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PREFERENCE OF PATHOGENIC BACTERIAL ASSOCIATED INFERTILITY IN SHEEP WITH SPECIAL REFERENCE TO CAMPYLOBACTER FOETUS AND ITS SENSITIVITY TO SOME ANTIMICROBIAL AGENTS

(With 7 Tables and One Figure)

By

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مدى تواجد البكتريا الممرضة المصاحبة للعقم فى الأغنام مع الأشارة الخاصة لميكروب كمبيلوبكتر فيتس وحساسيته لبعض المضادات الحيوية المختلفة

عبدالرحمن عبدالمجيد عبدالرحمن ، ضرار رفعت ابراهيم ، ايهاب محمود مجدى

استهدفت الدراسة إلى إلقاء الضوء على العدوى البكتيرية المسببة للعقم فى الأغنام وأجريت الدراسة على عدد 160 من النعاج (105 تعانى من الشياع المتكرر و 55 تعانى من اجهاضات) من مزارع أغنام فى محافظتي المنيا وأسيوط وقد أظهر الفحص البكتريولوجى للعينات المأخوذة من الرحم والأجنة المجهضة والدم الى عزل 16 عترة من ميكروب الكامبيلوباكتر بنسبة (10%) منها 12 عترة كامبيلوباكتر فيتس بنسبة (7,5%) و4 عترات كامبيلوباكتر جوجونى بنسبة (2,5%) وتم عزل 6 عترات من ميكروب البروسيلا من حالات الاجهاض فقط بنسبة (3,75%) كما تم عزل 15 عترة من ميكروب السلامونيلا بنسبة (9,4%) وكانت كالأتى 8 عترة سلامونيلا ابورتس بنسبة (5%) و5 عترات سلامونيلا دبلين بنسبة (3,1%) و2 عترة سلامونيلا انتريديز بنسبة (1,3%) كما اظهر الفحص البكتريولوجى لهذه العينات عن عزل 5 عترات من ميكروب الليستيريا مونوسيتوجينر بنسبة (3,1%) منها 4عترات من حالات الأجهاض بنسبة (7,2%) وعترة واحدة من حالات الشياع المتكرر بنسبة (95،%) واوضح اختبار الحساسية لميكروب كامبيلوباكتر فيتس الى حساسيته لكل من الكلورمنيفيكول والجنتاميسين وسيفروفلوكساسين واكسى تتراسيكلين ومقاومته لكل من البنسللين والنلاديكس اسيد الأيرثرومايسين وامبسللين هذا وقد تم مناقشة النتائج والأهمية الصحية للبكتريا المعزولة ومدى خطورتها على الصحة العامة.

SUMMERY

A total of 160 ewes were examined in present study having a history of infertility (55 abortions, and 105 repeat breeders). Samples for bacteriological examination including vaginal swabs, faetal fluid, liver and stomach content of aborted faeti, placenta and blood. All the isolates encountered in this investigation following use of culture on selective media, biochemical reaction and serological tests. A total of 16 (10%) isolates identified as being campylobacter spp. were recovered, of which 12 isolates from abortion cases with incidence of (21.8%) and 4 isolates from repeat breeder with incidence of (3.8%). Moreover 12 isolates out of the 16 isolates were identified as *C. fetus subsp fetus* with incidence of (7.5%) and 4 isolates as *C. jejuni* with incidence of (2.5%). The brucellae isolate was 6 (3.75%) from only aborted ewes The incidence of Ovine brucella by Rose Bengal plate test, Buffered acidified plate antigen test and Rivanol test were 3.75%, 3.125% and 2.5% respectvely. These strains were biotyped as Brucella melitensis biovar3. *Salmonellas spp* could be isolated from examined samples with percentage of (9.4%). Serotypeing of 15 isolates revealed 8 (5.0%) *S. abortus ovis* (O4, 12: c: 1, 6), 5 (3.1%) *S.dublin* (O1, 9, 12: g, p) and 2 (1.3) *S. enteriditis* (O1, 9, 12: g, m). Latex test was as a rapid serological test, revealed 12 isolates of 15 isolates were identified as Salmonella species with incidence of (80%). The overall incidence of *Listeria monocytogenes* isolated was 5 (3.1%). including 4 (7.2%) from abortion cases and 1 (0.95%) from repeat breeder case. Antibiotic sensitivity test for *Campylobacter fetus subsp fetus* was highly sensitive to Chloramephenical, Gentamycin, Ciprofloxacin and Oxytetracycline with incidence (90%), (80%), (70%) and (70%) respectively, while was resistant to Penicillin, Nalidixic acid, Erythromycin, and Ampcillin with incidence (100%), (100%), (80%) and (70%) respectively. The hygienic significant of the bacteria isolates were discussed.

