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STUDIES ON MICROBIAL INFECTION ASSOCIATED REPEAT BREEDER IN BUFFALOES AND COWS AND ITS SENSITIVITY TO DIFFERENT ANTIBIOTICS IN VITRO

(With 7 Tables)

By

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دراسات على العدوى الميكروبية المصاحبة لحالات الشياع المتكرر في الأبقار والجاموس وحساسية هذه الميكروبات لبعض المضادات الحيوية

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استهدفت الدراسة إلقاء الضوء على العدوى الميكروبية المختلطة (البكتيرية والفطرية) المصاحبة للشياع المتكرر في الأبقار والجاموس في محافظتي المنيا وأسيوط. اشتملت الدراسة على ١٢٠ حيوان (٥٠ جاموسة و٧٠ بقرة) تعانى من شيوع متكرر ٢٥ سليمة ظاهريا و٩٥ تعانى من التهابات رحمية مختلفة. أظهر الفحص الميكروبيولوجي إلى عزل ٢٠٠ عترة منها ٣٠ بنسبة (١٥%) من الحالات السليمة ظاهريا و ١٧٠ من حالات الالتهابات الرحمية بنسبة (٨٥%). وقد شملت العترات المعزولة على ١٦٠ عترة بكتيرية بنسبة (٨٠%) و ٢٥ عترة فطرية بنسبة (١٢,٥%) و١٥ عدوى مختلطة (بكتيرية وفطرية) بنسبة (٧,٥%). وكانت أهم العترات البكتيرية المعزولة هي أيشير يشيا كولاى بنسبة (١١,٩%) والكورينى بكتريم بيوجين بنسبة (٩,٤%) والميكروب العنقودي الذهبي بنسبة (٩,٤%) والميكروب السبحي فيجالز بنسبة (٨,١%) والميكروب السبحي بوفيز بنسبة (٧,٥%) والكورينى بكتريم بوفيز بنسبة (٦,٩%) والميكروب السبحي بيوجين بنسبة (٦,٩%) ومن الميكروبات اللاهوائية ميكروب الكلوستريديم بيرفرجينز بنسبة (٣,٧%) وميكروب ايروبكتريم لنتيم بنسبة (٣,١%) وقد شملت العزلات البكتيرية على عزل ٤٠ عدوى بكتيرية مشتركة بنسبة ٢٠%من اجمالى العترات المعزولة. وبالنسبة للعدوى الفطرية تم عزل فطر الاسبرجيلس والكنديدا بنسبة (٢٤%) لكل منهما وفطر البنسليم والفيوزاريم والخمائر بنسبة (١٢%) وفطر الميكور بنسبة (٨%) وكل من الابيذدا والروبيز بنسبة (٤%) من أجمالي الفطريات المعزولة. وبالنسبة للعدوى المختلطة (بكتريا وفطريات) نم عزل كل من أيشير يشيا كولاى مع الاسبرجيلس والميكروب العنقودي مع الاسبرجيلس وأيشير يشيا كولاى مع الفيوزاريم والميكروب السبحي مع كل من الخمائر والكنديدا والبروتيس مع الفيوزاريم بنسب مختلفة. وتم عمل اختبار الحساسية للعترات المعزولة باستخدام المضادات الحيوية المختلفة وقد وجد آن الأنروفلوكساسين والأوكس تتراسيكلين والجنتاميسين هم أكثر

المضادات الحيوية حساسية بينما كانت مقاومة لكل من نيومايسين وايرثرومايسين وامبسلين ومن خلال هذه الدراسة ينصح في حالات علاج الشيوع المتكرر أنه يجب الأخذ في الاعتبار العدوى المختلطة (البكتيرية والفطرية) لان استخدام المضادات الحيوية فقط يؤدى آلي نشاط الفطريات وعلى هذا ينصح باستخدام أدوية لها تأثير مزدوج على كل من البكتريا والفطريات بعد عمل اختبار الحساسية.

