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FUNGAL AGENTS ASSOCIATED WITH REPEAT BREEDING IN EGYPTIAN BUFFALO-COWS AND FIELD TRIALS FOR TREATMENT

(With 3 Tables and 1 Figure)

By

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العوامل الفطرية المصاحبة للشياع المتكرر في الجاموس المصري مع المحاولات الميدانية للعلاج

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تم دراسة تأثير الاستخدام الميداني لمحلول اللوتاجين لحالات الشياع المتكرر في الجاموس وكذا تأثيره على الفطريات المعزولة من نفس الحالات. فتم إختيار عدد ٧٠ حالة من الجاموس يتكرر شياعها بعد التلقيح بحوالي ٢٠-٢١ يوم. وأخذت مسحه مهبلية من جميع الحالات لعزل وتصنيف الفطريات المصاحبة لهذه الحالات(مع الأخذ في الإعتبار الشروط الأساسية لعدم تلوث المسحات أثناء الحصول عليها). وكذلك تم إختيار عدد ٣٠ حالة منهم للمتابعة بعد العلاج حيث أخذت مسحة مهبلية قبل وبعد العلاج. وأظهرت النتائج الآتي: تم عزل فطريات ذات أنواع مختلفة من بعض حالات الشياع المتكرر بنسب (٨٤,٢٩%) ، فتم عزل الأسبرجيلس بمعدل ١٨,٨٧% والفيوزيريم والدريكسليرا بنسبة واحدة وهي ٥,٦٦% لكل منهما . وأوضح التصنيف لهذه الأنواع من الفطريات أن معدلات الأسبرجيلس نيجر (٢٠,٧٥%) أعلى من معدلات الأسبرجيلس سيدوي والأسبرجيلس تامارى وكذلك الأسبرجيلس تيريس. بالإضافة إلى عزل وتصنيف دريكسليرا سبيسفيرا لأول مرة من حالات الشياع المتكرر. كذلك أظهرت النتائج أن السموم الفطرية المتحصل عليها من هذه الأنواع من الفطريات هي سموم الأفلا ب١ ، ب٢ (٨,٣٣%) وسيترينين (٣٣.٣٣%) من أصناف أسبرجيلس فليفس وكذا بنيسيليم سيترينيم على التوالي. كما أوضحت النتائج أن معدل الشياع المخصب بعد العلاج وصل إلى نسبة ٨٦,٢١% مع نسبة حمل ٨٤%. وفي ختام هذه الدراسة يمكن أن نتوصل إلى: أن السموم الفطرية تلعب دورا حيويا في إستمرارية ظاهرة الشياع المتكرر في الجاموس الناتجة من إلتهاب الجهاز التناسلي بهذه السموم بالإضافة إلى أن أستخدام محلول اللوتاجين بنسبة ٤% يبطل قدرة هذه السموم في إحداث تلوث وعمل إلتهابات والتي تؤدي إلى حدوث هذه الظاهرة في الجاموس.

SUMMARY

The field application of lotagen solution as an intrauterine infusion for repeat breeder buffalo-cows was studied through its effect upon the fungal isolates which commonly present in the cervico-vaginal swabs of repeat breeder animals. A total of 70 repeat breeder buffalo-cows were used in this study. Selection of these animals was based on the criteria that they had clinically normal genitalia as revealed by rectal palpation but failed to conceive. Out of those animals, 30 animals were used to detect the effect of intrauterine infusion of 4% lotagen solution. The obtained results revealed that the isolated fungal genera were Aspergillus (69.81%), Penicillium (18.87%), Fusarium (5.66%) and Drechslera (5.66%). The incidence of *Aspergillus flavus* (22.64%) and *A. niger* (20.75%) were higher than *A. sydowi*, *A. tamarii*, and *A. terrus*. Moreover, *Drechslera spicifera* was isolated for the first time from the repeat breeder buffalo-cows. The detectable mycotoxins were Aflatoxins B_1 and B_2 (8.33%) and Citrinin (33.33%) which were extracted from *A. flavus* and *P. citrinum* respectively. After treatment, the pregnancy rate was 84%.