Key words: *Ewes, abortion, repeat breeder, Campylobacter spp, Brucella spp. Listeria monocytogenes and Salmonella spp.*

INTRODUCTION

Many bacteria present in genital tract as saprophytes but under unfavorable conditions might become pathogenic and causes clinical or sub-clinical signs of endometeritis (AboEl-Ata, 1973). The role of specific aetiological agents such as *Brucella spp., Campylobacter spp., Leptospira spp., Listeria monocytogenes, Chlamydia psittaci, Coxiella burnetii, Mycoplasma bovigenitalium and Salmonella spp.* has been well

established, as the main causative of infertility in animals (Radostts *et al.*, 1995). *Listeria monocytogenes, Campylobacter fetus subsp. fetus, Yersinia pseudotuberculosis* and *Toxoplasma gondii* are probably representing the most common causes of abortion in sheep Jorgen *et al.* (2006).

Campylobacter fetus is divided into two subspecies: *Campylobacter. fetus subsp. venerealis and Campylobacter fetus subsp. fetus. Campylobacter. fetus subsp. fetus and C. jejuni* can cause late term abortions, stillbirths and weak lambs in sheep. Campylobacter is an inhabitant of the intestine of cattle and sheep and then invades the pregnant uterus of cows and ewes causing abortion (Laing, 1960). Campylobacteriosis is a true zoonotic disease occurring naturally in many domestic, laboratory and wild animals. It has been isolated from a variety of causes of abortion in domestic animals including sheep and goats (Anderson *et al.*, 1983). *Campylobacter* species are now recognized by veterinarians as cause of vibrionic infertility and abortion in cattle and sheep (Matin, 1989).

Brucella infection in farm animals is considered a great problem in most countries of the world, thus the early detection of brucella infection in a herd or flock is a pre-request for the successful control and elimination of one of the major problems considered to be a predisposing factor leading to infertility and sterility along with the possible transmission of infection to man (FAO/WHO, 1986 and Wasseif, 1992). Brucellosis still constitutes one of the major health problems in both man and animals in Egypt. It is the main cause of breeding failure and infertility of affected animals (EL-Gibaly, 1969; Shalaby, 1986 and Soliman, 1998). Ovine and caprine brucellosis still constitutes a serious problem in Egypt due to economic losses and their role in transmission of infection to cattle and buffaloes. Brucellosis in goats and sheep are manifested by abortion which occurs most frequently in the third or fourth month of pregnancy, arthritis and orchitis (Acha and Szyfres, 1991).

The accepted species of Brucella are *B. abortus* (biovars 1 to 9), *B. suis* (biovars 1 to 5), *B. melitensis* (biovars 1 to 3), B. ovis, B. canis and B. neotomae (Feketee *et al.*, 1992).

Brucellosis melitensis biovar3 is the prevalent strain in Egypt among sheep (EL-Gibaly, 1993).

Verma *et al.* (2000) isolated four *Brucella melitensis* biotype from 28 aborted ewes with incidence of (14.3%).

El-Hewairy *et al.* (2007) isolated Brucellosis melitensis biovar3 from five goats and two sheep by bacteriological examination, while could detected Brucellosis melitensis biovar3 from nine goats and eight sheep by RBPT and RIV tests

Salmonella abortus ovis causes a contagious infection disease with abortion in ewes accompanied with mortality of lambs (Jack, 1968, Pardon *et al.*, 1988 and Rubino *et al.*, 1993). *Salmonella dublin* can cause both enteritis and abortion in adult sheep, and the disease is often associated with metritis, anorexia, and loss of wool **(**Jack, 1971**).** *Salmonella abortus ovis* isolated in Egypt association with *S.typhinurium* and other untyped salmonella strains by of and Abdel-Ghani (1979).