SUMMARY

The present study aimed to throw light on the microbial infection and special mixed infection (bacterial and fungus) associated repeat breeder in buffaloes and cows. This work was carried out on 120 cervico-vaginal and uterine swabs collected from (50 buffaloes and 70 cows) suffering from repeat breeding (25 subclinically and 95 clinically infected cows from dairy farms at EL-Minia and Assiut Provinces. Bacteriological examination revealed that, 200 different microbial causative agents (30 isolates with incidence of 15% for subclinically and 170 isolates with incidence of 85% for clinically infected animals). The total microbial infection represented by 120 (60%) single bacterial isolates, 40 (20%) mixed bacterial isolates, 25 (12.5%) fungi isolates and 15 (7.5%) mixed infection (bacterial and fungi). The most common aerobic microorganisms isolates were E.coli 19 (11.9%) followed by Croynebacterium pyogenes 15 (9.4%). The most common mixed bacterial isolates were E.coli + Croynebacterium pyogenes + Proteus spp with incidence of 22% and Staphylococcus aureus + Croynebacterium bovis with incidence of 20%. 25 fungi isolates (12.5%) from total microbial isolates were found. The most important fungi isolates were Aspergillus spp (24%) and Candida (24%). Fifteen cases out of 120 repeat breeder cases (12.5%) proved to have mixed infection (bacterial and fungal). The most common mixed infection caused by E.coli + Aspergillus spp (33.3%). After Sensitivity test, the most active antibiotics were Enerofluxacin, Oxytetracycline, Gentamycin and Nalidixic acid. Most bacterial isolates were resistant to Neomycin, Erythromycin and Ampicillin. Sensitivity test revealed that most bacterial isolates, in this study, were highly sensitive to Enerofluxacin, Oxytetracycline, Gentamycin and Nalidixic acid and resistant to Neomycin, Erythromycin and Ampicillin.

Key words: *Bacteria – fungus – repeat breeder – Buffaloes – cows*

INTRODUCTION

Repeat breeding in animals has great economic importance as it causes increased calving interval, less number of offspring, decreased milk production and wastage of time and money on treatment. The repeat breeder has long been a problem world wide with an overall incidence rate of 10-25% (Bartlet *et al.,* 1986). In Egypt, the incidence of repeat breeder syndrome ranged from 64.44% to 71.5% from the other infertility problems in buffaloes (Atalla, 1984 and Osman, 1984). Cervicitis and endometeritis may be responsible for early embryonic death or repeat breeding problems which are mostly caused by increase in the number of the bacteria and /or in their virulence (Blanch, *et al.*, 1992). Many bacteria present in genital tract as saprophytes but under unfavorable conditions might become pathogenic and causes clinical or sub-clinical signs of endometeritis. Yousef (1984) isolated seven types of microorganisms from 10 normal cows and 10 (29.4%) different types of microorganisms from 34 cases of repeat breeder cows. Deka *et al.* (1985) concluded that 75% of cows with abnormal parturition were positive for uterine microflora, 50% were considered pathogenic and 50% as non-pathogenic. Dawson (1963) and Shouman *et al.* (1977) reported that Micrococcus citrus, Bacillus subtilis, anthracoid, Streptococcus feacalis, Staphylococcus aureus, E.coli, Proteus and Corynebacterium bovis are the most important isolates from apparently healthy uteri of cows, while Shouman *et al.* (1983) and Olson *et al.* (1984) reported that Corynebacterium pyogens, E.coli, Streptococcus pyogens, Staphylococcus aureus and Pasteurella are the main pathogens isolated from cow suffering from endometeritis and pyometra. Many authors recorded the relation between repeat breeder and fungal infection. Singh *et al.* (1993), Verma *et al.* (1999) and Megahed *et al.* (2000) isolated various fungal species from repeat breeder buffaloes as Aspergillus, Penicillium and Fusarium. However, most clinician, during their handling to this problem, paid their attention only to the bacterial infection with excessive use of antibiotic or antiseptic which may aggravate the case.

The objective of the present study was to investigate microbial infection (bacterial and fungi) associated repeated breeders in buffaloes and cows and to test the sensitivity of the bacterial agents to some different antibiotics.

MATERIALS and METHODS

Animals:

This study was carried out on 50 buffaloes and 70 cows (pluriparous and 4-8 years old) from dairy farms at EL-Minia and Assiut Provinces, Egypt. These animals were suffering from repeat breeding (they were served naturally 3-5 times at successive periods without conception).

