

HISTOPATHOLOGICAL CHANGES IN ENDOMETRITIS OF BUFFALOES CAUSED BY BACTERIAL INFECTION BY

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ABSTRACT

147 samples were collected from apparently healthy buffaloes and buffaloes suffering from various degrees of endometritis in dairy farms at Damietta and Dakahlia Governorates as well as from Mansoura abattoir. The isolated bacteria from healthy buffaloes were *E. coli* (20.5 %), *S. aureus* (15.4 %), *K. oxytoca* and *S. epidermidis* (10.3%), (7.7%) for each of , *P. mirabilis*, *M. nishinomiyoensis* and *E. faecalis* and (5.1%) for each of *P. vulgaris*, *P. rettegi*, *C. freundii* and *P. aeruginosa*. In endometritis cases, it was noticed that 30.2% of *E. coli*, (19.1%) *S. aureus*, (8.7%) *S. pyogenes*, (5.8) *A. bovis*, (5.2%) for each of *K. oxytoca*, *E. agglomerans* and *K. pneumoniae*, (4.6%) *P. aeruginosa*, (3.5%) for each of *P. vulgaris* and *C. freundii*, (2.3%) for each of *P. mirabilis* and *Salmonellae*, (1.8%) for each of *Y. pseudotuberculosis* and *A. pyogenes* and (1.2%) *E. faecalis*.

. Histopathological study to sample collected from healthy cyclic buffaloes revealed normal endometrium. Microscopic lesions of acute endometritis revealed stromal oedema, congestion of blood vessels, mild to severe neutrophilic infiltration and mild perivascular and periglandular fibrosis. In chronic endometritis, extensive stromal fibrosis around blood vessels and gland, and diffuse mononuclear cell infiltration mostly lymphocytes and plasma cells.

Sensitivity of the most prevalent isolates recovered from endometritis to 21 different types of antibiotics revealed that they were highly sensitive to enrofloxacin (96.38%), gentamicin (85.54%), chloramphenicol (83.13%), norfloxacin (80.72%) and piperacillin (78.31%), but, they were highly resistant to penicillin G (10.84%). Also, they were resistant to ampicillin (32.53%), streptomycin (36.14%), oxytetracycline (37.35%), SXT (39.7%) and lincospectinomycin (44.58 %).

INTRODUCTION

The etiology of endometritis is multi – factorial, making the disease very difficult to diagnose, treat or eradicate. The cause includes bacteria, virus and fungi. However, the majority of endometritis outbreaks in dairy herds are due to bacterial agents (*Foldi et al., 2006*). The sub-clinical endometritis generally remain undiagnosed till the culture isolation, antibiotic sensitivity test and biopsy examinations are performed, Agglutination tests are one of the most important tools for the diagnosis of infectious disease. Serological tests are used extensively for the identification of unknown organisms isolated from clinical specimens; for the detection of antibodies in serum obtained from animals suspected of having a disease and in epidemiological investigation. In many instances, final identification of unknown organism can be done only by serological tests where the presence of antibodies in animal’s serum may be indicative of an active infection or of past infection depending upon the titre against a particular antigen (*Sharma and Adlakha, 1996*).

This work is of great importance to direct the veterinarian’s attention for the subclinical cases that neglected without correct diagnosis and proper treatment. Mixed infections with bacteria must be taken in consideration upon dealing with endometritic problem.

MATERIAL AND METHODS

Table (1): Number of examined uterine samples taken from living and slaughtered buffaloes

Living animals			Slaughtered animals			Total
Apparently healthy	Endometritis cases	Sub-total	Apparently healthy	Endometritis cases	Sub-total	
25	46	71	15	61	76	147

As shown in table (1), a total of 147 samples were collected from apparently healthy buffaloes as well as buffaloes suffering from various degrees of endometritis in dairy farms at Damietta and Dakahlia Governorates as well as from Mansoura abattoir. Samples were taken from live as well as slaughtered animals. In live animals, lips of the vulva were carefully wiped with dry cellulose paper and set apart with the fingers of the left hand, while a sterile metal tube (46 cm. long and 1.5 cm. diameter) coating a sterilized swab, 61 cm. long and 0.3

cm. diameter (*Sinha et al., 1980*) was introduced carefully by the aid of the right hand within the vagina, then the swab was pushed forward and rotated well on the mucous membrane at the area of the external os uteri of the cervix and left for 1-2 minutes, then the swab was withdrawn inside the metal tube and transported to the laboratory in a transport media.

From slaughtered animals, genital organs were removed shortly after evisceration. Vagina was tied externally to avoid contamination during transportation of samples. Organs were placed in sterile containers and taken immediately to the laboratory. The genital tract was examined for presence of any pathological changes such as mucoid, mucopurulent or purulent exudates which might be present. Surface of uterus was touched by hot spatula and opened under complete aseptic precaution, and then a loopfull of uterine contents was directly cultured onto blood agar, MacConkey's agar, EMB agar, Baird Parker agar, Mannitol salt agar, Pseudomonas agar and Modified Edward's agar (**Oxoid 1998**). Other loopfull was directly transferred to pre-enrichment fluid media namely selenite F broth and incubated at 37°C for not more than 18 hours, then an inoculum was cultivated on different selective media including MacConkey's agar and S-S agar medium (*Ali and Ibrahim, 2001*). All the inoculated plates were incubated aerobically at 37°C for 24 hours. The suspected colonies were examined culturally, microscopically by Gram's stain as well as biochemically according to *MacFaddain (1980), Kocur (1986) and Carter et al. (1995)*.

In histopathological study, the obtained uteri were incised along the mid- dorsum , necropsy specimens from horns and body were taken and fixed in 10% formal saline solution, processed, sectioned, stained with Harri's Haematoxylin and Eosin (*Wilson and Gamble, 2002*) stains, and examined by light microscopy.

Antimicrobial susceptibility testing of the isolated bacteria was done according to *Bauer et al. (1996) and Quinn et al. (1994)* Slide agglutination (SAT) and Tube agglutination test (TAT, European method) were done to serum of infected animals (*Alton et al. 1975*).

RESULTS

Table (2): Endometritis in buffaloes regarding to the breeding history

Breeding history	Buffaloes	
	No. of cases	%
Difficult birth and birth help	9	19.57
Abortion	13	28.26
Retained placenta	11	23.91
Prolapse of vagina	3	6.52
Dead foetus	2	4.35
Unknown	8	17.39
Total	46	100

Table (3): Endometritis in buffaloes at different degrees of inflammation

Degree of inflammation	Buffaloes			
	Live		Slaughtered	
	No.	%	No.	%
1 st degree	9	19.57	11	18.03
2 nd degree	10	21.73	19	31.15
3 rd degree	18	39.13	21	34.43
Pyometra	9	19.57	10	16.39
Total	46	100	61	100

Table (4): Bacteria recovered from apparently healthy and endometritis cases of live buffaloes

