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MYCOTIC AND BACTERIAL AGENTS CAUSING REPEAT BREEDING IN THE BUFFALO-COWS AND TRIALS FOR THEIR TREATMENT

(With 6 Tables)

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العوامل الفطرية والبكتيرية المؤثرة على ظاهرة الشياع المتكرر في الجاموس ومحاولات علاجها

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استهدفت الدراسة استبيان انواع البكتريا والفطريات التي تؤدى إلى ظاهرة الشياع المتكـــرر في الجاموس وبعض محاولات علاجها. تم اجراء الدراسة على عدد ١٤٨ جاموسة وكـــانت الولادة طبيعية في جميعهم وكان الشياع يتكرر بانتظام من (٢٠ – ٢١ يوم) بـــالرغم مـــن تلقيحهم بطلائق عالية الخصوبة وفي الوقت المناسب. اظهر الفحص المـــهبلي والمســـتقيمي سلامة الأعضاء التناسلية ظاهريا. تم تجميع مسحات من عنق الرحم والمهبل بطريقة معقمة ونمت زراعتها على أوساط مختلفة للبكتريا والفطريات. اتضح من العـــزل أن ٦٢ حالـــة سالبة و٥٦ حالة بها عدوى بكتيرية فقط و٣٠ حالة بها عدوى مختلطة بكتيريـــة وفطريـــة. كانت أنواع البكتريا المعزولة في ٥٦ حالة هى ايشيريشيا القولون في ١٦ حالة وايشيريشـــيا القولون والكليسيلا في ١٤ حالة والكليسيلا في ٤ حالات وايشيريشيا القولـــون والمكـــروب العنقودى في ٨ حالات وسودوموناس في ٦ حالات والمكروب العنقـــودى فـــي ٨ حـــالات. اظهر اختبار الحساسية الذى تم اجـــراؤه أن الانروفلوكساســـين والاوكســـى تتراســـيكلين والجنتاميسين هم انشط المضادات الحيويـــة بنســـب ٨٥,٧ % و ٧٥,٠ % و ٦٩,٦% على الترتيب . تم علاج كل حالة بالمضاد الحيوي المناسب وتم التلقيح طبيعيا وتم اختبـــار الحمل بالجس عن طريق المستقيم عند ٤٢ يوم بعد التلقيح. أظهرت النتائج أن نسبة الحمل الكلية والفترة من العلاج للإخصاب وعدد التلقيحات اللازمة لكل إخصاب هـــى ٨٩,٢٨% و ١,١٦ + ٥,٣ يوم ٠,٦٢,١ + ٠,٢٥ تلقيحة على الترتيب . أما الثلاثون حالة التـــي بـــها عدوى بكتيرية و فطرية مختلطة فقد أظهر العزل الميكروبيولوجى وجود ايشيريشيا القولـــون و الاسبرجيلس في ١٢ حالة و ايشيريشيا القولون و الاسبرجيلس و الفيوزاريوم في ٤ حالات و ايشيريشيا القولون والبنسيللين في ٨ حالات والميكروب العنقودى والاسبرجيلس فـــي ٦ حالات . تم تقسيم هذه الحالات (٣٠ حالة) عشوائيا إلى ثلاث مجموعات (١٠ حالات في كل مجموعة) تم علاج المجموعة الأولى باللوجولز اليودي أسبوعيا لمدة ٣ أسابيع وعولجـــت

