

SEROLOGICAL ASSESSMENT OF THE OCCURRENCE OF *SALMONELLA ABORTUSOVIS*, *BRUCELLA SPP.*, AND *LISTERIA MONOCYTOGENES* AMONG EWES IN BABYLON PROVINCE, IRAQ

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ABSTRACT

The current study aimed to determine the infection rate of *Salmonella abortusovis*, *Brucella* spp, and *Listeria monocytogenes* among ewes in Babylon Province. For this purpose blood samples were collected from 50 aborted ewes, 15- 30 days after abortion, and 10 normal deliveries of ewes during the period from September 2021 to June 2022. The collected samples were evaluated by using ELISA. The results showed that from the aborted cases, 78% (39/50 cases) were positive for one or more of the diseases examined, while regarding the 11 normal deliveries, only 27% (3 out of 11) were found positive. Estimation of infection rate among the aborted ewes using Anti-*Salmonella abortusovis* antibody, anti-*Brucella* spp antibody and anti-*Listeria monocytogenes* antibody detected positive cases at rates of 66%, 22% and 12% respectively, and 25.6% of cases gave antibody titre against more than one etiology that was subjected in the current study. The current study reveals that 78.7% of abortions caused by *Brucella* spp., took place during the third semester.

Key words: Abortion, Brucellosis, Salmonellosis, Listeriosis

INTRODUCTION

Abortion is defined as the expulsion of an immature fetus (one or more) before the completion of the normal pregnancy period, which is either dead or remains alive for a period of less than 24 hours (Clothier *et al.*, 2020 and Deresa *et al.*, 2020). Abortion can be caused by either infectious or

noninfectious such as nutritional stress, which leads to decrease in the size or lysis of the corpus luteum and a decrease in progesterone level. The consumption of certain types of toxic plants leads to the closure of the binding site of Oosterodiol – B17 or has a direct toxic effect on the fetus (Radostits *et al.*, 2007).

Infectious abortion in ewes may be associated with viral, parasitic, fungal, or bacterial infections, which are the most important causes. *Brucella* spp., *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., and

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Chlamydia spp., are the main bacterial causes of ewe abortion (Blood *et al.*, 1989).

Many methods are used for diagnosis of bacterial abortions in ewes, including direct methods (smear staining, culture methods and genetic methods) or indirect methods (detection of humeral and cellular immune response against causative agents). ELISA consider the sensitive and specific test used for detection of humeral immune response (Latimer *et al.*, 2004). The current study is designed to examine the infection rate of *Salmonella abortusovis*, *Brucella* spp., and *Listeria monocytogenes* among ewes in Babylon province and investigating some epidemiological aspects related to these infections.

MATERIALS AND METHODS

The study was carried out during the period from September 2021 to June 2022, blood samples were collected under aseptic conditions into 5 ml sterile tubes from 60 ewes (50 from aborted ewes in a period from 15- 30 days after the abortion and 10 normal deliveries ewes) of the Babylon province then samples were transported in isothermal containers with ice ($4\text{ }^{\circ}\text{C} \pm 2$) for less than an hour to the Internal and Preventive Medicine Branch, College of Veterinary Medicine/Al-Qasim Green University,

Babylon, Iraq. The serum was obtained by centrifugation of blood samples at 3000 rpm for 15 min within 24 h of collection. It was then stored at -20°C to be used for detection of antibodies.

Serodiagnosis was done using the following ELISA kits:-

- a- Anti *Salmonella abortusovis* antibody: detected by use of ELISA kit (Creative Diagnostics- USA) and according to the manufacturer's instructions
- b- Anti *Brucella* Antibody: detected by use of ELISA kit (SVANOVIR®Brucella-Ab I-ELISA- Spain) (captures antibodies to *B. abortus* and *B. melitensis*.) and according to the manufacturer's instructions.
- c- Anti *Listeria monocytogenes* antibody: detected by use of ELISA kit (Mybiosource- UK) and according to the manufacturer's instructions.