Species of bacteria	Apparently healthy (25)		Degree of inflammation (46)									
	No	%	1 st degree		2 nd degree		3 rd degree		Pyometra		Total	
			No	%	No	%	No	%	No	%	No	%
<i>E. coli</i>	6	21.4	7	9.6	8	10.9	7	9.6	6	8.2	28	38.3
<i>S. aureus</i>	4	14.3	3	4.1	4	5.5	5	6.8	4	5.5	16	21.9
<i>S. pyogenes</i>	-	-	-	-	2	2.7	1	1.4	3	4.1	6	8.2
<i>A. bovis</i>	-	-	-	-	1	1.4	3	4.1	-	-	4	5.5
<i>K. oxytoca</i>	3	10.7	1	1.4	-	-	1	1.4	-	-	2	2.7
<i>C. freundii</i>	2	7.1	-	-	-	-	1	1.4	-	-	1	1.4
<i>P. mirabilis</i>	2	7.1	-	-	-	-	-	-	-	-	-	-
<i>S. epidermidis</i>	3	10.7	-	-	-	-	-	-	-	-	-	-
<i>M. nishinomiyoensis</i>	2	7.1	-	-	-	-	-	-	-	-	-	-
<i>P. aeruginosa</i>	2	7.1	-	-	-	-	1	1.4	1	-	2	2.7
<i>A. pyogenes</i>	-	-	-	-	-	-	-	-	1	-	1	1.4
<i>S. Entritidis</i>	-	-	-	-	-	-	1	1.4	-	-	1	1.4
<i>S. Typhimurium</i>	-	-	-	-	-	-	-	-	1	1.4	1	1.4
<i>E. agglomerans</i>	-	-	1	1.4	2	2.7	-	-	-	-	3	4.1
<i>K. pneumoniae</i>	-	-	1	1.4	1	1.4	2	2.7	-	-	4	5.5
<i>P. vulgaris</i>	1	3.6	1	1.4	1	1.4	1	1.4	-	-	3	4.1
<i>Y. pseudotuberculosis</i>	-	-	1	1.4	-	-	-	-	-	-	1	1.4
<i>E. faecalis</i>	2	7.1	-	-	-	-	-	-	-	-	-	-
<i>P. rettgeri</i>	1	3.6	-	-	-	-	-	-	-	-	-	-
Total	28	100	15	20.5	19	26.1	23	31.5	16	21.9	73	100

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Percentages were calculated according to total number of bacterial isolates (28) from healthy cases and (73) from diseased animals.

Table (5): Bacteria isolated from apparently healthy and endometritis cases of slaughtered buffaloes

Bacteria	Apparently healthy (15)		Degree of inflammation (61)									
	No.	%	1 st degree		2 nd degree		3 rd degree		Pyometra		Total	
			No.	%	No.	%	No.	%	No.	%	No.	%
<i>E. coli</i>	2	18.2	5	5	7	7	6	6	6	6	24	24
<i>S. aureus</i>	2	18.2	3	3	5	5	5	5	4	4	17	17
<i>S. pyogenes</i>	-	-	-	-	3	3	2	2	4	4	9	9
<i>A. bovis</i>	-	-	-	-	2	2	4	4	-	-	6	6
<i>K. oxytoca</i>	1	9.1	-	-	4	4	3	3	-	-	7	7
<i>C. freundii</i>	-	-	-	-	2	2	3	3	-	-	5	5
<i>P. mirabilis</i>	1	9.1	1	1	1	1	2	2	-	-	4	4
<i>S. epidermidis</i>	1	9.1	-	-	-	-	-	-	-	-	-	-
<i>M. nishinomiyoensis</i>	1	9.1	-	-	-	-	-	-	-	-	-	-
<i>P. aeruginosa</i>	-	-	1	1	-	-	2	2	3	3	6	6
<i>A. pyogenes</i>	-	-	-	-	-	-	-	-	2	2	2	2
<i>S. Kentucky</i>	-	-	-	-	-	-	1	1	-	-	1	1
<i>S. Typhimurium</i>	-	-	-	-	-	-	-	-	1	1	1	1
<i>E. agglomerans</i>	-	-	1	1	2	2	3	3	-	-	6	6
<i>K. pneumoniae</i>	-	-	3	3	2	2	-	-	-	-	5	5
<i>P. vulgaris</i>	1	9.1	-	-	1	1	2	2	-	-	3	3
<i>Y. pseudotuberculosis</i>	-	-	1	1	1	1	-	-	-	-	2	2
<i>E. faecalis</i>	1	9.1	1	1	1	1	-	-	-	-	2	2
<i>P. rettgeri</i>	1	9.1	-	-	-	-	-	-	-	-	-	-
Total	11	100	16	16	31	31	33	33	20	20	100	100

Percentages were calculated according to total number of bacterial isolates (11) from healthy cases and (100) from diseased animals

Table (6): Types of bacteria isolated from mixed infection in live buffaloes

Cases	No. of mixed bacterial isolates	Mixed infection								
		2 types	No.	%	3 types	No.	%	4 types	No.	%
Buffaloes	Apparently healthy (25)	• <i>E. coli</i> + <i>P. mirabilis</i>	4	28.6	• <i>E. coli</i> + <i>P. aeruginosa</i> + <i>P. mirabilis</i>	3	21.4	-	0	0.0
		• <i>E. coli</i> + <i>S. aureus</i>	2	14.3	• <i>E. coli</i> + <i>S. epidermidis</i> + <i>C. freundii</i>	3	21.4			
		• <i>S. aureus</i> + <i>K. Oxytoca</i>	2	14.3						
	Endometritis (46)	• <i>E. coli</i> + <i>S. Aureus</i>	8	17.8	• <i>E. Coli</i> + <i>S. aureus</i> + <i>K. pneumoniae</i>	9	20	• <i>E. coli</i> + <i>S. aureus</i> + <i>C. freundii</i> + <i>P. vulgaris</i>	4	8.9
		• <i>E. coli</i> + <i>P. vulgaris</i>	4	8.9	• <i>E. coli</i> + <i>S. pyogenes</i> + <i>A. bovis</i>	6	13.3	• <i>E. agglomerans</i> + <i>S. aureus</i> + <i>S. pyogenes</i> + <i>K. oxytoca</i>	4	8.9
		• <i>E. coli</i> + <i>A. bovis</i>	4	8.9						
		• <i>S. aureus</i> + <i>S. pyogenes</i>	4	8.9						
		• <i>S. aureus</i> + <i>P. aeruginosa</i>	2	4.4						

Percentages were calculated according to total number of mixed bacterial isolates recovered from apparently healthy and endometritis buffaloes (14, 45 respectively).

Table (7): Bacterial recovered from mixed infection in slaughtered animals

Cases	No. of bacterial isolates	Mixed infection									
		2 types	No.	%	3 types	No.	%	4 types	No.	%	
Apparently healthy (15)	4	• <i>E. coli</i> + <i>S. Aureus</i>	2	50							
		• <i>E. coli</i> + <i>E. faecalis</i>	2	50	-						
Endometritis (61)	64	• <i>E. coli</i> + <i>S. aureus</i>	6	9.4	• <i>E. coli</i> + <i>S. aureus</i> + <i>K. pneumoniae</i>	9	14.1	• <i>E. coli</i> + <i>S. pyogenes</i> + <i>S. aureus</i> + <i>P. vulgaris</i>	8	12.5	
		• <i>S. aureus</i> + <i>Y. pseudotuberculosis</i>	4	6.25	• <i>E. agglomerans</i> + <i>S. aureus</i> + <i>C. freundii</i>	6	9.4	• <i>E. agglomerans</i> + <i>S. pyogenes</i> + <i>P. mirabilis</i> + <i>K. oxytoca</i>	4	6.25	
		• <i>E. agglomerans</i> + <i>S. aureus</i>	4	6.25	• <i>E. coli</i> + <i>S. aureus</i> + <i>K. oxytoca</i>	6	9.4				
		• <i>E. coli</i> + <i>S. pyogenes</i>	4	6.25	• <i>E. coli</i> + <i>S.</i>	3	4.7				
		• <i>E. coli</i> + <i>P. aeruginosa</i>	4	6.25	• <i>E. coli</i> + <i>S.</i>	3	4.7				
		• <i>E. coli</i> + <i>A. pyogenes.</i>	2	3.1	Typhimurium + <i>P. Vulgaris</i>						
		• <i>S. aureus</i> + <i>A. bovis</i>									

Percentages were calculated according to total number of mixed bacterial isolates recovered from apparently healthy and endometritis of slaughtered buffaloes (4, 64 respectively).