المجموعة الثانية بمحلول اللوتاجين ٤% أسبوعيا لمدة ٣ أسابيع أما المجموعة الثالثة فتم علاجها بالجينوبيوتك المعلق في محلول الملح أسبوعيا لمدة ٣أسابيع. تم التلقيح طبيعيا بعد العلاج وتم تشخيص الحمل بالجس عن طريق المستقيم ٤٢يوم بعد التلقيح. أظهرت النتائج أن نسبة الحمل الكلية و الفترة من العلاج للإخصاب و عدد التلقيحات اللازمة لكل إخصاب هي ٦٦ ,٨٦% و ٨٥, ٢٦ + ٩, ٤ يوم و ٩٦, ١ + ٣٦, ٠ تلقيحة على الترتيب. من هذه الدراسة يمكن استخلاص أن استخدام المضادات الحيوية فقط في علاج ظاهرة الشياع المتكرر في الجاموس بدون عزل ميكروبي ربما يؤدى إلى قلة الاستجابة وذلك لان ٩, ٣٤% من الحالات المصابة بها عدوى بكتيرية وفطرية مختلطة ولان استخدام المضادات الحيوية فقط يؤدى إلى نشاط الفطريات. وعلى هذا ننصح باستخدام أدوية أو كيماويات لها تأثير مزدوج على كل من البكتريا والفطريات.

SUMMARY

The aim of this study was to investigate the microbial (fungi and bacteria) causes of repeat breeder syndrome in the buffalo-cows and selecting trials for treatment. This study was carried out on 148 buffalo-cows, that calved normally and had regular estrous cycles. They were served naturally 3 times by fertile buffalo-bulls at proper time without conception. Vaginal and rectal examinations revealed apparently normal genital organs. Cervico-vaginal swabs were collected under aseptic condition and cultured on different media for fungi and bacteria. 86 cases only (58.1%) had bacterial and/or fungal infections while other 62 cases (41.9%) were negative. Fungal and bacterial identifications revealed that, 56 (65.1%) cases had bacterial infection only (E. coli in 16 cases; E.coli + Klebsiela spp. in 14 cases, Klebsiela spp. in 4 cases; E. coli + Staphylococci spp. in 8 cases; Pseudomonas spp. in 6 cases and Staphylococci spp. in 8 cases). The most active 3 antibiotics after sensitivity tests were Enrofulxacin, Oxytetracycline and Gentamicin, which were active against 85.7, 75.0 and 69.6% from bacterial isolates respectively. Each case was treated by the most susceptible antibiotic. Treated animals were served naturally and pregnancy was diagnosed by rectal palpation 42 days post service. Total pregnancy rate, treatment conception interval (TCI) and service /conception (S/C) were 89.28, 16.1±3.5 days and 1.62±0.25 respectively. The other 30 (34.9%) cases had mixed infection (fungi and bacteria). E. coli + Aspergillus spp.; E. coli + Aspergillus spp. + Fusarium spp.; E. coli + Penicillium spp. and Staphylococci spp. + Aspergillus spp. were isolated in 12, 4, 8 and 6 cases respectively. These cases (30) were divided into 3 equal groups. 1st group was received lugol's iodine i.u. 3 times weekly interval. 2nd group was received lotagen (4%) 3 times weekly interval. 3rd group was

received Gynobiotic suspended in saline 3 times weekly interval. Treated animals were served naturally and pregnancy was diagnosed by rectal palpation 42 days post service. Total pregnancy rate, TCI and S/C were 86.66%, 26.85±4.9 days and 1.96±0.36 respectively. In conclusion: Uses of antibiotics alone in cases of repeat breeder buffalo-cows without microbial isolation may lead to low response that because 34.9 % of the infected cases had mixed fungal and bacterial infection and uses of antibiotics make the animals more susceptible to fungal infections. So, it is advisable, in treatment of repeat breeder, to use drugs or medicaments have both antibacterial and antifungal effects.

Key words: *Mycoticand bacterial agents, repeat breeding, buffalo-cows.*

INTRODUCTION

Buffaloes are of great agricultural importance in the developing countries in view of the fact that they provide meat and dairy products as well as power (Gordon, 1996). The fertility problem represents a major economic obstacle among breeding buffaloes (Selim *et al.*, 1998).

Cows and buffaloes which have regular estrous cycles and appear normal on routine clinical examination but failed to become pregnant following three or more breedings were considered as repeat breeder females (Bartlet *et al.*, 1986 and Megahed *et al.*, 2000). The repeat breeder has long been a problem world wide to dairymen, with an overall incidence rate of 10-25% (Bartlet *et al.*, 1986). The principal losses from repeat breeder syndrome are economic and represent 10-15% of total losses in cattle industry as a result of reduced breeding efficiency (Lafi and Kaneene, 1988).

