Damanhour Journal of Veterinary Sciences 5 (1), (2020) 11-13

Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

Hygienic Studies on Microbial Causes of Abortion in Sheep

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A B S T R A C T

A total of 250 samples of aborted foeti, vaginal discharge and placenta were collected from aborted ewes from Behera Governorate for Microbiological examination. Swabs from stomach and intestinal contents of aborted foeti as well as liver, spleen, and lungs were collected. The recorded results showed that the prevalence of infectious diseases causing abortion in examined aborted ewes where the highest prevalence was due to brucellosis followed by Salmonellosis then listeriosis. On contrary, all serum samples were found to be negative for presence of RVF antibodies. The bacteriological examination of aborted foeti, vaginal discharge and Placenta of aborted sheep revealed the isolation of Listeria spp. and Salmonella spp. with the incidences of 4.8% and 5.8%, respectively. While serological examination of serum samples revealed that the incidence of brucellosis and Rift Valley fever was 12.4 and 0.0 %, respectively. Polymerase chain reaction assay (PCR) was a valuable tool for direct and rapid diagnosis of Brucella melitensis in blood collected from aborted ewes.

Keywords: Studies, Microbial, Causes, Abortion, Sheep

1. Introduction

Sheep represent an important source of meat and milk production as human consumption in Egypt. High need of animal protein in Egypt increases year by year. So to overcome the problem of this deficiency, the maintenance of good fertility in herds is important because the reproductive health of animals is related to the nutritional needs of human population from meat, milk and wool for manufacturing purposes. These large farms met various problems especially the abortion problem which is initiated through various causes.

Abortion is caused by many factors as mechanical, chemical, nutritional, bacterial and mycotic causes. Bacterial abortion caused by Brucella melitensis, Campylobacter fetus, Listeria monocytogens, Salmonella spp., Escherichia coli, Leptospira, Staphylococcus aureus, Streptococci, Corynebacterium pyogens and Chlamydia spp. (Kholeaf et al., 1977, Butachaiah and Khera, 1982, Bajmocy et al., 1987, Plagemann, 1989 and Sargison et al., 2001).

Brucellosis is a zoonotic disease that causes abortion, fetus death and genital infections in animals and humans. The illness initially presents as fever and may later affecting various organs and tissues (Redkar et al., 2001). Brucellosis is considered one of the major problem affecting sheep and goats, producing many economic losses due to abortion and infertility (Butachaiah and Khera, 1982).

Sheep are mainly affected by Brucella melitensis (Wilson and Miles, 1975). Vibrionic abortion of sheep are characterized by abortion during the last half of gestation period, the disease is extremely sporadic. While Listeria monocytogens is a public health concern and affect human whose immune system are inefficient, and in pregnant women cause infant death, meningitis and abortion. In infected sheep, abortion occurred at early stages of pregnancy and stillborn or weak kids (Plagemann, 1989). Identification of Brucella melitiensis by isolation was time consuming and the cultures need to be handled with care because of the zoonotic potential. So PCR assay was used for confirmation of presumptive Brucella melitiensis isolates, allowing the rapid diagnosis and facilitated studies of microorganisms (William et al., 2004 and David et al., 2005). The aim of this study is to prove the microbiological causes of sheep abortion. This can be established through demonstration, isolation and identification of the bacterial agents. Also, use polymerase chain reaction (PCR) test to substitute the conventional cultural methods and rapid diagnosis of Brucella melitensis

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P ISSN: 2636-3003 EISSN: 2636-2996

Received: December 13, 2020; Received in revised form: December 19, 2020; accepted: December 20, 2020.

2. Materials and Methods

2.1. Study population:

The study was carried out in sheep farms located in North Coast and Desert Road, Egypt for a period of 6 months from April to October, 2020. The study population consisted of aborted ewes that were investigated for identification of infectious causes of abortion. Samples are transferred directly as soon as possible to the laboratory of Mariot Research Institute and Animal Health Research Institute.

2.2. Samples:

A total of 250 aborted ewes of local breeds were investigated. They were 2-5 years old small range – reared flocks (up to 200 animals). The dam and aborted fetuses or stillbirths were retained in a room and were manipulated with precautions for further investigations. Approximately, 5 ml of blood samples were obtained from the jugular vein for each aborted sheep using vacutainer test tubes. In addition, stomach contents of the aborted foeti, vaginal discharge and placenta of aborted ewes were obtained for the isolation of bacteria which cause abortion including Salmonella and Listeria. A questionnaire form was prepared for each aborted ewe involving; age, breed, type of housing, presence of separate kidding area, type of insemination, vaccination programs, frequency of previous abortions, stage of abortion and presence of other clinical signs.

3. *Serological and molecular detection of Brucella:*

3.1. Rose Bengal Plate Test (RBPT) was carried out according to Aldomy et al., (2009).

3.2. Multiplex PCR:

Positive RBPT samples were tested for further confirmation using a PCR assay that targeting the bcsp31 gene specific for genus Brucella, IS711 element downstream of the alkB gene specific for B. abortus, and the IS711 element downstream of BMEI1162 specific for B. melitensis (Probert et al., 2004).