Key words: *Buffalo-cow, Lotagen, Repeat breeder, Fungi*

INTRODUCTION

Egyptian buffaloes are multipurpose animals and can be considered as an important source of meat and milk, which are of a good taste and high quality. A cow or buffalo-cow that exhibits oestrus at normal intervals but fails to conceive to three or more services can be described as a repeat breeder animal. The repeat breeder animals constitute one of the biggest problems for the Veterinary gynecologist during his routine herd visit (Hartigan, 1995). The available treatments for a cow returning regularly to estrus without detectable problem are limited, but new skills and techniques extend the treatment possibilities beyond conventional hormonal therapy (Dawson, 1998).

The economic viability of a dairy herd is dependent upon normal reproduction in the farm animals. Reproduction may be affected by various factors, but the pathological changes in the reproductive tract caused by microorganisms appear to be the main factor for infertility (Krishnan et al., 1994). Fungal infections of the female reproductive tract of domestic animals have not received much attention in the past. However, with indiscriminate use of antibiotics and hormonal therapy,

mycobiotic infections are becoming more common (Giri et al., 1994 and Verma et al., 1999). They added that, the prolonged use of antibiotics or hydrocortisones should be avoided as it decreases the resistance of the hosts and makes them highly susceptible to fungal infection. The mode of transmission of fungi into the female genitalia may be either through coital transmission, inhalation or through haematogenous route (Smith, 1968 and Singh et al., 1991). The present study was carried out to identify the fungal isolates commonly present in the genitalia of the repeat breeding buffalo-cows. Moreover, the field application of Lotagen solution as an intrauterine infusion for treatment of such infections, was also evaluated.

MATERIALS and METHODS

A total of seventy repeat breeder buffalo-cows were included in this study. Those animals had a regular estrous cycle but failed to conceive after three natural or artificial inseminations. Selection of the repeat breeder buffalo-cows was based on the criteria that they had clinically normal genitalia as revealed by rectal examination, but failed to conceive. Out of the 70 examined buffalo-cows, 30 animals were used to detect the effect of intrauterine infusion of 150-200 ml (according to the size of the uterine horns) Lotagen solution 4% weekly for three weeks (Schering-Plough Animal Health) on the growth of the isolated fungi.

* **Collection of samples:**

The external genitalia were thoroughly washed with 0.01% potassium permanganate solution and the lips of the vulva were wipped with clean sterile cotton prior to sample collection. The protected sterilized cotton swab was carefully passed into vagina till portio-vaginalis under complete aseptic conditions. The sterile swab was then pushed out of its protective sheath and moved about gently around portio-vaginalis and external Os of the cervix. After retraction into its cover, the swab was gently removed. All swabs were brought on ice to the laboratory and kept at 4°C until processed. This technique was repeated weekly for three weeks after each intrauterine infusion for treatment of selected animals (n=30).

* **Heat and pregnancy diagnosis after treatment:**

The treated animals were observed for heat by their owners. The fertile heat was calculated as the animals which did not return to heat

after 21 days from natural insemination. Pregnancy diagnosis was carried out on day 60-75 post-insemination using recto-vaginal technique.

* **Isolation of Fungi:**

Swabs were collected from the examined animals before and after treatment. The swabs were cultured onto Sabouraud's dextrose agar medium (SDA) supplemented with chloramphenicol (50 mg/L). Three plates were used for each swab and were incubated at 28°C for 7-10 days. The observed fungi were isolated and examined for identification through macro- and microscopic studies according to different guidelines (Raper and Thom, 1949; Raper and Fennell, 1965; Domasch et al., 1980; and Nirenberg, 1989). The identified fungi were transferred on SDA slants and kept until physiological studies.