Table (8): Bacteria isolated from cases of endometritis in buffaloes

Bacterial isolates	Buffaloes					
	Apparently healthy		Endometritis		Total	
	No.	%	No.	%	No.	%
<i>E. coli</i>	8	20.5	52	30.2	60	28.1
<i>S. aureus</i>	6	15.4	33	19.1	39	18.4
<i>S. pyogenes</i>	-	-	15	8.7	15	7.1
<i>A. bovis</i>	-	-	10	5.8	10	4.7
<i>K. oxytoca</i>	4	10.3	9	5.2	13	6.1
<i>C. freundii</i>	2	5.1	6	3.5	8	3.8
<i>P. aeruginosa</i>	2	5.1	8	4.6	10	4.7
<i>A. pyogenes</i>	-	-	3	1.8	3	1.4
<i>Salmonellae</i>	-	-	4	2.3	4	1.9
<i>E. agglomerans</i>	-	-	9	4.8	9	4.3
<i>K. pneumoniae</i>	-	-	9	5.2	9	4.3
<i>P. vulgaris</i>	2	5.1	6	3.5	8	3.8
<i>Y. pseudotuberculosis</i>	-	-	3	1.8	3	1.4
<i>E. faecalis</i>	3	7.7	2	1.2	5	2.4
<i>P. mirabilis</i>	3	7.7	4	2.3	7	3.3
<i>S. epidermidis</i>	4	10.3	-	-	4	1.9
<i>M. nishinomiyoensis</i>	3	7.7	-	-	3	1.4
<i>P. rettgeri</i>	2	5.1	-	-	2	0.9
Total	39	100	173	100	212	100

Percentages were calculated according to total number of bacterial isolates recovered from apparently healthy and endometritis buffaloes (39, 173 respectively).

Table (9): Serotyping of isolated *Salmonellae*

Serotype	Salmonella serovars (4)				
	Antigenic formula			No.	%
	Somatic (o) Ag	Flageller (H) Ag			
		Phase I	Phase II		
<i>S. Typhimurium</i>	4, (5)	I	1, 2	2	50
<i>S. Enteritidis</i>	9, 12	gm	1, 7	1	25
<i>S. Kentucky</i>	8, 20	Iz	6	1	25

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Table (10): Serotyping of *E. coli* recovered from apparently healthy buffaloes and buffaloes suffering from endometritis

Serovar	Type of examined cases				Total (60)	
	Endometritis (52)		Apparently healthy (8)			
	No.	%	No.	%	No.	%
0167	12	23.07	2	0.25	14	23.33
0111	8	15.38	0	0.0	8	13.33
0125	7	13.46	0	0.0	7	11.66
0126	7	13.46	2	0.25	9	15.00
078	6	11.53	0	0.0	6	10.00
0119	5	9.61	1	0.13	6	10.00
0128	2	3.84	1	0.13	3	5.00
086	2	3.84	1	0.13	3	5.00
026	1	1.92	1	0.13	2	3.33
0114	2	3.84	0	0.0	2	3.33

percentages were calculated according to number of *E. coli* isolates recovered from endometritis (52) and apparently healthy (8) and total number of *E. coli* isolates (60).

Table (11): Results of agglutination tests of positive sera against the isolated bacterial species

Bacterial isolates	Buffaloes						
	No. of positive sera by slide agglutination (46)		Tube agglutination test				
	No.	%	1/20	1/40	1/80	1/160	1/320
<i>E. coli</i>	43	93.5	7	23	13	-	-
<i>S. Typhimurium</i>	12	62.1	8	4	-	-	-
<i>S. Entiritidis</i>	10	21.7	6	4	-	-	-
<i>P. aeruginosa</i>	14	30.4	2	8	4	-	-
<i>S. aureus</i>	41	89.1	-	-	16	15	10
<i>S. pyogenes</i>	20	43.5	-	12	8	-	-
<i>A. bovis</i>	18	39.1	3	10	5	-	-
<i>A. pyogenes</i>	16	34.8	5	11	-	-	-

Table (12): Susceptibility of different bacterial species recovered from endometritis in buffaloes to different antimicrobial agents

Antimicrobial agents	<i>S. aureus</i> (20)		<i>E. coli</i> (30)		<i>Salmonella</i> (4)		<i>S. pyogenes</i> (10)		<i>A. bovis</i> (8)		<i>P. aeruginosa</i> (8)		<i>A. pyogenes</i> (3)		Overall (83)	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Amikacine	12	60	19	63.33	2	50	6	60	2	25	5	62.5	1	33.3	47	56.63
Ampicillin	4	20	10	33.33	3	75	3	30	5	62.5	0	0.0	2	66.66	27	32.53
Cefeperezone	12	60	24	80	1	25	8	80	3	37.5	0	0.0	1	33.3	49	59.04
Cefotaxime	14	70	20	66.66	2	50	6	60	1	12.5	0	0.0	2	66.66	45	54.22
Chloramphenicol	17	85	26	86.66	3	75	8	80	7	87.5	5	62.5	3	100	69	83.13
Ciprofloxacin	16	80	25	83.33	3	75	7	70	7	87.5	1	12.5	1	33.33	60	72.29
Doxycycline	14	70	16	53.33	1	25	4	40	6	75	1	12.5	2	66.66	44	53.01
Enrofloxacin	19	95	29	96.66	4	100	10	100	8	100	7	87.5	3	100	80	96.38
Erythromycin	10	50	18	60	1	25	8	80	4	50	0	0.0	2	66.66	43	51.81
Flumequine	9	45	20	66.666	3	75	6	60	2	25	0	0.0	1	33.33	41	49.40
Flumox	15	75	18	60	3	75	8	80	4	50	1	12.5	1	33.33	50	60.24
Gentamicin	18	90	26	86.66	3	75	9	90	6	75	6	75	3	100	71	85.54
Lincospectinomycin	17	85	6	20	1	25	5	50	6	75	1	12.5	1	33.33	37	44.58
Neomycin	13	65	23	76.66	2	50	7	70	1	12.5	1	12.5	1	33.33	48	57.83
Nitrofurantion	6	30	24	80	3	75	6	60	3	37.5	4	50	2	66.66	48	57.83
Norofloxacin	17	85	28	93.33	3	75	9	90	3	37.5	6	75	1	33.33	67	80.72
Oxytetracycline	12	60	8	26.66	2	50	2	20	3	37.5	1	12.5	2	66.66	31	37.35
Pencillin G	0	00.0	0	00.00	1	25	4	40	2	25	0	0.0	2	66.6	9	10.84
Pipracillin	18	90	27	90	3	75	5	50	7	87.5	3	37.5	3	100	65	78.31
Streptomycin	16	80	10	33.33	1	25	2	20	1	12.5	0	0.0	0	0.0	30	36.14
SXT	11	55	8	26.66	2	50	5	50	4	50	2	25	1	33.33	33	39.76

Percentages were calculated according to number of tested bacterial isolates.