RESULTS

Out of 50 aborted ewes, 39 (78%) tested positive for one or more diseases, while 11 (22%) were ELISA negative for any of the investigated diseases. In contrast, among 10 ewes with normal deliveries, 3 (30%) tested positive, and 7 (70%) were negative (Table 1).

Table 1: Prevalence of examined diseases among aborted and normal deliveries ewes

Animals	Total No.	+ve for one or more examined diseases		-ve for any of examined diseases	
		No.	%	No.	%
Aborted	50	39	78	11	22
Normal deliveries	10	3	30	7	70
Total	60	42	70	18	30

Infection details of each disease separately was illustrated in Table (2). The results demonstrate that *Brucella* spp. was the most

commonly detected pathogen, followed by *Salmonella abortusovis* and *Listeria monocytogenes*.

Table 2: ELISA results for aborted and normal deliveries ewes.

Animals	Total No.	Anti <i>Salmonella abortusovis</i>		Anti <i>Brucella spp.</i> Antibody		Anti <i>Listeria Monocytogenes</i> antibody	
		No.	%	No.	%	No.	%
Aborted	50	11	22	33	66	6	12
Normal deliveries	10	1	10	2	20	0	0
Total	60	12	20	35	58.3	6	10

Results from Table (3) indicate that mixed infections were identified in 10 cases (25.6%), whereas 29 (74.3%) cases were confirmed to have a single pathogen.

Table 3: Frequency of mixed versus single infection among aborted ewes

Types of antibodies	Number of positive samples	Rate of positive samples (out of cases)
Anti <i>Brucella spp.</i> Antibody+ Anti <i>Salmonella abortusovis</i>	7	17.9%
Anti <i>Brucella</i> Antibody+ Anti <i>Listeria Monocytogenes</i> antibody	2	5.1%
Anti <i>Salmonella abortusovis</i> + Anti <i>Listeria Monocytogenes</i> antibody	1	2.5%
Total of mixed infection	10	25.6%
Single infection	29	74.3%
Total	39	100%

The relationship between abortion and the stage of pregnancy (Table 4)

- **Brucellosis:** Most cases (78.7%) occurred in the third trimester.
- **Salmonellosis:** Cases were distributed across the second (36.3%) and third trimesters (45.4%).
- **Listeriosis:** Primarily occurred in the first trimester (66.6%).

Table 4: The relationship between causative agents and the semester of abortion.

Type of infection	No.	cases in each Semester					
		1 st		2 nd		3 rd	
		No	%	No	%	No	%
Brucellosis	33	2	6	5	15.1	26	78.7
Salmonellosis	11	2	18.1	4	36.3	5	45.4
Listeriosis	6	4	66.6	0	0	2	33.3

The results indicate that recurrent abortions were observed in cases of Brucellosis and Salmonellosis, but not in Listeriosis.

Table 5: The relationship between abortions recurrence with infection type

Infection type	No	abortion recurrency	
		No	%
Brucellosis	33	4	12.5%
Salmonellosis	11	1	9.0%
Listeriosis	6	0	0%

DISCUSSION

The results of this study highlight the significant role of *Brucella spp.* in causing abortion in ewes, as evidenced by its high detection rate compared to *Salmonella abortusovis* and *Listeria monocytogenes*. This aligns with findings from previous research, which have consistently identified *Brucella spp.* as a leading cause of abortion in ruminants (Clothier et al., 2020; Deresa et al., 2020; Radostits et al., 2007; Hamzah et al., 2020).

Brucellosis was found to be the predominant infection, with 66% of aborted ewes testing positive for Anti-*Brucella spp.* antibodies. This finding is consistent with studies reporting high prevalence rates of *Brucella spp.* in aborted ewes (Arif et al., 2020; Al-Dabagh et al., 2014). *Brucella spp.* often causes late-term abortions in ewes, which aligns with our observation that the majority of abortions attributed to *Brucella spp.* occurred in the third trimester (Blood et al., 1989; Menzies, 2012; Mohmood et al., 2020). This may be due to the pathogen's interaction with erythritol, a compound found in the placenta that acts as a chemotactic factor for *Brucella spp.* (Roop et al., 2021).