Clinical examination:

Every animal was examined rectally and vaginally according to the scheme given by Zemjants (1970) and Roberts (1971). Animals were examined by one examiner and with the same conditions under which most veterinarians deal with such cases. According to the size, consistency of the tubular tract and presence of pathological discharge, the examined animals were classified into two categories either subclinically infected without apparent discharge and no palpable abnormalities in their tubular tract (rectal examination) or clinically infected in the uterus and/or cervix, with or without presence of vaginal discharge as in table 1

Table 1: Number of animals examined and reproductive status

Animal species	No. Examined	Repeat breeder			
		Subclinical		Clinically infected	
		No.	%	No.	%
Buffaloes	50	10	20.0	40	80
Cows	70	15	21.4	55	78.6
Total	120	25	20.8	95	79.2

Bacteriological examination:

A total number of 120 cervico-vaginal and uterine swabs were collected by vaginal swabs and a sterile aluminum tampon after sterilized in hot air oven at 160^0C for 2 hours for bacteriological examination according to Sharaf *et al.* (1963), Osman and Abou-Gabal (1975) and Zaki and Fouad (1963).

a) Isolation and Identification of isolates: - according to Bucchnan *et al.* (1975), Wilson and Miles (1985) and Cruickshank *et al.* (1980)

The vaginal swabs were taken from the external os using sterile gauze swabs, while uterine swabs were taken by aluminum tampons which passed into uterus and left inside the uterine lumen with frequent rotations for few seconds before withdrawal the samples. The obtained swabs were placed directly into screw-capped bottles containing sterile

nutrient broth for bacteriological examination (Erich and Morrow 1980). The screw-capped bottles containing the samples were incubated at 37^{0}C for 24 hours to enhance growth and multiplication of microorganisms.

For aerobic microorganism isolates, a loopful from each sample was streaked on MacConkey's agar plates, Blood agar plates, Nutrient agar plates, Violet Red Bile Glucose agar plates and Baired-Parker agar plates. These plates were incubated at 37^{0}C for 24-48 hours. Different colonies were picked up and purified by subculturing on selective media, then kept on nutrient agar sloops to identify the microscopical appearance, culture character, motility, biochemical and serological tests For anaerobic microorganisms isolates, a loopful from each sample was inoculated into Thioglycolate broth medium "Oxoid, GM10) and then streaked on to Cooked meat medium ("Mast DM 120"), Neomycin blood agar medium (neomycin sulphate solution was added to the media just before the addition of blood to make final concentration of 150ug/ml). The inoculated solid media was incubated anaerobically at 37^{0}C for 24-48 hrs by using (Gas-pack anaerobic jar "BBL-814-12"). Strick anaerobic isolates were examined and identified for microscopically appearance, culture character, motility, then transferred to cooked meat medium for other biochemical tests as described by Koneman *et al.* (1992)

For fungal isolates, swabs from the same samples were cultivated onto plates of Sabouraud, dextrose agar medium (SDA) supplemented with chloramephnicol (50 mg/l) and incubated at 28^{0}C for 7-10 days till fungal growth was observed. The growing fungi were identified based on their macro and microscopic characteristics the isolated fungi were identified according to Cruickshank *et al.* (1980), Domasch *et al.* (1980) and Nirenberg (1989).

b) Sensitivity test:

The important isolates were tested for sensitivity to some chemotherapeutic agents. One ml of 24hrs.-broth cultures was spread on the surface of nutrient agar. Antibiotic sensitivity discs were placed on the surface seeded agar. Plates were incubated aerobically at 37^{0}C for 24hrs. The sensitivity was judged according to the diameter of clearance zone around the discs according to Quinn *et al.* (1994). Ten different antibiotic discs, supplied by Oxoid were used. These antibiotics were Neomycin (30ug), Gentamycin (10ug), Chloramphenical (30ug), and Oxytetracycline (30ug), Nalidixic acid (30ug), Kanamycin (30ug), Ampicillin (10ug), Penicillin (10ug), Erythromycin (15ug), and Enerofluxacin (10ug).

RESULTS

Results of the present study are presented in Tables from 2-7.