* **Mycotoxin-Production potential of the isolated fungi:**

A total number of 53 isolated colonies during this investigation were screened for their ability to produce mycotoxins on yeast extract sucrose medium (YES). These fungi were *Aspergillus flavus, A. niger, A. sydowi, A. tamarii, A. terreus, Penicillium chrysogenum, P. citrinum, P. oxalicum, Fusarium moniliforme* and *Drechslera spicifera*. Erlenmeyer flasks (250 ml), each containing 50 ml of YES medium, were inoculated by the examined fungi and were incubated as stationary cultures at 28°C for 10 days. Three replicates of each isolate were analyzed. At the end of the incubation period, the contents of each flask were homogenized with 50 ml chloroform for 5 min. in a high speed blender. Extraction was repeated three times. The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate, filtered and dried to near dryness on a rotary evaporator. The residue was diluted with chloroform to one ml. The chloroform solution was analyzed for the presence of different mycotoxins using thin layer chromatography as described by Gimeno (1979).

RESULTS

The fungal isolates commonly present in the cervico-vaginal swabs of repeat breeder buffalo-cows as well as the effects of Lotagen as a treatment, are illustrated in Tables (1-3) and Figure (1). The results presented in Table (1) revealed that, out of 70 repeat breeder animals, 59 (84.29%) have yielded mycoflora in their swabs. The fungal isolates genera from animals subjected to treatment and follow-up were Aspergillus (69.81%), Penicillium (18.87%), Fusarium (5.66%) and Drechslera (5.66%) (Table 2). Among 53 fungal isolates, 12 (22.64%)

Aspergillus flavus, 11 (20.75%) *A. niger*, 6 (11.32%) *A. sydowi*, 6 (11.32%) *A. tamarii*, 2 (3.77%) *A. terreus*, 5 (9.43%) *Penicillium chrysogenum*, 3 (5.66%) *P. citrinum*, 2 (3.77%) *P. oxalicum*, 3 (5.66%) *Fusarium moniliforme* and 3 (5.66%) *Drechslera spicifera* were identified. The detectable mycotoxins Aflatoxins B_1 and B_2 (8.33%) and Citrinin (33.33%) were extracted from *A. flavus* and *P. citrinum* respectively.

The obtained results after application of lotagen solution as a treatment are shown in Tables (1,3) and Figure (1). The number of isolates decreased after infusion of lotagen.. Out of 30 treated animals, 29 responded for treatment but only one case did not respond completely to treatment after 3 times infusion with lotagen solution (Tables 3 and Figure 1), however, 86.21% from them exhibited fertile heat after treatment as well as 84 % pregnancy rate (Table 1).

DISCUSSION

Many investigators have reported that fungal infections of the bovine genital tract lead to infertility (Mishra et al., 1984; Singh et al., 1993 and Giri et al., 1994). For this reason, the principle aim of the present study was to evaluate the effect of intrauterine infusion of Lotagen solution on the fungal isolates from infertile or repeat breeder buffalo-cows.

In the present study, the overall prevalence of fungal genera in repeat breeder buffaloes were Aspergillus (69.81%), Penicillium (18.87%), Fusarium (5.66%) and Drechslera (5.66%). This is, somewhat, in agreement with the findings of Singh et al. (1993). They reported that about 9 different genera were isolated from repeat breeder buffaloes as Aspergillus (34.78%), Penicillium (4.35%), Fusarium (6.52%), and other genera like Candida, Mucor, Absidia, Rhizopus, Rhodotorula and Geotrium. The incidence of fungal isolates in this study was higher than that reported by the above researchers as well as that of Verma et al. (1999). The higher incidence of fungal isolates in the present study may be attributed to the site of sampling where, higher incidence may occurs if the clinical materials were not collected directly from the uterus (Giri, et al., 1994). In addition, it may be attributed to topographic variations, other ambient conditions (from hot humid to cold dry) and it is also possible that the incidence of this infection may vary from place to place.

The obtained results indicated that, there was clear-cut predominance of Aspergillus spp. which accounted for 69.81 % (37/53) of the isolates among repeat breeder cases. The lower incidence than that recorded in this study were 43.7% (Verma et al., 1999) and 34.78% (Singh et al., 1993). The incidence of Penicillium spp. and Fusarium spp. were 18.87% and 5.66% respectively. These results varied from that recorded by Singh et al. (1993) who reported that Penicillium spp. and Fusarium spp. were isolated from repeat breeder buffaloes at a percentage of 4.35% and 6.52% respectively. This variation may be attributed to the size of the population geographical location and hygiene measures. It is interesting to report that *Drechslera spicifera* was isolated for the first time from the genital tract (specially around portio-vaginalis and external cervical Os) of repeat breeder buffalo-cows. In the available literature, there is no report of its isolation from the genital tract.