This study aimed to identify the microbial (bacteria and fungi) causes of repeat breeder buffalo-cows and different trials for treatment.

MATERIAL AND METHODS

This study was carried out on 148 buffalo-cows in private farms at Kafr El-Sheikh province, Egypt. They calved normally and had regular estrous cycles. They were served naturally 3 times by fertile buffalo-bulls at proper times and not conceived. Vaginal and rectal examinations revealed apparently normal genital organs.

Cervico-vaginal swabs were collected under complete aseptic condition. Each swab was cultured in the Faculty of Veterinary Medicine, Alexandria University, Egypt, onto 3 plates of Sabouraud's dextrose agar medium (SDA) supplemented with chloramephnicol (50mg/L) and incubated at 28oC for 7-10 days. The isolated fungi were identified according to Domasch *et al.* (1980) and Nirenberg (1989).

The cases that had fungal infections were divided into 3 groups. 1st group received Lugol's iodine (150 ml) i.u. 3 times at weekly interval, 2nd group received Iotagen, 150 ml i.u. (4%, Schering-Plough Animal Health) 3 times at weekly interval and the 3rd group received 2 oblets of Gynobiotic suspended in 150 ml saline 3 times at weekly interval (each oblet contains 300,000 i.u. Nystatin and 1000,000 i.u. Chlortetracycline; Vetoquinol Veterinary Pharmaceuticals 70204 Lure Cedex France).

The same swabs were cultivated in nutrient broth and incubated overnight at 37oC then cultured on nutrient agar, blood agar and MacConkey's agar plates. Incubation was done at 37oC for 48 hours and suspected colonies were identified according to Koneman *et al.* (1992). Antibacterial susceptibility tests were performed on isolated organisms according to Quinn *et al.* (1994). Each case was treated by the most susceptible antibiotic.

Treated animals were served naturally and pregnancy was diagnosed by rectal palpation 42 days post service. The obtained results were statistically analyzed using Statistical Analysis System (SAS, 1987).

RESULTS

Results are presented in Tables 1-6.

The results of microbial isolation revealed that, 86 cases only (58.1%) had bacterial or mixed infections while other 62 cases (41.9%) were negative (Table 1).

Table 1: Results of microbial examination of the repeat breeder buffalo-cows

Type of infection	Cases No.	Percentages
Negative	62	41.9
Bacterial	56	37.8
Mixed bacterial and fungal	30	20.3
Total	**148**	**100**

Fungal and bacterial identifications revealed that, 56 (65.1%) cases had bacterial infection only (E. coli in 16 cases; E.coli + Klebsiela spp. in 14 cases, Klebsiela spp. in 4 cases; E. coli + Staphylococci spp. in 8 cases; Pseudomonas spp. in 6 cases and Staphylococci spp. in 8 cases) (Table 2).

Table 2: Bacterial species in the examined repeat breeder buffalo-cows with bacterial infection.

Bacterial species	Cases No.	Percentages
E. coli	16	28.6
E. coli + Klebsiela spp.	14	25.0
Klebsiela spp.	4	7.1
E. coli + Staphylococci spp.	8	14.3
Pseudomonas spp.	6	10.7
Staphylococci spp.	8	14.3
Total	**56**	**100**

The most active 3 antibiotics after sensitivity tests were Enrofulxacin, Oxytetracycline and Gentamicin, which were active against 85.7, 75.0 and 69.6% from bacterial isolates respectively (Table 4).

From the results of antibacterial sensitivity tests, each buffalo-cow with bacterial infection was treated intrauterine by the most susceptible antibiotic, as follows:

Enrofluxacin (Spectrama-Vet. Amoun Pharmaceutical Co. Egypt) 5 ml + 20 ml distilled water, 3 times at 3 days interval (32 cases).

