النعاج الحوامل بالمراحل الأخيره من الحمل (P<0.05). اما تكرار حالات الاجهاض بداء الكلاميديا فقد لوحظ انها تسبب الاجهاض مرة واحده فقط. كما لوحظت الاجسام الاشتمالية في مشيمه النعاج المجهضة والتي تميزت بشكلها الصغير الأرجواني عند صبغها بصبغة الكيمزا في حين انها ظهرت باللون الاحمر داخل هيولي الخلية عند صبغها بصبغة الزيل– نيلسن المحورة (MZN).