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Table (13): Interpretation of results of susceptibility of recovered bacteria from endometritis to different antimicrobial agents

Antimicrobial agents	S. aureus	E. coli	Salmonella	S. Pyogenes	A. bovis	P. aeruginosa	A. pyogenes	Overall (83)
Amikacine	S	S	S	S	R	S	MS	S
Ampicillin	HR	R	HS	R	S	HR	S	R
Cefepazone	S	HS	HR	HS	R	HR	HR	S
Cefotaxime	S	S	R	D	HR	HR	MS	S
Chloramphenicol	HS	HS	HS	HS	HS	S	HS	HS
Ciprofloxacin	HS	HS	HS	S	HS	HR	HR	S
Doxycycline	S	S	HS	R	HS	HR	S	S
Enrofloxacin	HS	HS	HS	HS	HS	HS	HS	HS
Erythromycin	S	S	R	HS	M.S	HR	S	S
Flumequine	R	S	HS	S	R	HR	R	MS
Flumox	HS	S	HS	HS	M.S	HR	R	S
Gentamicin	HS	HS	HS	HS	HS	HS	HS	HS
Lincospectinomycin	HS	HR	HR	M.S	HS	HR	R	R
Neomycin	S	HS	S	S	HR	HR	R	S
Nitrofurantion	R	HS	HS	S	R	MS	MS	S
Norofloxacin	HS	HS	HS	HS	R	HS	HR	HS
Oxytetracycline	S	HR	S	HR	R	HR	S	R
Pencillin G	HR	HR	HR	R	R	HR	S	HR
Pipracillin	HS	HS	HS	MS	HS	R	HS	HS
Streptomycin	HS	R	HR	HR	HR	HRH	HR	R
SXT	S	HR	R	MS	MS	R	HR	R

S: Sensitive (more than 50% and less than 75% of isolates were susceptible to antimicrobial agents).

Ms: Moderately Susceptible (50% of isolates were susceptible to antimicrobial agents).

HS: Highly sensitive (75% or more of isolates were susceptible to antimicrobial agents).

R: Resistant (more than 50% and less than 75% of isolates were resistant to antimicrobial agents).

HR: Highly resistant (more than 75% of isolates were resistant to antimicrobial agents).

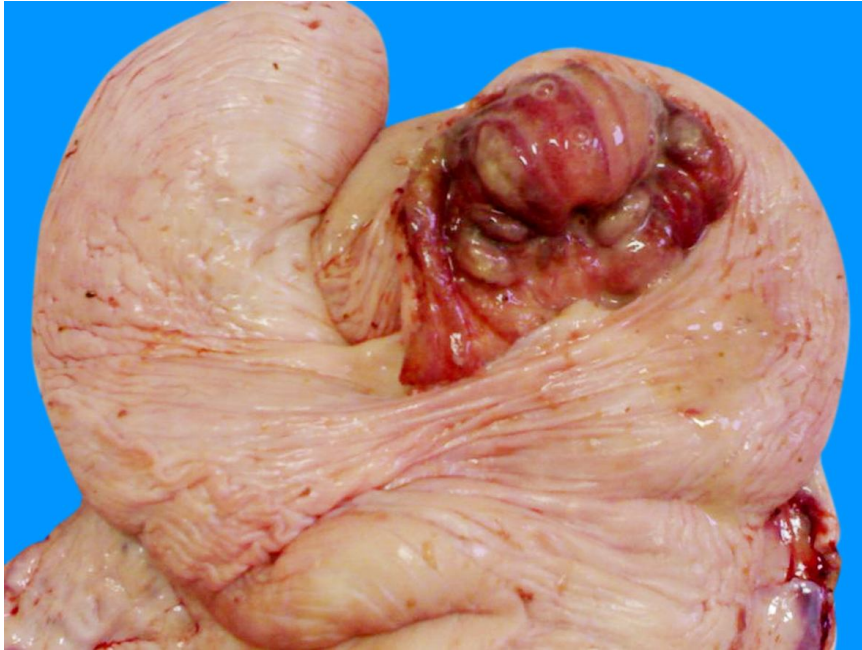


Photo (1): Uterus of a buffaloe with endometritis show mucopurulent exudates

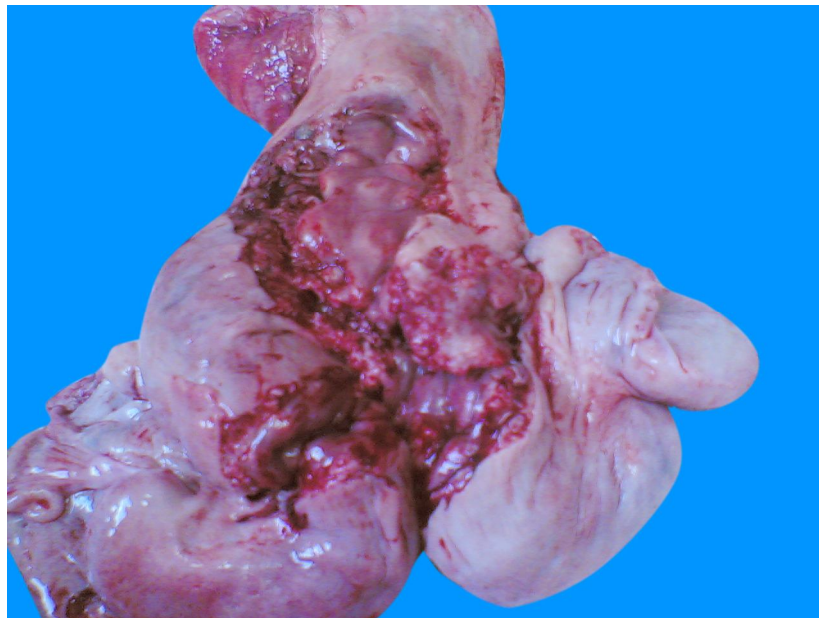


Photo (2): Uterus of a buffaloe with acute endometritis.