200 different microbial infections (25 microbial infections with incidence of 12.5% for subclinically infected and 175 microbial infections with incidence of 87.5% for clinically infected cases) were detected in this study. The total microbial infections were 120 (60%) for single bacterial isolates, 40 (20%) for mixed bacterial infection, 25 (12.5%) for fungal infection and 15 (7.5%) for mixed infection (bacterial and fungal). The incidences of different single bacterial isolates are shown in Table (3). The most common aerobic microorganisms isolates were E.coli 19 (11.9%) followed by Croynebacterium pyogenes 15 (9.4%) and Staphylococcus aureus 15 (9.4%). The most obligate anaerobic isolates found were Clostridium perfringenes with incidence of (3.7%) and Eubacterium lentum 5 (3.1%) for both subclinical and clinical cases. For mixed bacterial isolates, E.coli + Croynebacterium pyogenes + Proteus spp (22%) and Staphylococcus aureus + Croynebacterium bovis (20%) were prevalent as in Table (4). 25 fungal isolates with incidence of (12.5%) from total microbial isolates were detected (Table 5). The most common fungal isolates were 6 Aspergillus spp with incidence of (24%) and 6 Candida with incidence of (24%). E.coli + Aspergillus spp (33.3%) were the prevalent mixed infection found in all studied cases (Table 6).

Sensitivity test:

Sensitivity test: was carried out for estimation of the sensitivity of the 160 bacterial isolates to different antibiotics. The results of Sensitivity test are shown in Table (7). It was observed that most isolates 142 (88.75%) were found sensitivite to Enerofluxacin followed by Oxytetracycline 135 (84.37%), Gentamycin 125 (78.13%) and Nalidixic acid 118 (73.75%), while were resistant to Neomycin 132 (82.50%) followed by Erythromycin 130 (81.25%) and Ampicillin 128 (80.00%).

Table 2: Incidence and type of single and mixed infections of samples recovered from repeat breeder buffaloes and cows (subclinical and clinical cases)

Type of infection	No. of isolates		Repeat breeder			
			Subclinical (25)		Clinical cases (95)	
	No.	%	No.	%	No.	%
Single bacterial infection	120	60.0	15	7.5	105	52.5
Mixed bacterial infection	40	20.0	5	2.5	35	17.5
Fungal infection	25	12.5	2	1.0	23	11.5
Mixed bacterial and fungal	15	7.5	3	1.5	12	6.0
Total	200		25	12.5	175	875

Percentage infection relative to total number of microbial infection

Table 3: Incidence of total bacterial isolates from 120 cases of repeat breeder (subclinical and clinical cases) buffaloes and cows

Types of bacterial isolates	No. of isolates		Repeat breeder							
			Buffaloes				Cows			
			Subclinical (10)		Clinical (40)		Subclinical (15)		Clinical (55)	
A) Aerobic bacteria	No.	%	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus	15	9.4	1.0	0.63	4.0	2.5	1.0	0.63	9.0	5.6
Staphylococcus epidermidis	6	3.7	0.0	0.0	2.0	1.3	1.0	0.63	3.0	1.9
Streptococcus pyogenes	11	6.9	0.0	0.0	4.0	2.5	1.0	4.8	6.0	3.7
Streptococcus bovis	12	7.5	0.0	0.0	4.0	2.5	2.0	1.3	6.0	3.7
Streptococcus faecails	13	8.1	4.0	2.5	3.0	1.9	2.0	1.3	4.0	2.5
Klebsiella oxytoca	8	5.0	2.0	1.3	4.0	2.5	0.0	0.0	2.0	1.3
Croynebacterium bovis	11	6.9	0.0	0.0	3.0	1.9	2.0	1.3	6.0	3.7
Croynebacterium pyogenes	15	9.4	0.0	0.0	6.0	3.7	1.0	0.63	8.0	5.0
E.coli	19	11.9	2.0	1.3	6.0	3.7	3.0	1.9	8.0	5.0
Sarcina spp.	4	2.5	0.0	0.0	1.0	0.63	1.0	0.63	2.0	1.3
Citrobacter spp.	5	3.1	0.0	0.0	1.0	0.63	1.0	0.63	3.0	1.9
Pseudomonas spp.	9	5.6	2.0	1.3	2.0	1.3	1.0	0.63	4.0	2.5
Proteus spp.	8	5.0	1.0	0.63	3.0	1.9	0.0	0.0	4.0	2.5
Enterobacter aerogens	10	6.3	1.0	0.63	2.0	1.3	1.0	0.63	6.0	3.7
B) Obligate anaerobic										
Clostridium perfringenes	6	3.7	0.0	0.0	1.0	0.63	0.0	0.0	5.0	3.1
Eubacterium lentum	5	3.1	0.0	0.0	0.0	0.0	1.0	0.63	4.0	2.5
Bacteriodes spp.	3	1.9	0.0	0.0	1.0	0.63	0.0	0.0	2.0	1.3
Total	160		13	8.1	47	29.4	18	11.3	82	51.2