3.2.1. DNA extraction:

Extraction of DNA from blood was carried out according to the technique recommended by O'Leary et al. (2006).

3.3. Isolation and identification of Listeria:

The isolation of Listeria species is adopted according to Roberts and Green wood, (2003) using listeria selective enrichment broth (CM0862, Oxoid) supplemented with listeria selective enrichment agents (nalidixic acid, acriflavine and cyclohexamide) (SR0141, Oxoid) and oxford listeria selective agar (CM0856, Oxoid) supplemented with listeria selective supplement (SR0140, Oxoid). Presumptive Listeria spp. isolates were confirmed according to Gram reaction and biochemical identification (Singh and Prakash, 2008). The isolated and characterized L. monocytogenes strains were confirmed using Microbact Listeria 12L Kit system (Oxoid) according to the manufacturer recommendations. The

reference strain L. monocytogenes ATCC 7644 was used in all biochemical tests (Chye et al., 2004).

3.4. Isolation, identification and serotyping of Salmonellae:

Salmonella cultures from all samples were performed according to Collee et al. (1996). 0.1 ml from each BPW tube (after incubation) was transferred into a 10 ml Rappaport-Vasilliadis broth (RV broth, Difco, USA) and incubated at 42 °C for 24-48 hours. The RV broth samples were streaked onto Xylose- Lysine-Desoxycolate agar (XLD, oxoid) plates and incubated overnight at 37 °C. Typical colonies were picked and further tested by standard biochemical methods. Full identification of the Salmonella suspect isolates were done after matching the achieved morphological, biochemical and serological results against standard methods reported by Garrity (2001). The serological identification of the strains was carried out with Salmonella polyvalent O and H antisera in the Clinical Microbiology Department, Central Health Laboratories of Ministry of Health, and Egypt.

4. Results and Discussion:

Abortion of sheep constitutes the most important problem causes great economic implications in terms of milk yield, meat production and fertility of animals. Bacteria and fungi were usually associated with abortion of sheep. As shown in Table (1), the bacteriological examination illustrated that the Brucella melitensis was the most microorganism isolated from aborted sheep with the incidence of 21.4%. They mostly isolated from aborted foeti, placenta and vaginal discharge with the incidence of(10%, 7.1%and 4.3%, respectively from sheep),followed by Campylobacter fetus subsp. fetus, Campylobacter featus subsp.venerealis with the incidence of 11.4% and 7.1%, respectively in sheep. Also Listeria monocytogens, Salmonella typhimurium, Salmonella dublin, Escherichia coli and Staph.aureus were isolated from aborted foeti, placenta and vaginal discharge with an incidence 8.6%, 5.7%, 2.9%, 1.4% and 1.4%, respectively from sheep.These results agreement with Redman et al., (1963) who isolated campylobacter organisms with incidence of 14.7% from aborted ewe, Varga et al., (1990) recorded that abortion in sheep was caused in 18 flocks (78.3%) by C. fetus subsp. fetus and in 5 flocks (21.7%) by C. fetus subsp venerealis. Also agreement with Derbala and Ghazi (2001) they isolated Brucella melitensis from aborted sheep with the incidence of 18.9%, Leyla et al., (2003) who identifed Brucella melitensis from aborted featus with an incidence of 31%.While Plagemann (1989) isolated Listeria monocytogens, Salmonella typhimurium and Escherichia coli from aborted fetuse and placenta of aborted sheep Sargison et al., (2001) isolated E. coli from placenta of aborted sheep.

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The use of polymerase chain reaction (PCR), as shown in Fig. (1), revealed positive amplification of Brucella melitensis on 169bp and 792bp fragments on lane 1-2. While lane 3-4 indicates positive amplification of 383bp fragment of Aspergillus fumigatus. These results are in agreement with the results of (Bricker and Halling 1994, Ewalt and Bricker 2000) who used AMOS PCR technique as a diagnostic assay for identification and differentiation of Brucella melitensis from other type of Brucella spp.(Abortus, Melitiensis, Ovis, Suis) . David et al., (2005) recorded that PCR is a useful assay for detection and identifcation Aspergillus fumigatus , also is providing a good alternative to the time consuming isolation test normally used in laboratory routine .

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The target genes, sequence of the used primers and band sizes were tabulated in the following table 1

Target gene	Oligonucleotide sequence (5′ → 3′)	Band size (bp)
bcsp31, *Brucella* spp. (F)	5′ GCTCGGTTGCCAATATCAATGC ′3	223 **(Zerva et al., 2001)**
bcsp31, *Brucella* spp. (R)	5′ GGGTAAAGCGTCGCCAGAAG ′3	
BMEI1162 gene, *B. melitensis* (F)	5′ AACAAGCGGCACCCCTAAAA ′3	279 **(Mutnal et al., 2007)**
BMEI1162 gene, *B. melitensis* (R)	5′ CATGCGCTATGATCTGGTTACG ′3	
alkB gene, *B.abortus* (F)	5′ GCGGCTTTTCTATCACGGTATTC ′3	495 **(Song et al., 2019)**
alkB gene, *B.abortus* (R)	5′ CATGCGCTATGATCTGGTTACG ′3	