Listeriosis is called Circling Disease or Silage Sickness, is a disease of worldwide occurrence that can affect all ruminants as well as other animal species and human. It is, therefore, of zoonotic importance. The causative agent is usually *Listeria monocytogenes*; however ruminants, mainly sheep (Finley and Dennis, 1999). In a survey of an outbreak of listeria abortion in ewes Dennis (1966) indicated that 20 (50%) out of 40 ewes, while, Low and Renton (1985) succeeded in detecting *listeria monocytogens* in an outbreak of abortion involving 59 out of 196 pregnant ewes. Hassanein (1994) isolated 2 positive cases of *listeria monocytogens* from 178 cases of sheep with incidence of (1.1%).

The present study focused on the pathogenic bacteria associated with cases of infertility in sheep through isolation and identification of these bacteria with special reference to Campylobacter fetus subsp. fetus infection and its sensitivity to some different antimicrobial agents.

MATERIALS and METHODS

1- Animals:

A total of 160 ewes out of 600 were used in present study having a history of infertility (55 abortion, and 105 repeat breeders) collected from private farms in El-Mini and Assiut Governorates, illustrated in Table (1).

2- Samples:

Samples for bacteriological examination including vaginal swabs, faetal fluid, liver and stomach content of aborted faeti, placenta and blood. All samples were transmitted in an ice box to laboratory as soon as possible**.**

A- Vaginal swabs

Samples may be obtained by swabbing and or by washing the vaginal cavity. After cleaning the vulval region, the vaginal cavity is washed by infusing 10–20 ml of sterile phosphate buffered saline (PBS) into the cavity through a syringe attached to a sterile catheter. The fluid is sucked out and reinfused four to five times before being collected in a sterile flask containing enrichment medium.

B- Aborted fetuses and placentas:-

Specimens of the placenta of aborted ewes, stomach contents, lungs and liver of the fetus were taken under aseptic conditions and sent to the laboratory in a cooled insulated container (at 4–8°C). All samples should be inoculated directly on to broth media and then culture on selective medium.

C- Blood samples:

About of 10ml of whole blood was obtained in sterile test tubes. These samples were kept over night at 4°C to allow separation of serum, then removed by Pasteur pipette and centrifuged at 3000 rpm for 10 minutes. The clear serum was transferred into test tubes for each samples and labeled unless immediately used and stored at -20°C in deep freezer.

Isolation and identification of Campylobacter fetus subsp. fetus and *C.* jejuni according to (Bates, 1981):

Dark field and phase contrast preparations of samples from the placenta, fetal abomasum and uterine discharge appeared as an "S" shape with one or two spirals. Each sample is inoculated directly on to a selective medium Campylobacter selective medium (Skirrow, 1977) and Neomycin blood agar medium (neomycin sulphate solution) was added to the media just before the additions of blood to make final concentration of 150ug/ml. The plates are incubated at 25°C, 37°C and 42°C for 2-5 days by using (Gas-pack anaerobic jar "BBL-814-12"). It was used for production of anaerobiosis by using disposable hydrogen-carbon dioxide bags with socket. Microaerobic atmospheres of 5–10% oxygen, 5–10% carbon dioxide (and preferably 5–9% hydrogen) were provided (Baker platinum LTD, London). Colonies are slightly grey-pink, round, convex, smooth and shiny with a regular edge.

The organism was identified by their morphology. Culture smears were made and stained with Gram's and diluted Carbol fuchins. Motility of the organism was examined by the hanging-drop method. Several biochemical tests were applied according to (Konemann *et al.*, 1992 and Levett 1991). Catalase test, oxidase test, nitrate reduction, H_2S production test on TSI media and by using lead acetate. Catalase activity was checked by placing a drop of H_2O_2 on a slide and a loopful of the

isolate emulsified in it. The presence of gas bubbles within 10 seconds was considered as catalase- positive.

Growth temperature was checked by incubation of one plate of selective medium and one plate of blood agar at 25°C and another plate of each media at 42°C for 3 days. The plates were then checked for growth (Campylobacter fetus subsp. fetus does not grow at 42°C).

