Hygienic Studies on Microbial Causes of Abortion in Sheep
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ABSTRACT
A total of 250 samples of aborted foetii, 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 monocytogents, Salmonella spp., Escherichia coli, Leptospira, Staphylococcus aureus, Streptococci, Corynebacterium pyogenes and Chlamydia spp. (Kholeaf et al., 1977, Butachaliah and Khera, 1982, Bujmocy 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 (Butachaliah 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 monocytogents 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 melitensis 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 melitensis 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

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 identified 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 hsp31 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
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 fetus subsp. venerealis with the incidence of 11.4% and 7.1%, respectively in sheep. Also Listeria monocytogenes, 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 agree 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 10%. Redman et al., (1990) recorded that Campylobacter fetus subsp. fetus, Campylobacter fetus subsp. venerealis with an incidence of 31%. While Plagemann (1989) isolated Listeria monocytogenes, Salmonella typhimurium and Escherichia coli from aborted foeti 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 a 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. Also, H. I., Dawood, P. Z., and El-Elminy, M. S., (2018) Sero-diagnosis of brucellosis in sheep and humans in Assiut and El-Elminy governorsates, Egypt. International Journal of Veterinary Science and Medicine, 6, 63-67. Abubakar M., Arshed M.J., Hussain M., Elshimah-ul-Haq and Ali Q., (2010) Serological evidence of Brucella abortus prevalence in Punjab province, Pakistan-a cross-sectional study. Transbound Emerg Dis, 57, 443-447.

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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) |
|-------------|----------------------------------|---------------|
| bscp1, B. abortus spp. (F) | 5’ GCTCGGTTCAGCATACTGATGC 3’ | 223 (Zerva et al., 2001) |
| alkB, B. melitensis spp. (F) | 5’ CAGCGGTATGATCGTTACG 3’ | 279 (Mintal et al., 2007) |
| alkB, B. abortus spp. (R) | 5’ GCCGCTTCTATACGCTGATTCG 3’ | 495 (Song et al., 2019) |

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. % | |
| Listeria spp. | 9 | 3.6 | 5 | 2.0 | 20 | 9 | 7.6 |
| Salmonella spp. | 7 | 2.8 | 3 | 1.2 | 4 | 1.6 | 4 | 5.6 |
| Total | 16 | 6.4 | 3 | 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. % | 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 (7): Prevalence of diseases causing abortion in ewes in relation to type of housing

| Disease | Type of housing | Total (n=250) |
|---------|----------------|---------------|
| | Opened (n=174) | Closed (n=76) | |
| | No. % | No. % | No. % | No. % |
| Brucellosis | 18 | 10.3 | 17 | 11.1 | 31 | 12.4 |
| Listeriosis | 5 | 2.9 | 7 | 9.2 | 12 | 4.80 |
| Salmonellosis | 7 | 4.0 | 6 | 7.9 | 13 | 5.20 |

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

| Disease | Separate kidding area (n=44) | Absence (n=206) | Total (n=250) |
|---------|---------------------------|----------------|---------------|
| | 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 (n=216) |
|---------|-------------------------|---------------|
| | 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 bscp31 gene specific for genus Brucella (223 bp), IS711 element downstream of BMEI162 (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 Lanes 2/10: Positive Brucella genus specific for bscp31KDa gene. Lanes 2/6 and 8/10: Positive B. melitensis strains for BMEI162 gene. Lane 7: Positive B. abortus strains for alkB gene and B. melitensis strains for BMEI1162 gene (mixed infection).