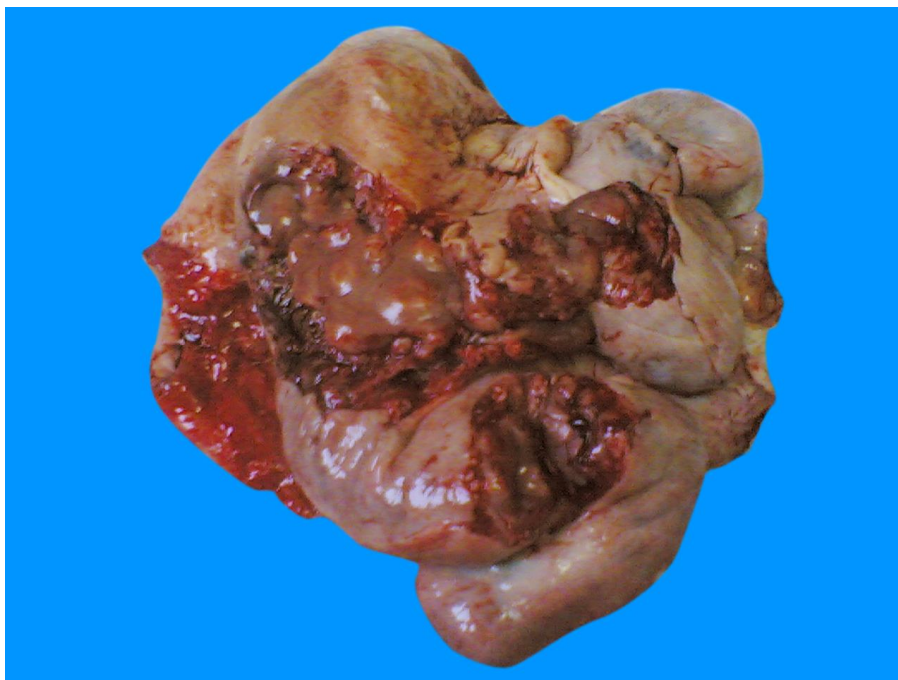


Photo (3): Uterus of a buffalo with chronic endometritis

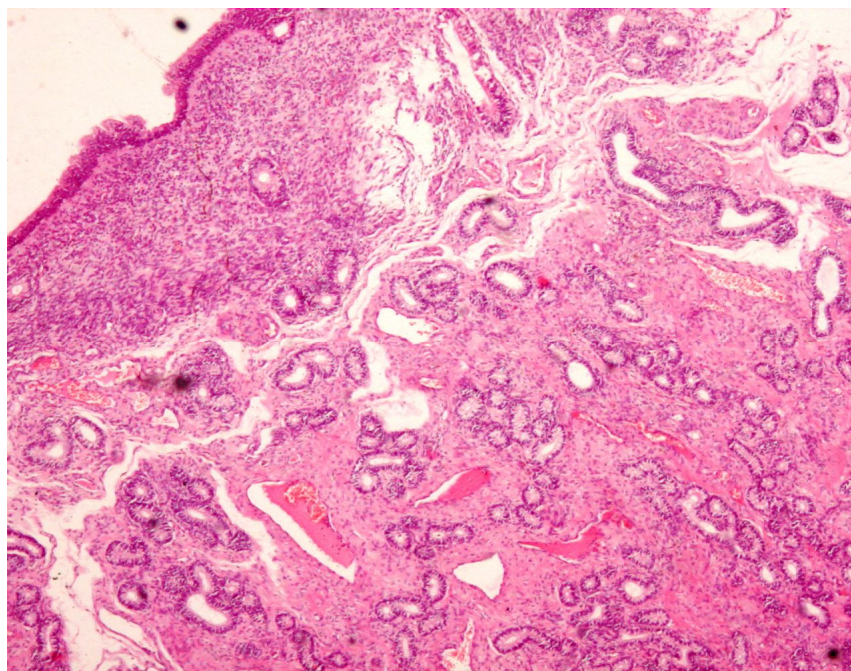


Photo (4): Tissue section in uterus of a buffalo showed normal endometrium (H&E, X52)

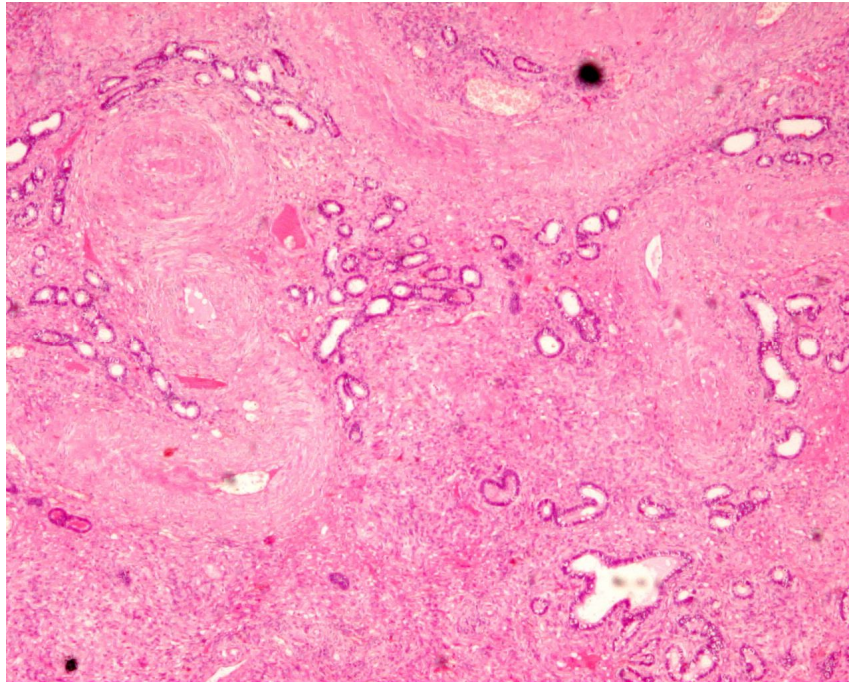


Photo (5): Endometrium showed stromal fibrosis and periglandular fibrosis (H&E, X52).

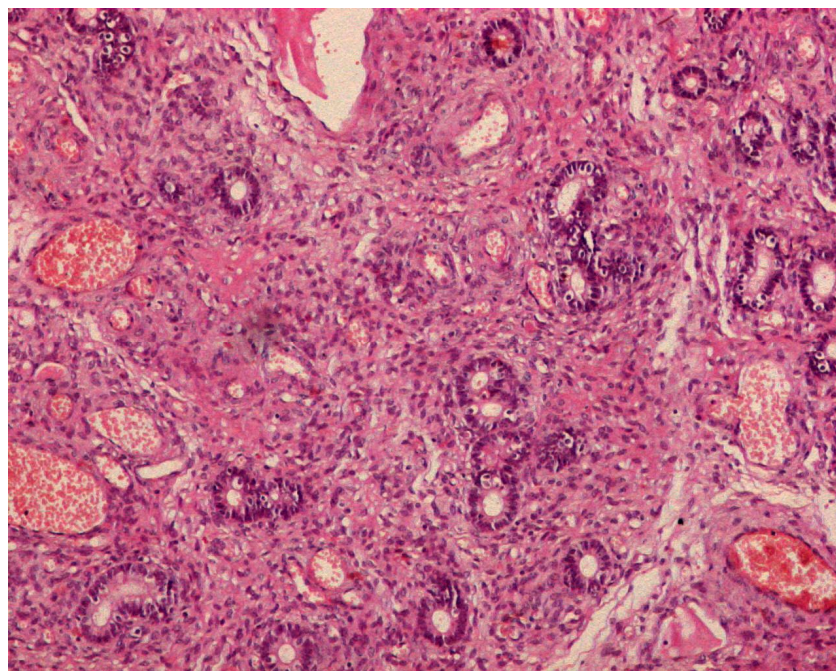


Photo (6): Endometrium showed severe congestion and periglandular leukocytic infiltration with fibrosis (H&E, X130)

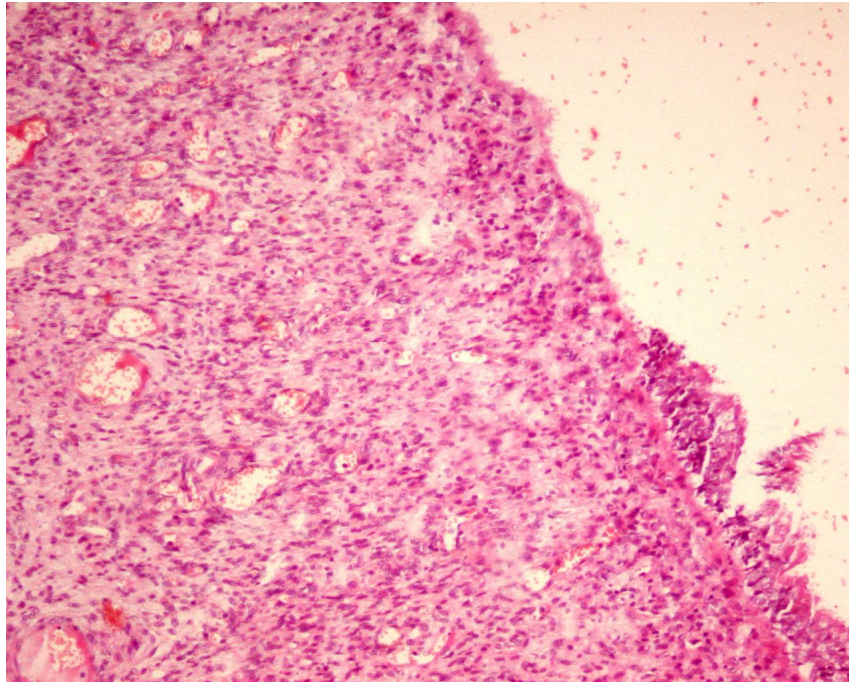


Photo (7): Endometrium showed desquamation of the epithelial lining besides, congestion and leukocytic infiltration (H&E, X130)