Isolation and identification of Brucella spp.

1- Culture:

Isolation, identification and typing of isolated Brucella strains were done according to the methods recommended by Alton *et al.* (1988). Samples were direct cultured on the surface of two plates of Brucella selective medium (Difco Laboratories, Detroit, Michigan, USA) at 37°C by using (Gas-pack anaerobic jar "BBL-814-12"). It was used for production of an atmosphere of 10% CO_2 tension automatically. All culture plates were examined for brucella growth at 4^{th} day and daily for 10 days till 14 days. Suspected colonies were further identified and sub cultured on brucella agar slopes.

Identification of Brucella isolated was according to morphological characters, microscopically examination and reaction with positive sera. For microscopical examination using a smear on a clean air-dried slide, heat fixed, stained with Modified Ziehl-Neelsen stain and examined for the presence of Brucella-coccobacilli.They were also biochemically characterized as described by Farrell (1974), Buchanon and Gibbons (1974), Morgan, *et al.* (1978), Alton *et al.* (1988) and Carter (1995).

2- Serological tests for brucellosis:

a- Buffered acidified plate antigen test (BAPAT): The test was carried out according to Alton *et al.* (1988)

b- Rose Bengal plate test (RBPT): The test was carried out according to Morgan *et al.* (1978)

c - Rivanol test (Riv.T): The test was carried out according to Alton *et al.* (1988)

Antigens:

Antigen for standard Buffered acidified plate antigen test (BAPAT), Rose Bengal plate test (RBPT) and Rivanol test (Riv.T) were obtained from Veterinary Vaccines and Sera Research Institute, Abbasya, Cairo, Egypt.

Isolation and identification of Listeria spp.:

Selective enrichment method.

This method recommended by Curtis *et al.* (1989).

Samples of aborted foeti were macerated and thoroughly homogenized under aseptic conditions with sterile distilled water. One ml of the homogenized specimens was immersed in 9ml of listeria enrichment broth and incubated at 30°C for 48 hours and cultured on to plates of listeria aesculin selective agar. Colonies were picked up and purified by subculturing on same selective media, then kept on nutrient agar sloops for identification on basis of microscopical appearance, culture character, motility, biochemical and serological tests.

Isolation and identification of Salmonella:

1- Culture

A loopful from each sample was streaked on to MacConkey' s agar plates and S.S agar incubated at 37°C for 24-48 hours. Different colonies were picked up and purified by subculturing on selective media, then kept on nutrient agar sloops for identification of microscopical appearance, culture character, motility, biochemical identification according to (Edward and Ewing, 1972, Buchanan and Gibons, 1974 Cruickshnk *et al.*, 1982, Speck, 1984 and Wilson and Miles, 1985).

2- Latex test:

The suspected colonies were subjected to latex test according to Mitrutina and Tendetnik (1994) as rapid test for diagnosis and confirm that the isolated colonies were salmonellae

3- Serological identification of Salmonella

Typing of *Salmonella* isolates was applied to identify the serovars of the isolated colonies using polyvalent and monvalent antisera. "O" and "H" antigens as well as the phase of the organism were detected by using Agglutination sera test according to the modified Kauffmanns and White scheme described by (Mcwhorter *et al.,* 1977, Baily and Scott 1990, Brenner 1994 and Quinn *et al.*, 1994). Suspected culture was mixed thoroughly with a drop of saline on clean slide. A small drop of polyvalent Salmonella antisera was mixed thoroughly with the bacteria suspension by tilting the slide for one minute. Positive agglutination was recognized by formation of fine granules or large aggregate, delayed or partial agglutination was considered as negative or false agglutination. Cultures which gave positive results were similarly tested using monovalent group for determination of specific "O" antigen and within group of "H" antigen both phase, 1 and phase 11. The sera used were purchased from Wellcome Research Laboratories Beckengham, England. The serological tests were carried out in Ministry of health laboratories in Cairo as well as Serology Unit, Animal Health Institute, Giza, Egypt

