

## MICROBIOLOGICAL STUDIES ON ENDOMETRITIS IN FARM ANIMALS

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### SUMMARY

The present study deals with the bacterial causes of endometritis in farm animals. The most prevalence isolated organisms were; *E. coli* (35%) in cows, (60%) in ewes and (50%) in goats, *Klebsiella oxytoca* (30%) in cows, *S. aureus* (25%) in cows, (50%) in ewes and (30%) in goats. *S. pyogenes* (30%) in cows, (40%) in ewes and (20%) in goats. Other organisms were e.g *Pseudomonas aeruginosa*, *Salmonella spp.*, which isolated in descending percentages. Serological identification of *E. coli* isolates revealed "11" different serogroup and 2 strains were untypable. *Pseudomonas aeruginosa* were typed into 6 serogroups and 5 strains were untypable also *Salmonella spp.* were serotyped to 4 serovars.

Antibiogram of the most prevalent isolated from endometritis were done against 11 different

antibiotics. Moreover, the antiseptic effect of betadin and potassium permanganate. on the isolated organisms were recorded and the results described in details.

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### INTRODUCTION

Cattle and sheep are an important domestic animals for meat production in Egypt. There are a great demand to throw more light on these animals specially their reproductive capacity and related problems. Endometritis due to bacterial infection is the most common cause of infertility in bovine and ovine (Coleman et al., 1985; Borsberry and Dobson, 1989; Hussein et al., 1993 and Katoch et al., 1997).

Endometritis may be responsible for early embryonic death or repeat breeding problem are

mostly caused by bacterial infection (Abo El-Ata, 1973 and Krishan Murthy et al., 1974, Abd El-Rahiem et al., 1988 and Blanch et al., 1992).

The use of different antibiotics and disinfectants in the treatment of endometritis and evaluation of its effect to different isolates has been studied by several authors (Pateria, 1992; Hussein et al., 1993 and Thurmond et al., 1994).

The aim of this work was to study the of bacterial isolates from uteri of apparently healthy and diseased cows, ewes and goats. Also complete identification of the isolated strains biochemically and serologically specially the most prevalence pathogenic organisms and studying the effect of different antibiotics against the most pathogenic bacteria to choose the most effective drug, also study the action of some disinfectants used in the field as betadine and potassium permanganate.

## MATERIAL AND METHODS

A total of 80 samples from cows, 40 samples from each of ewes and goats uteri were investigated during the present study including 60 cases suffering from different stages of endometritis of each cow, ewes and goats. While 40 samples from apparently healthy uteri (20 samples from cow uteri and 10 samples from ewe uteri and 10 from goat uteri). They were obtained from Giza Abattoirs. The surface of uterus was touched by hot spatula and opened under complete aseptic pre-

caution then a loopfull of uterine content was directly cultured onto blood agar, MacConkey's agar. Other loopfull is directly transferred to pre-enrichment fluid media i.e selenite broth (Oxoid) and incubated at 37°C for not more than 18 hours and then an inoculum was cultivated on differential selective media including MacConkey's agar, and S. S. agar media.

All inoculated were incubated plates aerobically at 37°C for 24 hours. The suspected colonies were examined culturally, morphologically as well biochemically as described by Carter (1984) and Collins and Cummins (1986).

### Serological identification

- A) Serological identification of *Salmonella* using O-antisera were employed in the identification of somatic antigen by slide agglutination technique, while bacto-salmonella H. antisera were employed in the identification of Flagellar-H-antigen by the tube agglutination technique (Difco).
- B) Polyvalent and monovalent *E. coli* antisera (Behring) antisera used were 8 polyvalent antisera and (43) monovalent antisera.
- C) Isolated strains of *Pseudomonas aeruginosa* were serotyped using *Pseudomonas* antisera for somatic antigen "O" according to Srinivasan (1977) and Homma (1982). Antisera used were 3 polyvalent (I, II, III) and (14) monovalent antisera (A to N groups) obtained from "Denka Seiken" Laboratory.

### In vitro drug sensitivity test

In vitro drug sensitivity test of all the bacterial isolates was done by employing the standard disc technique according to Cruikshank et al. (1975) against (11) different chemotherapeutic agents on Mueller Hinton agar plates and the zone of inhibition was measured in millimeter.