Salmonella abortusovis was detected in 22% of aborted ewes, which is lower than some studies but still notable. For example,

Habrun et al., (2006) and Wirz-Dittus et al., (2010) reported higher prevalence rates of *Salmonella abortusovis*. Variations in detection rates could be due to differences in diagnostic methods, geographic locations, or sample sizes. The presence of *Salmonella abortusovis* in aborted ewes emphasizes the need for continued surveillance and control measures to manage this pathogen (Giannati-Stefanou et al., 1997 and Roshan et al., 2018).

Listeria monocytogenes was detected in 12% of aborted ewes, which is relatively lower compared to other studies (Ayoub et al., 2020). Listeriosis is known to cause abortion primarily in the first trimester (Gojam & Tulu, 2020 and Mikaeel & Al-Saeed, 2020), which aligns with our observation of its distribution. The differences in detection rates may be attributed to the sensitivity of the diagnostic assays or the epidemiological context of the studied populations.

Our study also found that 25.6% of the cases had antibodies against more than one pathogen, suggesting either co-infection or residual antibodies from previous infections or vaccinations. This complexity underscores the challenge of accurately diagnosing the cause of abortion based solely on serological tests (Nielsen & Yu, 2010 and Tizard, 1988). Indirect ELISA tests, as used in this study, cannot differentiate between current infections and past exposures, which may lead to overestimation of active infections.

The relationship between the trimester of pregnancy and the causative agents revealed that *Brucella spp.* is predominantly associated with abortions in the third trimester, while *Listeria monocytogenes* is more commonly associated with the first trimester. This is consistent with the literature, which indicates different patterns of abortion timing based on the causative agent (Nooruldeen et al., 2021 and Aghwan et al., 2021).

Recurrent abortions were noted, particularly with *Brucella* spp. and *Salmonella abortusovis*, indicating that either repeated exposure or insufficient immune response from previous infections could be factors. This highlights the need for effective vaccination and control strategies to prevent recurrent cases (Blasco, 1997 and Esmaeili *et al.*, 2021).

In conclusion, this study reinforces the importance of identifying and managing the major bacterial pathogens associated with abortion in ewes. Continued research and improved diagnostic techniques are essential for better understanding and controlling these infections

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التقييم المصلي لحدوث عدوى السالمونيلا ابورتوس أوفيس والبروسيليا وليستيريا مونوسيتوجينيس في النعاج بمحافظة بابل العراق

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هدفت الدراسة الحالية إلى معرفة معدل انتشار السالمونيلا ابورتوسوفيس وبروسيليا spp وليستيريا مونوسيتوجينيس في النعاج في محافظة بابل، ولهذا الغرض تم جمع عينات الدم من ٥٠ نعجة مجهزة و ١٠ نعجات ولادة طبيعية في الفترة من ١٥-٣٠ يوم بعد الإجهاض خلال الفترة من سبتمبر ٢٠٢١ إلى يونيو ٢٠٢٢ وتم إجراء اختبارات ELISA. أظهرت النتائج أن ٢٢٪ (١١ من ٥٠) من حالات الإجهاض كانت سلبية لثلاثة اختبارات استخدمت في الدراسة الحالية بينما كانت ٧٨٪ (٣٩ من ٥٠) إيجابية. تم الكشف عن الأجسام المضادة لـ Salmonella abortusovis و Brucella spp و Listeria monocytogenes بنسب ٦٦٪ و ٢٢٪ و ١٢٪ على التوالي، و ٢٥,٦٪ من الحالات تعطي عيار الأجسام المضادة ضد أكثر من مسبب واحد من الأمراض التي خضعت للدراسة الحالية. تكشف الدراسة الحالية أن ٧٨,٧٪ من حالات الإجهاض الناجمة عن Brucella spp حدثت في الفصل الدراسي الثالث. الإجهاض السابق لا يحمي النعاج من الإجهاض مرة أخرى.