Sensitivity test for Campylobacter fetus subsp. fetus

Determination of the antibiogram of *Campylobacter fetus subsp. fetus* to antimicrobial agent, it was carried according to (Lannette *et al.*, 1985 and Perelman *et al.*, 1991). Ten different antibiotic discs, supplied by Oxoid LTD, London, England, (Oxoid Manual, 1982). These antibiotics were Penicillin (1.5 I.U) Nalidixic acid, Streptomycin (10ug) Ciprofloxacin (5ug), Chloramephenical (30ug), Oxytetracycline (30ug), Erythromycin, Gentamicin, Amoxicillin (10ug) and Ampicillin. One ml of 24hr. broth cultures was spread on the surface of blood agar. Antibiotic sensitivity discs were placed on the surface seeded agar. Plates were incubated anaerobically at 37°C for 24hr. The sensitivity was judged according to the diameter of clearance zone around the discs

RESULTS

Table 1: Number and percentage of examined ewes.

Total No. of studied ewes	Healthy status	No. of infected cases	
		No.	%
600	Abortion	55	9.2
	Repeat breeder	105	17.5
	Total	160	26.7

Table 2: Biochemical characteristics of isolated Campylobacter strains.

Characteristics	*C. fetus subsp fetus*	*C. jejuni*
Catalase reaction	+	+
Oxidase	+	+
Motility	+	+
H2S production		
TSI	-	+
Lead acetate	+	+
Growth		
at 25 ^{0}C	+	-
at 37^{0}C	+	+
at 42^{0}C	-	+
Growth in:		
3.5%NaCL	-	-
1% glycine	+	+

Table 3: Type and percentage of different bacterial isolates from 160 ewes (55 abortions and 105 repeat breeders).

Isolates	Positive	Healthy status

	isolates		Abortion (55)		Repeat breeder (105)	
	No.	%	No.	%	No.	%
Campylobacter spp.	16	10	12	21.8	4	3.8
C. fetus subsp fetus	12	7.5	10	18.2	2	1.9
C. jejuni	4	2.5	2	3.6	2	1.9
Brucella melitensis biovar3	6	3.75	6	10.9	0	0.0
listeria monocytogens	5	3.1	4	7.2	1	0.95
Salmonella spp.	15	9.4	11	20.0	4	3.8
S. abortus ovis	8	5.0	6	14.5	2	1.9
S.dublin	5	3.1	3	5.4	2	1.9
S. enteriditis	2	1.3	2	0.0	0	0.0
Total	42	26.3	33	54.5	9	8.6

Percentage of positive isolates calculated according to the total number of samples
Percentage of each isolates was calculated according to the type of healthy status

Table 4: Serological tests used for detection of *Brucella melitensis* biovar3.

No of examined samples	Serological tests								
	RBPT		BAPAT		Rivanol test				
160	+ve	%	+ve	%	1/25	1/50	1/100	1/200	1/400
	6	3.75	5	3.125	-	1	1	1	1

RBPT = Rose Bengal plate test
BAPAT= Buffered acidified plate antigen test

Table 5: Result of Latex test for *Salmonella.*

No. of samples	No. of isolates	Latex test			
		Positive		Negative	
		No.	%	No.	%
160	15	12	80	3	20

Percentage of positive case was calculated according to the number of *Salmonella* isolates

Table 6: Serotyping of *Salmonella* isolates.

Source of samples	No. of cases	No. of isolates		*S.abortus ovis*		*S.dublin*		*S. enteriditis*	
		No.	%	No.	%	No.	%	No.	%
Abortion	55	11	20	8	14.5	3	5.4	0	0.0
Repeat breeder	105	4	3.8	0	0.0	2	1.9	2	1.9
Total	160	15	9.4	8	5.0	5	3.1	2	1.2

Percentage of each strain was calculated according to the number of each case

Table 7: Antibiotic sensitivity test for Campylobacter fetus subsp. fetus isolated.