Oxytetracycline (Pan-terramycine, Pfizer-Egypt, S.A.E. Cairo ARE) 30 ml, 3 times at 3 days interval (17 cases).

Gentamicin sulphate (Gentamicin 5%, Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) 5 ml + 20 ml distilled water, 3 times at 3 days interval (7 cases).

Following the treatment the total pregnancy rate (TPR), treatment conception interval (TCI) and services per conception (S/C) were 89.28, 16.1±3.5 days and 1.62±0.25 respectively (Table 5).

The other 30 (34.9%) cases had mixed infection (fungi and bacteria). E. coli + Aspergillus spp.; E. coli + Aspergillus spp. + Fusarium spp.; E. coli + Penicillium spp. and Staphylococci spp. + Aspergillus spp. were isolated in 12, 4, 8 and 6 cases respectively (Table 3).

Table 3: Bacterial and fungal species in the examined repeat breeder buffalo-cows with mixed bacterial and fungal infection

Isolates	Cases No.	Percentages
E. coli + Aspergillus spp.	12	40.0
E. coli + Aspergillus spp. + Fusarium spp.	4	13.3
E. coli + penicillium spp.	8	26.7
Staphylococci spp. + Aspergillus spp	6	20.0
Total	**30**	**100**

Following treatment the TPR, TCI and S/C were 90.0%, 26.66±5.1 days and 1.89±0.27 respectively in the 1st group (Lugol's iodine treated group), 90.0%, 29.66±3.7 days and 1.89±0.19 in the 2nd group (lotagen treated group) respectively and 80.0%, 23.87±3.5 days and 2.13±0.31 in the 3rd group (Gynobiotic treated group) respectively (Table 6).

Table 4: The most active antibiotic after the sensitivity tests against bacterial isolates from 56 repeat breeder buffalo-cows with bacterial infections .

Type of antibiotic	Sensitive isolates No.	Percentages
Enrofluxacin	48	85.7
Oxytetracycline	42	75.0
Gentamicin	39	69.6
Ampicillin	13	23.2

Table 5: Reproductive parameters of repeat breeder buffalo-cows with bacterial infections after treatment by the most susceptible antibiotic (means ± SME)

Antibiotic	Cases No.	TCI (days)	1st SPR	Total pregnancy rate*	S/C
Enrofluxacin	32	15.6±3.2 [b]	81.25% [a]	93.75% [a]	1.47±0.17 [b]
Oxytetracycline	17	15.5±2.8 [b]	70.58% [b]	82.35% [b]	1.85±0.22 [a]
Gentamicin	7	20.0±5.3 [a]	71.40% [b]	85.71% [ab]	1.83±0.19 [a]
Total	**56**	**16.1±3.5**	**76.78%**	**89.28%**	**1.62±0.25**

Means in the same column carry different letters are significantly different ($P < 0.05$)
*Total pregnancy rate = Pregnancy rate after 3 services
1st SPR = First service pregnancy rate.

Table 6: Reproductive parameters of repeat breeder buffalo-cows with mixed bacterial and fungal infections (means ± SME)

TREATMENTS	Cases No.	TCI (days)	1st SPR	Total pregnancy rate*	S/C
Lugol's iodine	10	26.66±5.1 [ab]	60.0% [a]	90.0% [a]	1.89±0.27 [b]
Lotagen	10	29.66±3.7 [a]	50.0% [b]	90.0% [a]	1.89±0.19 [b]
Gynobiotic	10	23.87±3.5 [b]	60.0% [a]	80.0% [b]	2.13±0.31 [a]
Total	**30**	**26.85±4.9**	**56.66%**	**86.66%**	**1.96±0.36**

Means in the same column carry different letters are significantly different ($P < 0.05$)
*Total pregnancy rate = Pregnancy rate after 3 services.