Description of cycling conditions was presented in the following table 2

Steps	Temperature	Duration	No. of cycles
Initial PCR activation step	95°C	3 min.	1 hold
Denaturation	95°C	90 sec.	35 cycles
Primer annealing	65°C	1 min.	
Extension	72°C	2 min.	
Final extension	72°C	5 min.	1 hold
Cooling	Hold at 4°C till further processing		

Table (3): Prevalence of infectious diseases causing abortion in examined aborted ewes

Disease	Aborted ewes (n=250)	
	Positive	%
Brucellosis	31	12.4
Listeriosis	12	4.8
Salmonellosis	13	5.20
Rift Valley fever	0	0.0

Table (4): Rate of isolation of Listeria and Salmonella from different samples of aborted ewes

Isolated Bacteria	Site of isolation						Total	
	Aborted foeti (n=250)		Vaginal discharge (n=250)		Placenta (n=250)			
	No.	%	No.	%	No.	%	No.	%
Listeria spp.	9	3.6	5	2.0	5	2.0	19	7.6
Salmonella spp.	7	2.8	3	1.2	4	1.6	14	5.6
Total	16	6.4	8	3.2	9	3.6	33	13.2

Table (5): Prevalence of diseases causing abortion in ewes in relation to age groups

Disease	Age groups (years)						Total (n=250)	
	< 2 (n=54)		2 - < 3 (n=83)		≥ 3 (n=113)			
	No.	%	No.	%	No.	%	No.	%
Brucellosis	5	9.3	8	9.3	18	15.9	31	12.4
Listeriosis	0	0.0	4	4.8	8	7.1	12	4.80
Salmonellosis	1	1.9	4	4.8	8	7.1	13	5.20

Table (6): Prevalence of diseases causing abortion in ewes in relation to breeds

Disease	Breeds						Total (n=250)	
	Rahmani (n=79)		Baladi (n=98)		Barki (n=73)			
	No.	%	No.	%	No.	%	No.	%
Brucellosis	12	15.2	13	13.3	6	8.2	31	12.4
Listeriosis	4	5.1	5	5.1	3	4.1	12	4.80
Salmonellosis	6	7.6	3	3.1	4	5.5	13	5.20

Table (6): Prevalence of diseases causing abortion in ewes in relation type of housing

Disease	Type of housing				Total	
	Opened (n=174)		Closed (n=76)			
	No.	%	No.	%	No.	%
Brucellosis	18	10.3	13	17.1	31	12.4
Listeriosis	5	2.9	7	9.2	12	4.80
Salmonellosis	7	4.0	6	7.9	13	5.20

Table (7): Prevalence of diseases causing abortion in ewes in relation water source

Disease	Water source				Total	
	Canal (n=54)		Pipes (n=196)			
	No.	%	No.	%	No.	%
Brucellosis	16	29.6	15	7.7	31	12.4
Listeriosis	12	22.2	15	7.7	27	10.8
Salmonellosis	9	16.7	4	2.0	13	5.20

Table (8): Prevalence of diseases causing abortion in ewes according to presence of separate kidding area

Disease	Separate kidding area				Total	
	Presence (n=44)		Absence (n=206)			
	No.	%	No.	%	No.	%
Brucellosis	8	18.2	23	11.2	31	12.4
Listeriosis	6	13.6	6	2.9	12	4.80
Salmonellosis	6	13.6	7	3.4	13	5.20

Table (9): Prevalence of diseases causing abortion in ewes according to application of disinfection procedures

Disease	Disinfection procedures				Total	
	Presence (n=34)		Absence (n=216)			
	No.	%	No.	%	No.	%
Brucellosis	2	5.9	29	13.4	31	12.4
Listeriosis	0	0.0	12	5.6	12	4.80
Salmonellosis	1	2.9	12	5.6	13	5.20

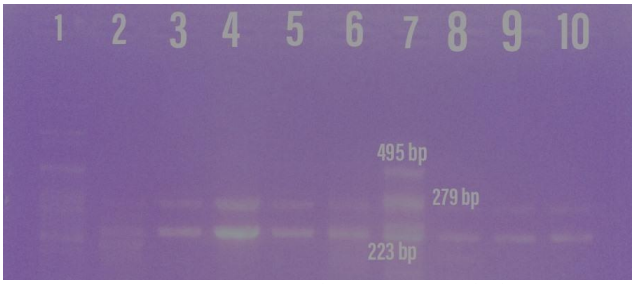

Photo (1): Conventional PCR products of bcsp31 gene specific for genus Brucella (223 bp), IS711element downstream of BMEI1162 (279 bp) specific for B. melitensis and IS711 element downstream of the alkB gene (495 bp) specific for B. abortus isolated from the whole blood samples of aborted ewes.
Lane 1: 50 bp molecular weight DNA ladder with a size range of 50-1500bp
Lane 2□10: positive Brucella genus specific for bcsp 31KDa gene.
Lane 2□6 and 8□10: Positive B. melitensis strains for BMEI1162 gene.
Lane 7: Positive B. abortus strains for alkB gene and B. melitensis strains for BMEI1162 gene (mixed infection).