Antibacterial agent	*Campylobacter fetus subsp fetus*. (n=10)					
	S		I		R	
	No.	%	No.	%	No.	%
Penicillin (1.5 I.U)	0	0.0	0	0.0	10	100
Ampcillin(10ug)	2	20	1	10	7	70.0
Streptomycin(10ug)	4	40	5	50	1	10
Erythromycin (15ug)	0	0.0	2	20.0	8	80.0
Amoxicillin(10ug)	3	30	2	20	5	50
Ciprofloxacin (10ug).	7	70	2	20	1	10
Chloramephenical (30ug)	9	90	1	10	0	0.0
Oxytetracycline(30ug)	7	70	2	20	1	10
Gentamycin (10ug)	8	80	0	0.0	2	20
Nalidixic acid (30ug)	0	0.0	0	0.0	10	100

+++ = Highly sensitivity, ++ = Intermediate sensitivity R = - Resistance

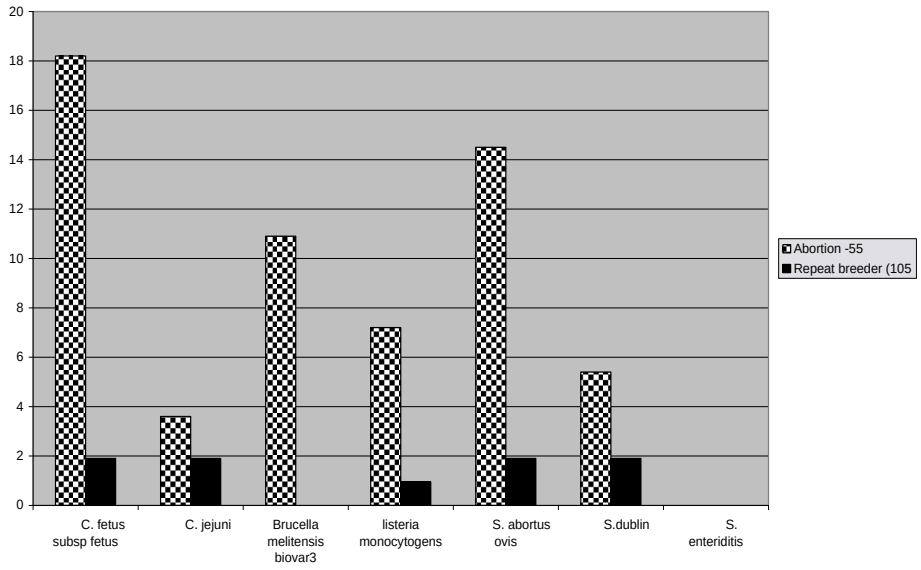

Fig. 1: Percentage of bacterial isolate from abortion and repeat breeder cases

DISCUSSION

Bacterial pathogens were the most prevalent cause of abortion. Several of the abortifacients were zoonotic microorganisms, for example *Listeria monocytogenes, Campylobacter fetus subsp. fetus, Yersinia pseudotuberculosis* and *Toxoplasma gondii*. The identified

microorganisms probably represent the most common causes of abortion in Danish sheep (Agerholm *et al.,* 2006).

The present work, as an investigation trial focused on bacteria causing infertility in sheep. 160 ewes out of 600 were show clinical sings. Out of these, 55 suffered from abortions with a percentage of (9.2%) and 105 suffered from repeat breeding with a percentage of (17.5%) as in table (1)

Campylobacter fetus subsp. fetus and Campylobacter *jejuni*

There are several species within the Campylobacter genus (C.fetus and C.jejuni) that are considered pathogenic to both human and animals (Vargas *et al.,* 2003). C. fetus is divided into subspecies and may be found in the genital tract causing infertility or sporadic abortion (Maclaren and Agumbah, 1988). Infection with Campylobacter fetus subsp. fetus and Campylobacter *jejuni* results in abortions in late pregnancy or stillbirths. Identification of the species involved is important because in some areas, *C jejuni* is as common as *C fetus*, all the isolates encountered in this investigation detected using of culture on selective media, biochemical reaction and serological tests. From direct smears, it appears as an "S" with one, two or several spirals. The isolates were positive to catalase reaction, oxidase, motility, growth at 25°C and 37°C but not growth on 42°C, production of H_2S on lead acetate but not on TSI (Table, 2). These results agreed with Eaglesome and Garcia (1992), Taylor (1992), Elham (2000), Vargas *et al.* (2003) and Mona and Hassan (2004). A total of 16 (10%) isolates identified as being campylobacter were recovered, of which 12 isolates from abortion cases with incidence of (21.8%) and 4 isolate from repeat breeder with incidence of (3.8%). Moreover 12 isolates out of the 16 isolates were identified as *C. fetus subsp fetus* with incidence of (7.5%) and 4 isolates as *C. jejuni* with incidence of (2.5%) Table (3). These results are nearly in agreement with those recorded by Elham (2000) but differed in the incidence of the species, where isolated *Compylobacter. fetus* and *Compylobacter jejuni* were 3.3% and 8.3% respectively. Mona and Hassan (2004) recovered 13 isolates of campylobacter species from aborted foeti and vaginal mucous of farm animals included 6 bovine origin, 2 of ovine origin, 2 from caprine origin and 3 from camels in agreement with results of the present study.