Betadin product of Nile Therapeutic Company was used at concentrations of 4%, 10%, 20% while potassium permanganate was used at different concentrations of 0.1%, 0.2%, 0.5%. They were used as antiseptic solution against most pathogenic bacterial isolated from cases of endometritis of farm animals in vitro 24 hours. Bacterial

broth cultures containing  $3 \times 10^8$  bacterial/ml were prepared via matching by MacFerland opacity tube and addition of different concentration of each disinfectant to each tube of bacterial culture and cultured onto MacConkey's agar (Gram -ve bacteria) or onto nutrient agar for Gram +ve bacteria and *Pseudomonas aeruginosa*.

### RESULTS

The bacteriological finding in examination of apparently healthy uteri of cattle, ewes and goats were revealed (11) different types of microorganisms as shown in table (1).

Table (1): Most prevalence microbes isolated from apparently healthy uteri of cattle, ewes and goats.

Species of bacteria	Cattle (20)*		Ewes (10)*		Goats (10)*	
	No.	%	No.	%	No.	%
<i>Escherichia coli</i>	5	25	3	30	3	30
<i>Klebsiella oxytoca</i>	4	20	2	20	1	10
<i>Staph. aureus</i> 4	4	20	2	20	3	30
<i>Citrobacter freundii</i>	3	15	1	10	1	10
<i>Pseudomonas aeruginosa</i>	2	10	3	30	3	30
<i>Proteus mirabilis</i>	2	10	2	20	3	30
Non haemolytic Strept.	2	10	3	30	1	10
<i>Proteus vulgaris</i>	2	10	1	10	1	10
<i>Providencia alacifaciens</i>	2	10	-	-	-	-
<i>Serratia spp.</i>	2	10	-	-	-	-
<i>Providencia rettgeri</i>	1	5	-	-	-	-

\* Number of examined cases.

The results of the present investigation as shown in table (2) illustrated the presence of many bacterial pathogens among endometritis of infected cattle, ewes and goats showed high rate of isolation. The most predominant facultative anaerobic organisms were *E. coli*, *Staph. aureus*, *Klebsiella oxytoca*, *Strept. pyogenes*, *P. aeruginosa*, *Citrobacter freundii*, *Salmonella haemolytic* *Strept. A. pyogenes*, *untyped Corynebacterium*, *Serratia*, *Listeria monocytogenes* in an incidence of (31.7), (21.5%), (16.6%), (10%), (9.9%), (8.3%), (4.9%), (3.3%) and (1.6%) of each of the remaining strains.

On the other hand, *Salmonella species*, *L. monocytogenes*, *C. freundii*, *E. coli* combined with *S. aureus* or *E. coli* with unidentified *Corynebacterium*, *S. aureus* combined with *Strept. pyogenes*, *Proteus mirabilis* and *Serratia* also recovered, also represented the less frequent percentages.

Bacteriological examination of uteri of ewes and goats are shown in table (3). It was clear that more than one organism were isolated from each uterus. There are 8 different types of microorganism were recovered from different

Table (2): Most prevalence microbes isolated from endometritis in cattle.

Species of bacteria	Endometritis* (60)						Total incidence
	Catarrhal		Chronic		Pyometra		
	No.	incidence	No.	incidence	No.	incidence	
<i>Escherichia coli</i>	7	11.7	6	10	6	10	31.7
<i>Klebsiella oxytoca</i>	6	10	4	6.6	-	-	16.6
<i>Staph. aureus</i>	4	6.6	5	8.3	4	6.6	21.5
<i>Citrobacter freundii</i>	3	5	2	3.3	-	-	8.3
<i>Pseudomonas aeruginosa</i>	-	-	2	3.3	4	6.6	9.9
<i>Strept. pyogenes</i>	-	-	-	-	6	10	10
<i>Salmonella</i>	-	-	1	1.6	2	3.3	4.9
<i>Haemolytic Strept.</i>	-	-	2	3.3	-	-	3.3
<i>A. pyogenes</i>	-	-	-	-	1	1.6	1.6
<i>Untyped Corynebacterium</i>	-	-	1	1.6	-	-	1.6
<i>Serratia</i>	-	-	1	1.6	-	-	1.6
<i>Listeria monocytogenes</i>	-	-	1	1.6	-	-	1.6
Rate of isolation	-	33.3	-	41.2	-	38.1	-

\* Number of examined cases.

Incidence calculated according to number of examined cases.

Table (3): Most prevalence microbes isolated from endometritis in ewes and goats.