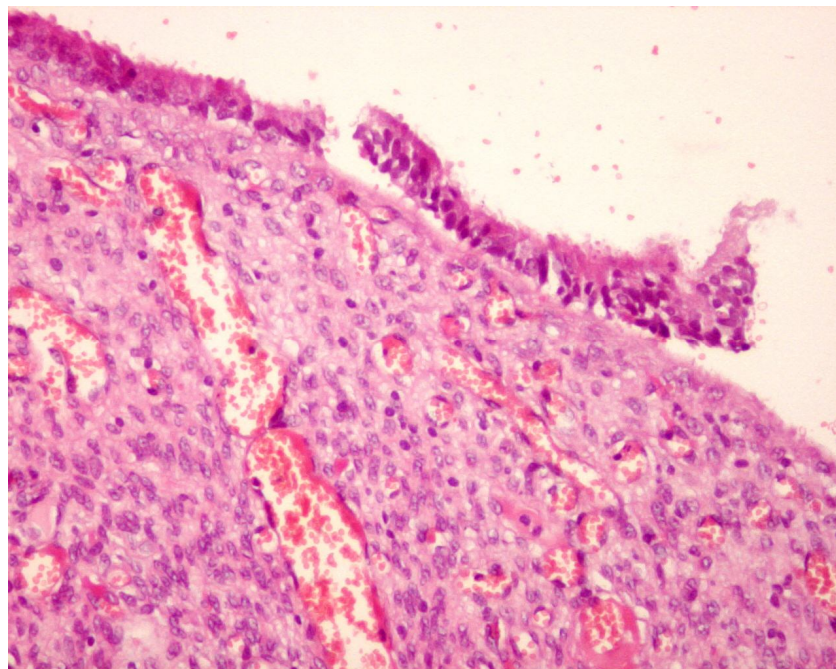


Photo (8): Endometrium showed partial desquamation of the epithelial lining besides, severe congestion and leukocytic infiltration mainly neutrophils (H&E, X130)

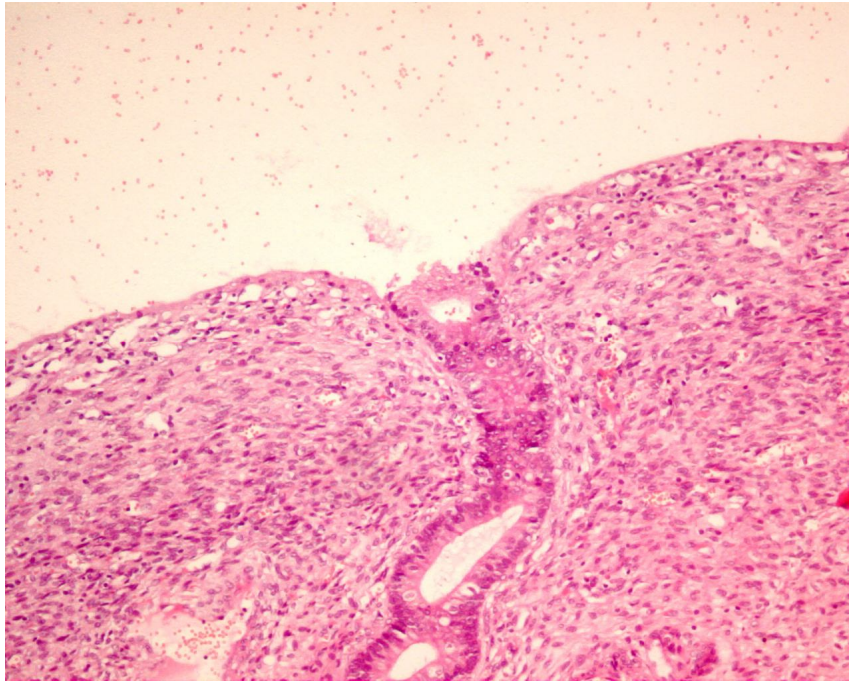


Photo (9): Endometrium showed ulceration of the epithelial lining with leukocytic infiltration (H&E, X 130)

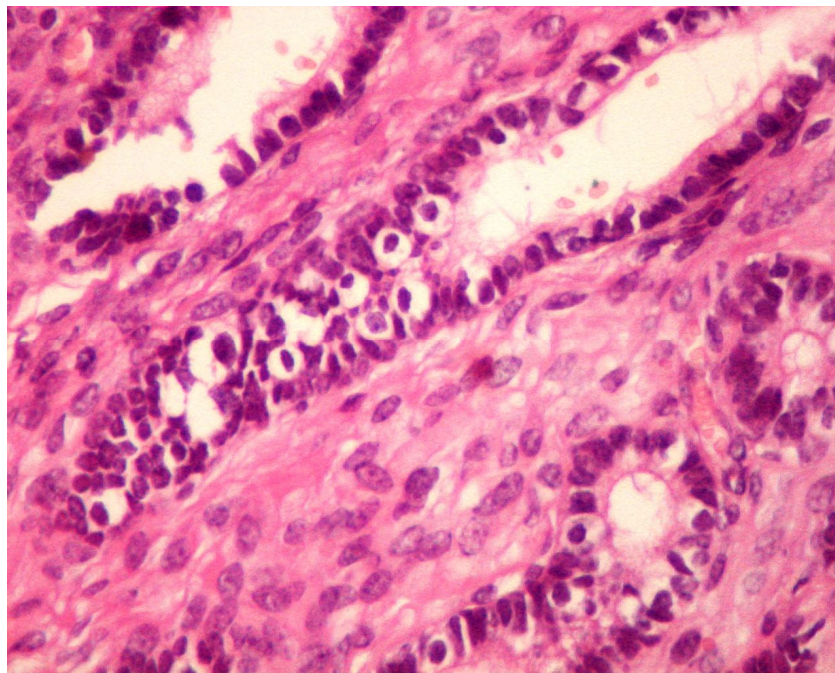


Photo (10): Endometrium showed stromal fibrosis infiltrated with leukocytes mainly neutrophils (H&E, X 520)

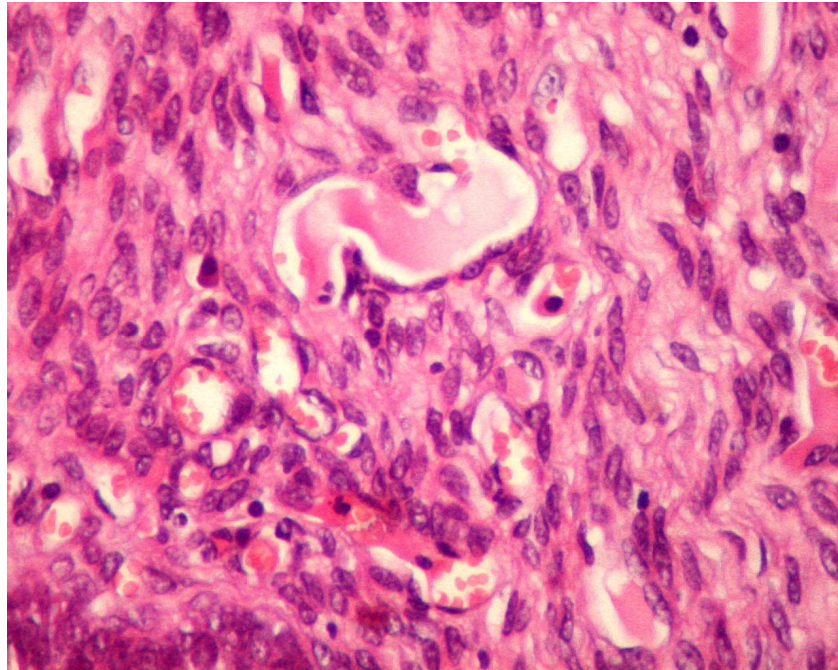


Photo (11): Endometrium showed round cells beside plasma cells (H&E, X520)

(Chronic endometritis)