Percentage isolates relative to total number of bacteria isolates

Table 4: Type and incidence of mixed bacterial infection (samples recovered from 120 cases of repeat breeder buffaloes and cows)

Types of bacterial isolates	No. of isolates		Repeat breeder			
			Subclinical		Clinical	
	No.	%	No.	%	No.	%
E.coli + Streptococcus pyogenes + Streptococcus faecails	6	15.0	1	2.5	5	12.5
Staphylococcus aureus + Croynebacterium bovis	8	20.0	0.0	0.0	8	20.0
E.coli + Croynebacterium pyogenes + Proteus spp.	9	22.5	0.0	0.0	9	22.5
Pseudomonas spp. + Proteus spp.+ Sarcina spp	5	12.5	1	2.5	4	10.0
E.coli + Citrobacter spp + . Streptococcus faecails	4	10.0	1	2.5	3	7.5
Enterobacter aerogens + Klebsiella oxytoca	3	7.5	2	5.0	1	2.5
Croynebacterium pyogenes + Streptococcus bovis	5	12.5	0	0.0	5	10.0
Total	40		5	12.5	35	87.5

The percentage was calculated in relation to the total number of mixed bacterial infections.

Table 5: Incidence of fungal isolates from different cases of repeat breeder (subclinical and clinical cases) buffaloes and cows

Types of fungal isolates	No. of isolates		Repeat breeder							
			Buffaloes				Cows			
			Subclinical (10)		Clinical (40)		Subclinical (15)		Clinical (55)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus spp	6	24.0	0	0.0	2	8.0	1	4.0	3	12.0
Penicillium	3	12.0	0	0.0	1	4.0	0	0.0	2	8.0
Candida	6	24.0	1	4.0	2	8.0	1	4.0	2	8.0
Mucor	2	8.0	0	0.0	1	4.0	0	0.0	1	4.0
Fusarium	3	12.0	0	0.0	1	4.0	0	0.0	2	8.0
Absidia,	1	4.0	0	0.0	0	0.0	0	0.0	1	4.0
Rhizoous	1	4.0	0	0.0	0	0.0	0	0.0	1	4.0
Yeast	3	12.0	1	4.0	1	4.0	0	0.0	1	4.0
Total	25		2	8.0	8	32.0	2	8.0	13	52.0

Percentage isolates relative to total number of fungal isolates

Table 6: Incidence of mixed infection (bacterial and fungal) of samples recovered from different cases of repeat breeder buffaloes and cows

Mixed infection isolates	No. of mixed isolates		Repeat breeder							
			Buffaloes				Cows			
			Subclinical		Clinical		Subclinical		Clinical	
	No.	%	No	%	No	%	No	%	No	%
Staphylococci spp. +Aspergillus spp.	4	26.7	0	0.0	1	6.7	1	6.7	2	13.3
E.coli + Aspergillus spp.	5	33.3	0	0.0	2	13.3	1	6.7	2	13.3
E.coli + Fusarium spp.	3	20.0	0	0.0	1	6.7	0	0.0	2	13.3
Proteus spp. + Fusarium	1	6.7	0	0.0	1	6.7	0	0.0	0.0	0.0
Yeast + Streptococcus faecails +Candida	2	13.3	0.0	0.0	1	6.7	0.0	0.0	1	6.7
Total	15		0.0	0.0	6	40.0	2	13.3	7	46.7

The percentage was calculated in relation to the total number of mixed infection (bacterial and fungal).