Species of bacteria	Ewes (30)*				Goats (30)*				Total incidence		
	Catarrhal No.	Chronic incidence	Pyometra No.	Pyometra incidence	Catarrhal No.	Chronic incidence	Pyometra No.	Pyometra incidence			
<i>Escherichia coli</i>	6	20	4	13.3	5	16.6	1	3.3	2	6.6	26.5
<i>Staph. aureus</i>	3	10	2	6.6	3	10	1	3.3	2	6.6	19.9
<i>Strept. pyogenes</i>	1	3.3	2	6.6	2	6.6	1	3.3	3	10	16.5
<i>Actinomyces pyogenes</i>	-	-	-	-	1	3.3	-	-	2	6.6	9.9
<i>Klebsiella oxytoca</i>	2	6.6	-	-	1	3.3	-	-	-	-	3.3
<i>Salmonella</i>	1	3.3	-	-	1	3.3	1	3.3	-	-	6.6
<i>Pseudomonas aeruginosa</i>	3	10	-	-	2	6.6	-	-	3	10	16.6
<i>Haemolytic Strept.</i>	2	6.6	-	-	1	3.3	-	-	-	-	3.3
Rate of isolation		59.8		26.5		59.7		53		13.2	39.8

\* Number of examined cases.

Table (4): Serological identification of *E. coli*, *Pseudomonas aeruginosa* and *Salmonella serovars*.

<i>Escherichia coli</i>		<i>P. aeruginosa</i>		<i>Salmonella serovars</i>	
Serogroup	No.	Serogroup	No.	Serogroup	No.
O125	10	K	5		
O167	7				
O1	6	M	4	<i>S. typhimurium</i>	4
O86	5				
O151	5	N	3		
O6	5			<i>S. saintpauli</i>	1
O111	5	G	3		
O126	4				
O157	2	J	2	<i>S. abortus ovis</i>	1
O153	1				
O119	1	N	2		
Untyped	2	Untyped	5		
Total	53	--	24		6

Table (5): In vitro drug sensitivity pattern of the bacterial isolated from uteri of cattle, ewes and goats.

Name of Antibiotics	Conc (mg)	<i>E. coli</i>	<i>Staph.aureus</i>	<i>Strept.pyogenes</i>	<i>Salmonella serovars</i>	<i>Non H.Strept.</i>	<i>P. aeruginosa</i>
Enrofloxacin	5	S	S	S	S	S	S
Oxalimicacid	2	R	S	S	R	S	R
GentamicinI	10	S	S	S	R	R	S
Streptomycin	15	R	R	R	R	R	R
Erythromycine	25	R	R	R	R	R	R
Amoxycillin	30	R	R	R	S	R	R
Oxytetracyclin	30	R	S	R	S	S	R
Nalidixicacid	30	R	S	S	R	S	R
Cefadroxil	30	R	R	R	R	R	R
Chloramphenicol	30	R	R	S	S	R	R
Colistin sulphate	50	R	R	R	R	R	S

Table (6): Effect of disinfectants on most pathogenic isolated organisms in vitro.

Disinfectant	Conc	Time	<i>P. aeruginosa</i>	<i>Staph. aureus</i>	<i>Strept pyogenes</i>	<i>E. coli O157</i>	<i>E. coli 78</i>	<i>E. coli O111</i>	<i>S. typhimurium</i>
Betadine	4%	2h	+ve	-ve	-ve	-ve	+ve	+ve	+ve
	10%	2h	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	20%	2h	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Potassium permanganate	0.1%	2h	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	0.2%	2h	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	0.5%	2h	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Betadine	4%	24h	+ve	-ve	-ve	-ve	+ve	+ve	+ve
	10%	24h	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	20%	24h	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Potassium permanganate	0.1%	24h	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	0.2%	24h	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	0.5	24h	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Betadine	4%	28h	+ve	-ve	-ve	-ve	+ve	+ve	+ve
	10%	28h	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	20% <sup>4</sup>	28h	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Potassium permanganate	0.1%	28h	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	0.2%	28h	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	0.5%	28h	-ve	-ve	-ve	-ve	-ve	-ve	+ve

N. B.: Salmonella killed after 72h with Pot. permanganate.

+ve: Bacterial growth.

-ve: No bacterial growth.

degree of endometritis. The most prevalent organisms were *E. coli*, *S. aureus* in a percentage of 50% in ewes and goats, *Strept pyogenes* isolated in a percentage of 40% from ewes and 30% from infected uteri of goats, *A. pyogenes*, *Klebsiella oxytoca*, *Salmonella* serovars., *Pseudomonas aeruginosa*, *Haemolytic Strept.* were isolated in descending percent.