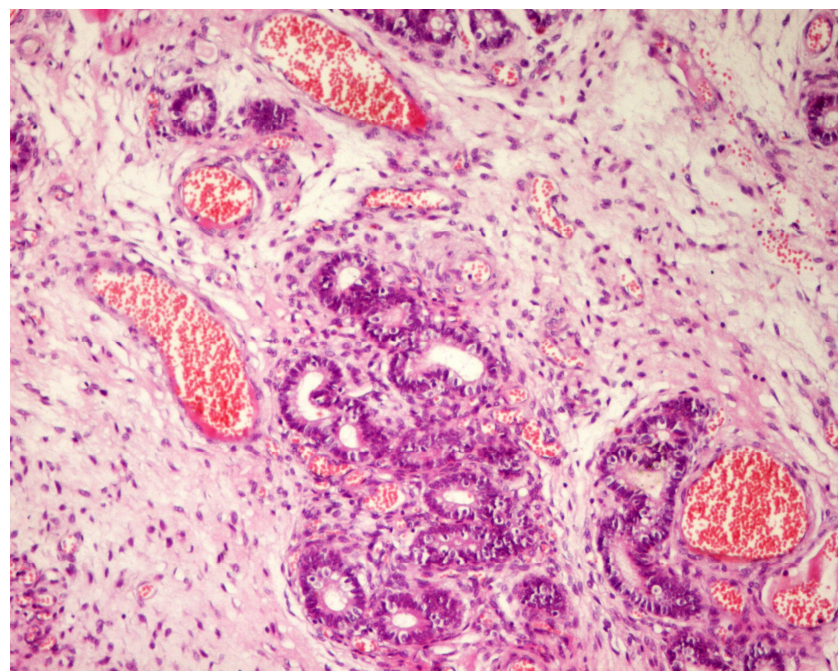


Photo (12): Endometrium showed severe congestion beside perivascular fibrosis and periglandular fibrosis infiltrated with leukocytes (H&E, X 330)

(Chronic endometritis)

DISCUSSION

In the present investigation, it is appeared that the diseased genitalia of buffaloes were classified into 4 degrees of endometritis according to (character of the vaginal mucus) as follows (0) clear or translucent mucus; (1) mucus containing flecks of whit or off - white pus (2) discharge containing < 50 ml exudate containing \leq 50% white or off - white mucopurulent material; and (3) discharge containing > 50 ml exudates containing purulent material, usually white or yellow but occasionally sanguineous (*Williams et al., 2005 and Sheldon, 2007*). Healthy uterus has its own saprophytic bacteria but under unfavorable conditions might become pathogenic and cause clinical or sub-clinical sings of endometritis (*Gunter et al., 1955; Dawson, 1950, Roberts, 1971 and AboEl-Ata, 1973*).

When looking carefully into results obtained in tables (2 &3) it is noticed that the history of difficult birth and birth help, abortion, retained placenta, fluid discharge, prolapse of vagina, dead foetus, in addition to failure of conception were most prevalent symptoms appearing in the examined buffaloes suffering from chronic endometritis. These observations are in accordance with the finding obtained by *Millar and Ras (1952)* who noticed that fluid discharge especially during oestrus and failure for conception are the most pronounced symptoms and also bacteriological examination aided in proper diagnosis of endometritis. *Roberts (1986)* reported that, stillbirth, abortion, dystocia, postpartum metritis and laceration or lesions of the uterus, cervix, vagina and vulva could be followed by a persistent endometritis.

Bacterial contamination of the postpartum uterus was a frequent finding which by itself didn't disturb the anatomical and histological restoration of tubular genital tract. The improper balance between uterine infection and intrauterine antimicrobial self-defense mechanisms, however, often resulted in complication such as puerperal metritis, clinical endometritis, pyometra and subclinical endometritis (*Földi et al., 2006*).

In the obtained results, high incidence of the 3rd degree of chronic endometritis of the uterus. This result nearly in agreement with *Abo El – Ata, (1973)* and *Ali and Ibrahim (2001)*.

From the results obtained in table (4), different types of bacteria were isolated from normal buffaloes. *E. coli* (21.4%), *S. aureus* (14.3%), (10.7%) for each of *S. epidermis* and *K. oxytoca*, (7.1%) for each of *C. freundii*, *P. mirabilis*, *M. nishinomiynois*, *P. aeruginosa* and *E. faecalis*, (3.6%) for each of *P. vulgaris* and *P. rettgeri*. These results nearly agree with

Zaki et al.(1962); Radoslavov (1975); El Deeb (1990); Hussein et al. (1993); Zerbe et al.(2001); Abdel Mola (2003); Hassab El-Naby and El Ekhnawy (2004); and Abdel Rahman and Ibrahim (2007).

Regarding to the bacterial isolates recovered from endometritis cases in buffaloes, *E.coli* represented (38.3%), followed by *S.aureus* (21.9%), *S. pyogenes* (8.2%), (5.5%) for *A .bovis* and *K. pneumoniae* , (4.1%) for *E. agglomerans* and *P. vulgaris*, (2.7%) for *K. oxytoca* and *P. aeruginosa*, (1.4%) for each of *C. freundii*, *A. pyogenes*, *S. Enteritidis*, *S. Typhimurium* and *Y. pseudotuberculosis*. Most of these isolates were also isolated by many authors (**Metwelly, 2001; Abd El Mola, 2003; Rizk and Bkhiet, 2003; Idrees, 2004; Prajapati et al, 2005; Maarouf and El-Bealawy, 2005; and Yavari et al., 2007**).

As shown in table (5), presence of bacterial isolated from apparently healthy uteri and those with varying degrees of endometritis of slaughtered buffaloes showed nearly the same isolates recovered from live animals with little variation in their prevalence.

In tables (6&7), 2 types, 3 types and 4 types of mixed isolates were recovered from different positive samples, *E. coli* shared in most of the mixed infection. These results nearly agree with **Hassab El-Naby and El Ekhnawy (2004), and Metwelly (2002)** who isolated mixed culture from *E. coli* + *Klebsiella* spp. (25%) and *E. coli* + *staphylococci* spp. (14.3%), moreover, **Shouman et al., (1977)** isolated *E. coli* with *Proteus rettgerii* and *Citrobacter*. Also, **Williams et al. (2005)** isolated *E. coli* with non haemolytic streptococci. **Abdel Rahman and Ibrahim (2007)**, and **Azab et al. (2006)** isolated *E. coli* from many cases of mixed infection of the uterus.

When looking carefully into the results obtained in (Table 8), *E. coli* was the most common organism isolated from cases of endometritis in buffaloes followed by *S. aureus* and *S. pyogenes*. These results agree with (**Giriffin, 1974; Noakes et al., 1991; Thurmond et al., 1993; Anjaneyulu et al., 1999; Sayed and Shehata, 2001; and El-Azab and Maarouf, 2002**) who reported that the most important pathogens in case of endometritis were *E. coli*, *staphylococci* and *streptococci*. Also, recovery of *E. coli*, *S. aureus* and *S. pyogenes* were similar to the previously reported by **Dohmen et al. (1995) and Fredriksson et al. (1985)**.

Recent researches indicate that uterine infection is predominated by *E. coli* in the first week postpartum and *A. pyogenes* in the second week is associated with subsequent endometritis (**Gilbert et al., 2007; and Williams et al., 2007**).

In table (9), serological typing of 4 isolates of salmonellae revealed 3 serovars namely *S. Typhimurium* (2), *S. Enteritidis* (1) and *S. Kentucky* (1). These results are in accordance with **Ali and Ibrahim (2001)** who serotyped 6 isolates of Salmonella recovered from endometritis and the results revealed 3 serovars namely *S. Typhimurium* (4), *S. Saintpaul* (1) and *S. Abortus ovis* (1).