Inflammation of the reproductive tract (specially portio-vaginalis during natural mating) due to fungal infection, may be responsible for causing infertility. Also, mycotoxins of fungi in the genital tract of repeat breeder animals are spermicidal to spermatozoa (Saxena and Ishaque, 1977). Therefore, either there may be death of the sperm or death of the embryo in the female genital tract (Giri et al., 1994). Furthermore, aflatoxins which produced by isolated fungi compete with oestradial-17β for oestrogen receptor but not for progestin receptors (Singh et al., 1993).

Therefore, it can be concluded that mycotoxins of fungi play a significant role in causing repeat breeding and infertility problems in buffaloes.Moreover, the intrauterine infusion of 4% Lotagen solution could be indicated to counteract such sort of genital infections successfully.

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Table (1): Distribution of the results

Items	n	%
* Total examined animals.	70	
* No. of positive samples.	59	84.29
* No. of negative samples.	11	15.71
* No. of animals used for treatment and follow-up.	30	
* No. of isolated species.	10	
* No. of isolated colonies.	53	
* Treatment response.	29/30	96.67
After 1st dose	17/30	56.67
After 2nd dose	10/30	33.33
After 3rd dose	2/30	6.67
* Non-response for treatment.	1/30	3.33
* Fertile heat after treatment.	25/29	86.21
* Pregnancy rate.	21/25	84.00

Table (2): Mycotoxin-production potential of the isolated fungi.

Fungal species	No. of tested isolates	No. of toxigenic isolates	Mycotoxins detected
Aspergillus flavus	12 (22.64 %)	1 (8.33 %)	Aflatoxins B_1 & B_2
A. niger	11(20.75 %)	-	-
A. sydowi	6 (11.32 %)	-	-
A. tamarii	6 (11.32 %)	-	-
A. terreus	2 (3.77 %)	-	-
* Total Aspergillus sp.	69.81 %		
Penicillium chrysogenum	5 (9.43 %)	-	-
P. citrinum	3 (5.66 %)	1 (33.33 %)	Citrinin
P. oxalicum	2 (3.77 %)	-	-
* Total Penicillium sp.	18.87 %		
Fusarium moniliforme	3 (5.66 %)	-	-
Drechslera spicifera	3 (5.66 %)	-	-
Total	53	2	

Table (3) Treatment response.

Fungal species	No. of animals before treatment	No. of animals after treatment
Aspergillus flavus	7	-
A. niger	5	1
A. sydowi	3	-
A. tamarii	3	-
A. terreus	1	-
Penicillium chrysogenum	3	-
P. citrinum	3	-
P. oxalicum	1	-
Fusarium moniliforme	2	-
Drechslera spicifera	1	-
Total	30	1

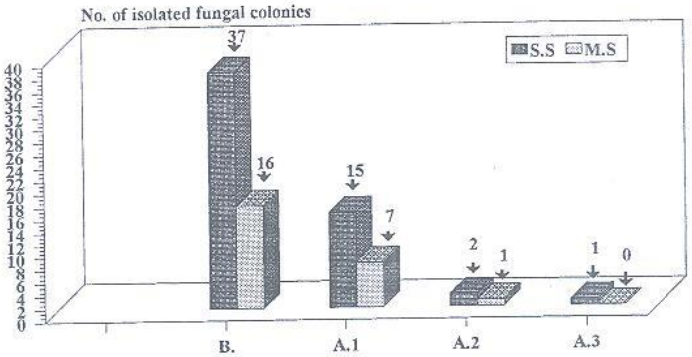

Figure 1. Effect of treatment upon isolated fungal colonies.

S.S = Single samples.
M.S = Mixed samples (A. flavus with A.terreus and A.flavus with A.niger).
B. = before treatment. A.1 = After first dose of treatment.
A.2 = After seconde dose of treatment. A.3 = After third dose of treatment.