DISCUSSION

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). The main cause of repeat breeder syndrome in buffaloes is the genital tract infection (Roberts, 1971). The drug of choice for treatment of repeat breeder syndrome depends on types of the present organisms, the duration and extent of the tissue damages when uterine infusions are used (Hardenbrook, 1958). The same author added that, volume of the fluid injected intrauterine was variable with the intent of the clinician, the drug used, the stage of estrous cycle and the size of the uterus. The effect of genital infection on the fertility is through the damage of gametes and/or through adverse effect on the development of embryos as well as endometritis that may impair implantation (Rasbech, 1950).

In this study, 62 out 148 repeat breeder buffaloes were negative for bacterial or fungal isolation, 56 cases had bacterial infections only while other 30 cases had mixed bacterial and fungal infections (Table 1).

The isolated bacterial species in 56 bacterially infected repeat breeder buffaloes were E. coli (16 cases), E. coli + Klebsiela spp. (14 cases), Klebsiela spp. (4 cases), E. coli + Staphylococci spp. (8 cases), Pseudomonas spp. (6 cases) and Staphylococci spp. (8 cases) (Table 2).

These results are in agreement with that of Selim *et al.* (1998). They isolated E. coli in 8 out 11 repeat breeder buffaloes and Pseudomonas acrogenosa in other 3 cases. Also, Gunter *et al.* (1955) recorded that, Pseudomonas aerogenosa, Enterococci and E. coli were mainly saprophytic in normal genital tract while in infertile cows, the strains were primarily pathogenic. The most important bacterial isolates from buffaloes with chronic endometritis were E. coli and Pseudomonas aerogenosa (Shouman *et al.*, 1983). Messier *et al.* (1984) isolated

Pseudomonas aerogenosa in 2 out 6 repeat breeder cows and E. coli from cows with metritis and normal cows. The most prevalent strain isolated from cows with endometritis was E. coli and the most virulent strains when inoculated in mice was Pseudomonas aerogenosa (Safronova *et al.*, 1991). Also, Jacob *et al.* (1995) isolated Staphylococci spp. from clinical cases of endometritis.

Ramakrishna (1996) found that, 46 out 60 samples of cervico-vaginal discharge from repeat breeder cows were culture positive. He added that, the most common isolate was E. coli followed by Staphylococci, Streptococci, Corynebacterium, Proteus and Pseudomonas. Nguyen Van Thanh (1997) found that, 9 buffaloes with metritis were proved that all infected with E. coli, Staphylococci and Streptococci, 7 with Salmonella, 2 with Pseudomonas and one with Corynebacterium. Also, Metwelly (2001) recorded that, the most common bacterial isolates from cows suffered from endometritis were E. coli and Staphylococci spp.

In this study, the most active 3 antibiotics against the bacterial isolates were Enrofluxacin, Oxytetracycline and Gentamicin. They act against 85.7%, 75.0% and 69.6% bacterial isolates respectively (Table 4). These results are in agreement with finding of Jacob *et al.* (1995). They recorded that, isolated staphylococci from cases with endometritis were sensitive to treatment with Gentamicin. Ramakrishna (1996) found that, bacterial isolates from repeat breeder cows were sensitive to Gentamicin (89.1%) and tetracycline (58.6%). Also, Selim *et al.* (998) showed that, the isolated Pseudomonas spp. from repeat breeder buffaloes appeared to be sensitive to Ampicillin, Gentamicin and Chloramphnicol. However, Metwelly (2001) recorded that, the in-vitro antimicrobial susceptibility of bacterial isolates from cows with endometritis proved that, Enrofluxacin, Oxytetracycline, Gentamicin and Ampicillin were the most active (96.0, 89.0, 85.0 and 85.0% respectively).

The pregnancy rate after the first sevice in animals treated by Enrofluxacin, Oxytetracycline and Gentamicin were 81.25%, 70.58% and 71.40% respectively, while, The total pregnancy rates were 93.75, 82.35 and 85.71% respectively. The lowest S/C (1.47±0.17) was obtained in cases treated by Enrofluxacin, while the highest S/C (1.85±0.22 and 1.83±0.19) were obtained in cases treated by Oxytetracycline and Gentamicin respectively (Table 5).