Serological typing of (53) strains of *E. coli*, they proved to be belonging to 11 different serogroups in addition to 2 strains which were untypable as illustrated in table (4), also serological identical

of (24) strains *Pseudomonas aeruginosa* revealed 6 serogroups and 5 strains were untyped. Serotyping of *Salmonella Spp.* revealed 3 serovars namely, *S. typhimurium* (4), *S. Saintpaul* (1) and *S. abortus ovis* (1).

Antibiograms using some isolated strains from the predominant bacteria are shown in table (5).

The result of using Betadine and potassium permanganate as antiseptic against most pathogenic strains isolated from endometritis are shown in table (6).

## DISCUSSION

In the present investigation, it is appeared that the diseased genitalia of (cattle, sheep, goat) were classified to 3 degrees of endometritis, (Catarrhal, chronic, pyometra). Similar findings were reported by many authors in cows (Roberts, 1971; Miller et al., 1980 and Abo El-Ata, 1973) in cows and buffaloes.

In this study , 11 different types of bacteria were detected from 20 samples of normal uteri of cattles and 20 samples from normal uteri of sheep and goats. These bacteria were *E. coli* 25% in cattle, 30% sheep and goats. *Klebsiella oxytoca* (20%) in both species, also *Staph. aureus*, *Citrobacter. Pseudomonas aeruginosa*, *Providenica alacifaciens*, *Providenica rettgeri*, *Proteus vulgaris*, *Proteus mirabilis*, *non haemolytic Strept. and Serratia* were isolated in descending percent as shown in table (1). Similar observations have also been reported in cows and buffaloes by Abo El-Ata (1973), Miller et al. (1980), Markusfeld (1984) and Dass and Paranjape (1987).

The results of the study as shown in table (2) have verified the presence of many bacterial pathogens among endometritis of infected cattle and showed high isolation rate. The most predominant facultative anaerobic organisms were *E. coli* (31.7%), *Staph. aureus* (21.5), *Klebsiella oxytoca* (16.6%), *Strept pyogenes* (10%), *Pseudomonas aeruginosa* (9.9%), *Citrobacter freundii* (8.3%), *Salmonella*

*serovars* (4.9%), *Haemolytic Strept.* (3.3%), *A. pyogenes* (1.6%), *Untyped coryne bacterium* (1.6%), *Serratia* (1.6%), *Listeria monocytogenes* (1.6%). These results similar to those obtained by Laila et al. (1987) and Prabhudas et al. (1989). They recorded that *E. coli* was the chief isolate from endometritis in she-camels and cattle respectively. Some researchers found that *Staph. aureus*, *Strept. pyogenes*, *A. pyogenes*, *untyped Corynebacterium*, *Klebsiella* and *Proteus* from repeat breeder cows and buffaloes, Shoman et al. (1977); Awad and El-Hariri (1980); Yousef (1984) and Takacs et al. (1990). Many authors recorded that *Staphylococci*, *Pseudomonas spp.* and *Coryne bacterium spp.* were the most frequently recovered bacteria from the bovine uterus, Elliott et al. (1968) and Hussein et al. (1990) and Enany et al. (1990) but Hussein et al. (1993) stated that the main causes of endometritis were *Staph. epidermidis*, *Micrococcus luteus*, *Proteus morganii*. Many bacterial pathogens were isolated from endometritis of infected she camel were *E. coli*, *Strept pyogenes*, *Staph. aureus*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Corynebacterium pyogens*. Badr (1997) isolated *A. pyogenes*, *E. coli*, *Streptococcus spp*, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus* from buffaloes with retained fetal membranes. Gandotra et al. (1992) and Goswami et al. (1992) reported that *E. coli* had affinity for urinary tract epithelium and smooth muscles as well as for progesterone. The adherence via K. antigen seemed to be important for colonization of the pathogen during



development of pyometra. On the other hand, these results are in agreement with Roberts (1971) and Hegazy et al. (1979) who recorded that the main etiological cause of endometritis in cattle and she camels were *Corynebacterium*, *Staphylococci*, *Streptococci*, *E. coli*, *Klebsiella oxytoca*, *Proteus* and *Serratia*. Whereas, Goswami et al. (1992) reported that the diphtheroides formed the most predominant group from infected genital tract of buffaloes.