Table 7: The results of Sensitivity test for the isolated microorganisms

Type of antibiotics	Degree of sensitive					
	Sensitive		Intermediate		Resistant	
	No.	%	No	%	No	%
Neomycin (30ug),	10	6.25	18	11.25	132	82.50
Gentamycin (10ug)	125	78.13	15	9.37	20	12.50
Chloramphenical (30ug)	107	66.87	25	15.63	28	17.50
Oxytetracycline (30ug)	135	84.37	17	10.63	8	5.00
Nalidixic acid (30ug)	118	73.75	32	20.00	10	6.25
Kanamycin (30ug)	85	52.13	28	17.50	47	29.37
Ampicillin (10ug)	30	18.75	12	7.50	128	80.00
Erythromycin (15ug)	13	8.12	17	10.63	130	81.25
Penicillin (10ug)	45	28.13	36	22.50	79	49.37
Enerofluxacin (10ug).	142	88.75	10	6.25	8	5.00

DISCUSSION

In the present study, 200 different microbial infections (30 microbial infections with incidence of (15%) from subclinical and 170 microbial infection with incidence of (85%) from clinical cases) were isolated. Healthy uterus has its own saprophytic bacteria but under unfavorable conditions might become pathogenic and causes clinical or sub-clinical signs of endometritis (Gunter *et al.*, 1955, Dawson, 1950 and Roberts, 1971 and AboEl-Ata, 1973). According to the present findings, it was clear that infection with single bacterial isolate prevailed among repeat breeding cows with palpable abnormalities in their genitalia upon rectal examination (52.5%) followed by the mixed bacterial infection (17.5%). In agreement with the present results,

Yousef (1984) isolated seven types of bacteria from 10 normal cows and 10 (29.4%) different types of bacteria from 34 cases of repeat breeder cows. Deka *et al.* (1979) reported that 36 (51.4%) out of 70 infertile cows were positive for pathogenic bacterial infection compared with only 6 (20%) out of 30 were positive for non-pathogenic bacteria from apparently normal infertile cows which reported by Metwelly (2002) who isolated 56 (65.1%) positive cases for bacteria from 148 buffalo-cows suffering from repeat breeding. HassabEl-Naby and El-Ekhnawy (2004) reported that mixed and single bacterial infection in repeat breeding cows were 50.8 and 49.1% respectively, while it is lower than obtained by Awad *et al.* (1977). Osman *et al.* (1984) reported that many bacteria were isolated from the healthy and diseased genitalia of buffalo cows and mixed infections were frequently isolated.

In the present investigation, the most predominating aerobic microorganisms isolates were E.coli 19 (11.9%) followed by Croynebacterium pyogenes 15 (9.4%) and Staphylococcus aureus 15 (9.4%). Similar isolates were found and isolated from the uteri of cows with history of repeat breeding, retained placenta and metritis (*Gunter et al.*, 1955, Namboothripad and Raja, 1976, Zafrucas, 1976, Osman, 1984, Eduvie *et al.*, 1984, El-Azab *et al.*, 1988 and Ramakrishna, 1996). Staph.epidermidis, Anthracoid and E.coli were the most bacterial infection isolated from cervices of typical repeat breeder cows and buffaloes (El-Azab *et al.*, 1980, Messier *et al.* 1984, Selim *et al.* 1998 and Hassab El-Naby and El-Ekhnay 2004).

It is noteworthy that E.coli, Citrobacter, Proteus, Staphylococci, Pseudomonas spp. And Klebsiella spp were isolated from the uteri of normal cows and buffaloes (Zerb, *et al.*, 2001 and Metwelly 2002). Some workers believe that the uterus of the cows at the time of first inseminations is nearly always sterile while several others described that repeat breeding is mainly due to the presence of subclinical infections in the uterus form the opportunistic uterine microflora (Javed and Khan 1991).

E.coli + Croynebacterium pyogenes + Proteus spp (22%), Staphylococcus aureus + Croynebacterium bovis (20%) and E.coli + Streptococcus pyogenes + Streptococcus faecails (15%) were the most encountered mixed infections isolated. This was in conformity with previous reports pointed that E.coli with Proteus and Citrobacter (Shouman *et al.*, 1977), E.coli + Klebsiella with incidence of 25.0% and E.coli + Staphylococci with incidence of 14.3% (Metwelly, 2001) as well as E.coli + Strep. facalis + Klbs.pneum, E.coli + Staph.aureus +

Proteuss .pp, Strept. agalactiae + Staph.aureus and Pseud.aeruginosa + Strept.faecalis (Hassab El-Naby and El-Ekhnawy, 2004) were isolated from repeat breeder cases.