These results are in agreement with that of Abdel-Gawad *et al.* (2000). They reported that, the conception rate in repeat breeder

buffaloes treated by Gentamicin was 67.85±8.18%. Also, Metwelly (2001) obtained total conception rates of 79.59, 73.07 and 67.69% in cows with endometritis and treated by Enrofluxacin, Gentamicin and Oxytetracycline respectively. He added that, the lowest S/C (1.30±0.22) was obtained in cows treated by Gentamicin while, the S/C were 1.64±0.11 and 1.50±0.23 in cows treated by Enrofluxacin and Oxytetracycline respectively.

Thirty animals out of 148 repeat breeder buffaloes proved to have mixed bacterial and fungal infections. These results are nearly similar to that of Sinha *et al.* (1980). They recorded that, 17 out 58 repeat breeder buffaloes proved to have fungal infections. The isolated fungal species in this study were E. coli + Aspergillus spp.; E. coli + Aspergillus spp. + Fusarium spp.; E. coli + Penicillium spp. and Staphylococci spp. + Aspergillus spp. were isolated in 12, 4, 8 and 6 cases respectively (Table 3). These results are in agreement with finding of Singh *et al.* (1993). They isolated 9 different fungal species from repeat breeder buffaloes as Aspergillus (34.78%), Penicillium (4.35%), Fusarium (6.52), Mucor, Candida, Absidia, Rhizopus, Rhodotorula and Geotrium. While, Verma *et al.* (1999) found that, the incidence of Aspergillus spp. infection in repeat breeder buffaloes was 43.7%. However, Megahed *et al.* (2000) recorded that, the isolated fungal spp. from repeat breeder buffaloes were Aspergillus spp, Penicillium spp., Fusarium spp. and Drechslera specifera with percentages of 69.81, 18.87, 5.66 and 5.66% respectively.

Infections of the female genital tract of domestic animals have not received much attention in the past. However, Giri *et al.* (1994) and Verma *et al.* (1999) recorded that, with indiscriminate use of antibiotics and hormonal therapy, mycotic infections are becoming more common. They added that, using antibiotics or hydrocortisone for long periods must be avoided as they decrease the host resistance and make them highly susceptible to fungal infections.

Ttreatment of repeat breeder buffaloes with mixed bacterial and fungal infections by Lugol's iodine, Lotagen and Gynobiotic resulted in total pregnancy rates of 90.0, 90.0 and 80.0% respectively with average 86.66% (Table 6). These results are in agreement with that of El-Naggar *et al.* (1983). They recorded that, lotagen (Methyl condensation product of Metacresol Sulfonic acid + Formaldhyde) is the common disinfectant used in the treatment of endometritis in cows and buffaloes after Lugol's iodine. They added that, the conception rate in repeat breeder buffaloes treated by lotagen was 66.6% while in that treated by lugol's iodine was 59.9%. Abboud *et al.* (1989) showed that, the use of lugol's iodine was

efficient in treatment of repeat breeder buffaloes. The first service conception rate in repeat breeder buffaloes treated by Lugol's iodine was 37.83% (Bakr, 1996). Malinowski *et al.* (1998) used Gynobiotic successfully in treatment of postpartum disorders in cows. Megahed *et al.* (2000) concluded that, the fungi mycotoxins play an important role in causing repeat breeding and infertility problems in buffaloes. They added that, uses of intrauterine infusion of 4% Lotagen in buffaloes with fungal infection resulted in total pregnancy rate of 84.0%.

It could be concluded that, uses of antibiotics alone in cases of repeat breeder buffalo-cows without microbial isolation may lead to low response that because 34.9 % of infected cases had mixed fungal and bacterial infection and uses of antibiotics make the animals more susceptible to fungal infections. So, it is advisable, in treatment of repeat breeder, to use drugs or medicaments have both antibacterial and antifungal effects.

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