The problem of endometritis among ewes and goats was microbiologically screened. An array of bacteria encountered from 30 samples of each ewes and goats with endometritis is depicted in table (3). *E. coli* isolated in percentage of (49.9%) from ewes and (26.5%) from goats *Staph. aureus* (33.2%) from ewes and (19.9%) from goats, *Strept. pyogenes* (23.2%) from ewes and (16.5%) from goats, *Pseudomonase aeruginosa* (16.6%) from each ewes and goats, *A. pyogenes* (6.6%) from ewes and (9.9%) from goats, *Klebsiella oxytoca* (6.6%), (3.3%) from ewes and goats respectively. *Haemolytic Strept.* (6.6%) from ewes and (3.3%) from goats, *Salmonella serovars* (3.3%) from ewes and (6.6%) from goats. Similar results were opinion of Katoch et al. (1997), they isolated *Actinomyces pyogenes*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *E. coli* and *Bacillus spp* from endometritis of sheep and goats.

In the present work, serological techniques have

also been used to investigate the serovars of isolated *E. coli*, *Pseudomonas* and *Salmonella* as shown in table (4), serotyping of (53) isolates of *E. coli* revealed 11 different "O" serogroups of *E. coli* and 2 strains were untypable, the most prevalent serogroups were O125, O167, O1, O86, O151, O6, O111, O126, O157, O153, O119. The same results were recorded by many workers as a causative agents of genital disorders in camels (Awad et al., 1978; Hegazy et al., 1979 and Enany et al., 1990).

Table (4) indicates the serological typing of 24 isolates of *Pseudomonas aeruginosa* and they belonged to 6 different serogroups and 5 strains were untyped. Similar results were recorded by Hanafy (1996) who isolated *P. aeruginosa* serogrouped as HLK and M. from water samples of poultry and animals farms.

Serological typing of 6 isolates of *Salmonella*, they belonged to 3 different serovars, 4 strains were: *S. typhimurium*, *S. saintpaul* (1) strain and *S. abortus ovis* (1) strain.

Results of antibiotic sensitivity test using 3 representative isolates of each tested organism are shown in table (5). *E. coli* strains were highly sensitive to enrofloxacin and gentamicin, *Staph. aureus* and *Streptococcus pyogenes* strains were sensitive to enrofloxacin, oxalinic acid, gentamicin and nalidixic acid. *Staph. aureus* were sensitive to oxytetracyclin but *Strept. pyogenes* were

sensitive to chloramphenicol.

*Salmonella* isolates were sensitive to enrofloxacin, amoxicillin, oxytetracycline and chloramphenicol. Strains of *non haemolytic Strept.* were sensitive to enrofloxacin, oxalin acid and oxytetracycline nalidixic acid, *Pseudomonas aeruginosa* strains were sensitive to enrofloxacin, gentamicin and colistin sulphate. Similar results were obtained by many authors, Awad and El-Hirri (1980), Gandotra et al. (1992), Goswami et al. (1992), Pradhan et al. (1999) and Anjaneyulu et al. (1999). They reported that enrofloxacin gentamicin and chloramphenicol were found to be highly effective in the isolates of endometritis in bovine. Whereas Hussein et al. (1993) stated that tetracycline and ampicillin the most effective in treatment of repeat breeder syndrome. On the contrary Pradhan et al. (1999) recorded that most of uterine bacterial isolates were resistance to oxytetracycline. Other drugs like erythromycin, streptomycin, Cefadroxil were found to be the least effective drugs, also *E. coli* and *Pseudomonas aeruginosa* were found to the most resistance organism which were completely resistant to 9 and 8 antibiotics respectively out of 11 tested antibiotic. These results agree with those reported by Gandotra et al. (1992).

The bactericidal effect of betadin and potassium permanganate are shown in table (6). It was clear that all the bacteria were killed with betadin 20% and potassium permanganate in concentration of 0.5% within 2 hours except *Salmonella typhimuri-*

*um* was resisted the action of potassium permanganate till 48 hours and killed after 72 hours.

The medicament using betadin has a selective therapeutic effect for most pathogenic strains isolated from endometritis. Iodine has an antibacterial action via diffusion into the bacterial cell, which where interfere with the vital metabolic reaction. Many authors were used lugol's iodine in treatment of endometritis in cows (Abd El-Mohgny, 1988 and Hussein et al. 1993).

It can be concluded from the results of the present study that the best medicament used to treat the endometritis via apply antibiogram and using antiseptic betadin or potassium permanganate upon the living animals.

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