In the present study, 25 fungal isolates (12.5%) from total microbial isolates (23 (11.5%) from the clinically diagnosed repeat breeder cows and 2 (1.0%) from subclinically affected animals) were identified. Some of the affected animals studied here subjected to treatment with repetitive doses of disinfectants and antibiotics. Intra-uterine infusion of disinfectants as well as antibiotics suppresses natural defense mechanisms (Frank *et al.,* 1983). Also, excessive prolonged intra-uterine infusion of antibiotics in treatment of chronic endometritis is usually followed by establishment of fungi and yeasts in the genital tract of mares and cows (Cited after Ramoun *et al.,* 2002).

The most common fungal isolates found in this study were Aspergillus spp (24%), Candida (24%), Penicillium (12%) and Fusarium (12%). These results are in accordance with previous studies (Sinha *et al.,* 1980, Singh *et al.,* 1993 and Verma *et al.* 1999) but with lower incidences. Megahed *et al.* (2000) isolated Aspergillus spp., Penicillium spp., Fusarium spp. and Drechslera spp. with incidences of 69.81%, 18.87%, 5.66% and 5.66%, respectively.

For the mixed infection (bacterial and fungal), 15 mixed infections (7.5%) from the total microbial infection were found. These results are lower than that obtained by Sinha *et al.* (1980) who recorded 17 (29.3%) mixed infections out of 58 repeat breeders and Metwelly (2002) isolated 30 (34%) cases mixed infections (bacterial and fungal) from 148 buffalo-cows suffering from repeat breeding.

E.coli + Aspergillus spp (33.3%), Staphylococci spp. +Aspergillus spp (26.7%), E.coli + Fusarium spp (20%) were the most mixed infections isolated here. Similarly, Metwelly (2002) isolated 12 (40%) for (E.coli + Aspergillus spp), 4 (13.3%) for (E.coli + Aspergillus spp + Fusarium spp), 8 (26.7%) for (E.coli + Penicillium spp) and 6 (20.0%) for (Staphylococci spp. +Aspergillus spp) from repeated breeder buffalo cows.

Sensitivity test:

The most active antibiotics against the bacterial isolates were Enerofloxacin (88.75%) followed by Oxytetracycline (84.37%), Gentamycin (78.13%) and Nalidixic acid (73.75%). Similarly, Vicek and Savobodova (1985) reported that the bacterial isolates were susceptible to Oxytetracycline and Chloramphenicol. Similar results were obtained by Megahed (1986), Ramakrishna (1996). In accordance

to our results, Metwally (2001) found that, the in-vitro antimicrobial susceptibility of bacterial isolates from cows with endometeritis to Enrofluxacin, Oxytetracycline, Gentamycin and Ampicillin were 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 Cifrofloxacin followed by Gentamicin 115 (74%) and Chloramphenicol (67%). Hassab El-Naby and El-Ekhnawy (2004) concluded that the bacteria causing repeat breeding in cattle and buffaloes were more sensitive to Enerofloxacin, Gentamicin and Chloramphenicol while other antibiotics have moderate to less effectiveness against most pathogens. The majority of bacterial isolates were resistant to Neomycin (82.50%), Erythromycin (81.25%) and Ampicillin (80.00%). These results were nearly similar to those obtained by Awad *et al.* (1977) and Megahed (1986). Refaat (1980) reported that the isolated bacteria from buffaloe-cows suffering repeat breeding were moderately sensitive to Erythromycin. Karwani and Aulakh (2004) found that isolates from repeat breeder cattle and buffaloes were resistant to Penicillin, Ampicillin, Neomycin and Naledixic acid with varying degrees of drug resistance.

The present findings, in addition to the aforementioned reports are of great importance to direct the veterinarians' attention for the subclinical cases that neglected without correct diagnosis and proper treatment. Keeping in view the present findings and cited statement reported here, it is suggested that, of the repeat breeding animals, clinical examination along with isolation of micro-organisms and sensitivity test be routinely performed to ascertain the cause and prognosis of the case. Mixed infections with bacteria and fungus must be taken in consideration upon dealing with repeat breeding problem.

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