Brucellosis

Control of brucellosis depend on the use of efficient sero-diagnostic tests. However, no single test is capabale of identifying all positive cases (Morgan *et al.,* 1978). Ovine and caprine brucellosis still

constitutes a serious problem in Egypt due to economic losses and their role in transmission of infection to cattle and buffaloes. Brucellosis in goats and sheep are manifested by abortion which occurs most frequently in the third or fourth month of pregnancy, arthritis and orchitis (Acha and Szyfres, 1991). Isolates of brucellae encountered were small, non-motile, non-sporing, Gram-negative bacilli or coccobacilli appear single, but may be in pairs or small groups as described by Buchanon and Gibbons (1974) and Carter (1995). In the present study, the incidence of brucellae isolates was 6 (3.75%) from only aborted ewes (Table, 3). This results are agreement with Moorthy and Singh (1982) who isolated brucellae from ewes with incidence of (3.2%), while higher than that recorded by Verma *et al.* (2000) who isolated brucellae from ewes with incidence of (2.45%). Bassiony and Ibrahim (1997), Samaha *et al.* (2002), Ding (1993) and Revviriego *et al.* (2000) reported similar results but lower than that reported by Mosalam (1993) who isolated *Brucella melitensis biovar3* with incidence of (22.22%) from aborted foeti of sheep. Dawood (1977) isolated Brucella melitensis biovar3 from sheep and goats with incidence of (20.63%) and (19.23%) respectively. The serological tests of ovine Brucella by Rose Bengal plate test, Buffered acidified plate antigen test and Rivanol test were 3.75%, 3.125% and 2.5% respectively (Table, 4). The difference in the incidence of infection detected by three serological tests is probably due to the difference in the sensitivity of these tests and /or the vaccination status of tested animals. These strains were biotyped as *Brucella melitensis biovar3*. These results are higher than obtained by Montasser (1999) who isolated *Brucellosis melitensis* biovar3 from sheep by using serological tests, CFT, SAT, BAPAT, RBPT and Rivonol test with incidence of 1**.**18%, 1.3%, 1.61%, o99% and 0.95%, respectively, while lower than that reported by Shalaby *et al.* (2003) who isolated *Brucella melitensis* type 3 from sheep with incidence 9.8% by using Rose Bengal Plate Test (RBPT) as a rapid screening quantitative test and 8.06% by using Rivanol test (Riv.T). In accordance, El-Hewairy *et al.* (2007) detected *Brucellosis melitensis* biovar3 from sheep by using RBPT, STAT and RIV test with incidence of 5.3%, 3,3% and 5.3%, respectively**.**

***Salmonellas* spp**

Isolates of *Salmonella* were non-lactose fermented with H_2S on MacConkey agar and S.S agar, haemolytic on blood agar, gram negative and short rods. The isolates were urease and oxidase negative, released H_2S on triple sugar iron (TSI). These results were in agreement with