In table (10), serotyping of *E. coli*, revealed 10 different "O" serogroups of *E. coli*, the most prevalent serogroups were 0167, 0111, 0125, 0126, 078, 0119, 0128, 086, 026 and 0114. These results agree with **Ali and Ibrahim (2001)** who mentioned that the most prevalent serogroups of *E. coli* recovered from many cases of endometritis were 0125, 0167, 01, 086, 0151, 06, 0111, 0126, 0157, 0153, and 0119. The same results were recorded by many workers as causative agents of genital disorders in camels (**Awad et al., 1978; Hegazy et al., 1979 and Enany et al., 1990**).

As shown as in table (11), 46 sera from buffaloes infected with endometritis were examined for antibodies using (SAT). All examined sera were considered as positive when showing any degree of flocculation with the (SAT) (**Bruno et al., 1999**). Positive sera were subjected to (TAT) according to **Alton et al., (1988)**.

In high titers of TAT (1/160 and 1/320), the cytoplasmic protein can detect true positively between 88.8% - 100%, while in low titre of TAT the positively against cytoplasmic protein not exceeded 83% (**Topley and Wilson, 1998**).

It is important to state that the highest antibodies titres were showed with *E. coli* and *Staphylococcus aureus*.

Foley and Lynne (2008) mentioned that smaller number of serotypes of salmonella were significantly associated with animal disease including *S. Typhimurium*, *S. Enteritidis*, *S. Newport*, *S. Heidberg* and *S. Montevideo*.

Sensitivity of the isolated bacteria to antimicrobial agents as shown in tables (12&13) commonly used in endometritis therapy against *S. aureus*, *E. coli*, Salmonella, *S. pyogenes*, *A. bovis*, *A. pyogenes* and *P. aeruginosa*. revealed high susceptibility to enrofloxacin, gentamicin, and chloramphenicol. These results were in agreement with **Awad and El-Hariri (1980), Gandotra et al. (1992), Goswami et al. (1992), Pradhan et al. (1993), Anjaeneyulu et al. (1999), Ali and Ibrahim (2001), Abd El-Mola (2003), and Hassab El Naby and El Khnawy (2004)**. While, **Abd-El Rahman and Ibrahim (2007)** found that the most effective.

antibiotics were enrofloxacin, gentamicin and oxytetracycline. *Prajapati et al. (2005)* found that the most effective antibiotics were gentamicin followed enrofloxacin and chloramphenicol. *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%). It was found that these pathogens were highly resistant to penicillin G, ampicillin, streptomycin and oxytetracycline. They also found that isolates from repeat breeder cows and buffaloes were resistant to penicillin, ampicillin, neomycin and nalidixic acid with varying degree of drug resistance. *Ocal et al (2004)* revealed that gentamicin is a drug of choice in treatment of bovine uterine infection, the other antibiotics have moderate to less effectiveness against most pathogens such as oxytetracycline, amoxicillin and streptomycin, while, *Konigssonk (2001)* mentioned that oxytetracycline has no effect in the treatment of endometritis if used alone except if added to other antibiotic or synthetic hormone. *Yavari et al. (2007)* found that all isolates from clinical postpartum endometritis in dairy cows showed resistance to penicillin. *Pradhan et al. (1993)* recorded that most of uterine isolates were resistant to oxytetracycline. *Ali and Ibrahim (2001)* found that most of uterine bacterial isolates were resistant to erythromycin and streptomycin.

When looking to photographs, the apparently healthy uterus didn't show any pathological changes in the endometrium. The same result was observed by *Gani et al., (2008)*. *Studer and Morrow (1978)* reported that the presence of neutrophils in and beneath the surface epithelium, around glandular duct and scattered in superficial stroma during oestrus phase of cycle in normal endometrium, while, in luteal phase inflammatory cells are due to pathology of endometrium.

The microscopic lesions noticed in the present study were also supported by *Sar et al. (1996)*. They observed in acute endometritis stromal edema, congestion of blood vessels and mild to severe neutrophilic infiltration. They were due to acute inflammatory changes in the endometrium. The histopathological findings of chronic endometritis in the present study were in confirmation with those reported by *Sar et al. (1996)*, *Bajaj (2002)*, *Prajapati et al. (2005)* and *Azab et al. (2006)*. Moderate to severe periglandular fibrosis in chronic endometritis plays an important role in the reduction of uterine milk and alteration in protein synthesis, consequently early embryonic death, infertility and repeat breeding as mentioned by *Gonzalez et al. (1985)*.

The cystic dilatation of endometrial gland of varying sizes and numbers are atrophic changes and might be due to retention of endometrial secretion. It appears that chronic inflammatory changes causes extensive damage to uterine tissue with formation of pus in the clinical cases of endometritis, also atrophy of gland due to pressure of fibrous tissue proliferation in endometrial stroma (*Prajapati et al., 2005*).

This study recommends that:

All postpartum animals should be investigated by culture and sensitivity test in 1st week to exclude postpartum complications which may cause infertility in animals and affects animals reproduction.

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التغيرات الهستوباثولوجية لالتهاب الرحم فى الجاموس المسبب بالعدوى البكتيرية

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الملخص العربى

نتيجة لزيادة أهمية الجاموس كوحدة إنتاجية استهدفت هذه الدراسة التركيز على البكتيريا الموجودة فى الجهاز التناسلى السليم بالمقارنة مع حالات التهاب الرحم والذى يمكننا من تقييم دور البكتيريا على الجهاز التناسلى للجاموس وإجراء اختبار الحساسية لاختيار المضاد الحيوى الأكثر تأثيراً.

طبقاً لهذه الدراسة تم جمع ١٤٧ عينة من الجاموس السليم والمصاب بالتهابات رحمية صديدية أو صديدية مخاطية حيث تم أخذ مسحات من عنق الرحم والمهبل لـ ٧١ جاموسة (٢٥ سليمة و٤٦ تعاني من التهاب فى الرحم) من محافظتى الدقهلية ودمياط بالإضافة إلى ذلك تم أخذ مسحات من الرحم لـ 76 جاموسة (١٥ سليمة، ٦١ بها التهابات رحمية) بمجرى المنصورة - محافظة الدقهلية.

٣%)، أظهرت نتائج الفحص البكتريولوجى أن ميكروب الاشرشيا كولاى هو السائد فى حالات الإصابة بالتهابات الرحم فى الجاموس (٣٠,٢%) يليها المكروب العنقودى الذهبى بنسبة (١٩,٣%)، ميكروب سترىتوكوكس بيوجين (٨,٧%).

أظهر التصنيف السيرولوجى لسلاسل الاشرشيا كولاى المعزولة ١٠ عترات سيرولوجية اعتماداً على الانتيجين الخلوى كالاتى: O114, O26, O86, O128, O119, O78, O126, O125, O111, O167. وأيضاً تم تصنيف العترات المعزولة من السالمونيلا إلى سالمونيلا تيفيميوريم، سالمونيلا انترينيدس و سالمونيلا كنتاكى.

كما تمت دراسة حساسية المعزولات البكتيرية للمضادات الحيوية المختلفة باستخدام اختبار الحساسية وقد لوحظ أن أقوى هذه المجموعات التى تؤثر على الممرضات المسببة لالتهاب الرحم هى الانروفلوكساسين، الجينتاميسين، الكلورامفينيكول، النورفلوكساسين، بيبيراسلين يليها سيبروفلوكساسين، فلوموكس، اميكاسين، نيوميسين، نيتروفورنتين، سيفوبيرازون، سيفوتاكسيم، والدوكسيسيكلين، الاريثرومايسن والفلوميكوين كما كانت الميكروبات مقاومة لـ (ستربتوميسين، الامبيسيلين، السلفاميسازول ترايميثوبريم، الأوكسى تتراسيكلين، اللينكوسبتينو ميسين) وكانت الميكروبات ذات مقاومة عالية لـ البنسلين ج .