Engler (1988), Brenner (1994) and Turutoglu *et al.* (2000). Data presented in Table (3) revealed that *Salmonellas* spp could be isolated from examined samples with percentage of 15 (9.4%). Latex test was as a rapid serological test; revealed 12 isolates of 15 isolates were identified as salmonella species with incidence of 80% (Table, 5). These results are in agreement with Pardon *et al.* (1988) and Bourgogne, (1998) and lower than that obtained by Soumaya *et al.* (2004) who isolated 252 serotypes of *Salmonella* from 377 different samples obtained from aborted ewes, fetuses with incidence of 66.8% belonged to *S. abortus ovis* (92%), *S.dublin* (4%) and *S. enteriditis* (0.8%). Latex test is a rapid serological test, where 92.8% of 252 isolates were identified as *Salmonella* species, Sereotyping of 15 isolates revealed 8 (5.0%) *S. abortus ovis* (O4, 12: c: 1, 6), 5 (3.1%) *S.dublin* (O1, 9, 12: g, p) and 2 (1.3) *S. enteriditis* (O1, 9, 12: g, m) as in Table (6).

Listeria monocytogenes

Microscopical examination of samples Listeria appears as small, Gram-positive rods, nonperforming, catalase-positive, which are sometimes arranged in short chains. In direct smears they were coccoid, Flagella are produced at room temperature but not at 37°C. Hemolytic activity on blood agar has been used as a marker to distinguish *Listeria monocytogenes* among other *Listeria species*. This is in agreement with George (2002). Infection by *L. monocytogenes* has been reported to be increasing in incidence and may be as high as 52% in farm animals (Rebhun, 1995). The present study recorded that the overall incidenc of *Listeria monocytogenes* was 5 (3.1%) including 4 (7.2%) from abortion cases and 1 (0.95%) from repeat breeder cases (Table, 3). These results higher than that obtained by Fedio and Jackson (1992) who estimated an attack rate of 0.4%, Lida *et al*. (1991) and Hassanein, (1994) reported similar results while lower than obtained by Dennis (1966) and Low and Renton (1985).

Antimicrobial sensitivity test: -

The extensive use of antibiotics as growth promoters and prophylactic agents for disease control in veterinary medicine has undoubtedly been responsible for large numbers of bacteria that have become resistant to different antibiotics. Results of the antibiotic susceptibility pattern of *Campylobacte fetus subsp fetus* are clearly shown in Table (7). *Campylobacter fetus subsp fetus* were highly sensitive to, Chloramephenical (90%), Gentamycin (80%), Ciprofloxacin (70%) and Oxytetracycline (70%). while was resistant to Penicillin (100%), Nalidixic acid (100%), Erythromycin (80%), and

Ampcillin (70%). These results are in agreement with those of many authors. Vicek and Savobodova (1985) reported that the bacterial isolates from cases of repeat breeder were susceptible to Oxytetracycline and Chloramphenica. Also Ramakrishna (1996) found that, bacterial isolates from repeat breeder cows were sensitive to Gentamycin (89.1%). Elham (2000) found that *Campylobacter fetus subsp fetus* isolated from aborted fetus and cervical swabs of ewes was sensitive to Gentamycin and sulfamethoxazol while resistance to Ampicillin, Nalidixic acid, and Erythromycin. Metwelly (2001) recorded that, the in-vitro antimicrobial susceptibly of bacterial isolates from cows with endometeritis were Enrofluxacin, Oxytetracycline, Gentamycin and Ampicillin with incidence of 96.0, 89.0, 85.0 and 85.0%, respectively. Karwani and Aulakh (2004) reported that, out of total 155 isolates from repeat breeder cattle and buffaloes maximum isolates 146 (94%) were found sensitive to Ciprofloxacin followed by Gentamicin 115 (74%) and Chloramphenicol (67%). Also these results are in agreement with that obtained by Hassab El-Naby and El-Ekhnay (2004) who mentioned that bacteria causing repeat breeding in cattle and buffaloes, were more sensitive to Enerofloxacin, Gentamicin and Chloramphenicol. These results are nearly similar to those obtained by Awad *et al.* (1977) and Megahed (1986**)** who recorded that the bacterial isolates were resistant to Erythromycin and Penicillin. Refaat (1980) reported that the bacterial isolates from buffaloe-cows suffering from repeat breedingr were moderately sensitive to Erythromycin and Karwani and Aulakh (2004) who found that isolates from repeat breeder cattle and buffaloes showed resistance to Penicillin, Ampicillin, Neomycin and Naledixic acid with varying degree of drug resistance.

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