دراسة البكتريا الطبيعية لرحم الجاموس والابقار أثناء دورة الشبق وفى بعض اضطرابات المهبل

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أجريت الدراسة على ٦٠ مسحة مهبلية من حيوانات سليمة وغير عشار بواقع ٣١ مسحة من أبقار ، و٢٦ من جاموس . ثم الاستنبات الجزرنومى على مجموعة متباينة من المنابت حتى يمكن عزل اكثر أنواع ممكنه .

اثبتت نتائج الفحص البكتيرى عن وجود حالتين سلبيتين من الجاموس بنسبة ٦ر٩٪ . بينما كانت الابقار ايجابية . وأمكن عزل أنواع متعددة من البكتريا اهمها المكورات العنقودية الذهبية ، المكورات السبحية البقرية ، مكورات مسحية محللة الدم الجزئى (الفا) ، الوتدية البقرية ، أنواع من عصيات وتدية غير مقسمة عصيات القولون ، بكتريا مولدة الهواء (ايروباكتر) ، شبيهات الجمر ، والخمائر .

وقد اكدت نتائج البحث حقيقة هامة ، هى ان نسبة البكتريا المعزولة اثناء طور الشبق تكون اقل كثيرا عن نسبتها اثناء الاطوار الاخرى فى الجاموس والابقار . وقد نوقشت هذه الحقيقة بالتفصيل مع ذكر تعليلاتها المعملية . كما لوحظ ان الحيوانات التى تعانى من خمول المهبل تحمل نسبة عالية من بكتريا الرحم عند مقارنتها بتلك المصابة بالحويصلة المهبلية .

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THE MICROFLORA OF THE UTERUS OF NON-PREGNANT BUFFALOES AND COWS DURING THE OESTROUS CYCLE AND IN SOME OVARIAN DYSFUNCTIONS

(With Three tables)

By

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SUMMARY

Uterine samples from 60 vaginal swabs were collected from healthy normal bovine(31 cows and 29 buffaloes). Out of the total examined samples, 2 buffaloes proved to be sterile while all cows samples proved to be bacteriologically positive.

A variety of microorganisms were isolated from non-pregnant buffaloes and cows. The most important isolants were: Staph. aureus, Staph. epdermidis, Sarcina lutea, Str. bovis, alphahaemolytic streptococci, C. bovis, unclassified corynebacterium, E.coli, aerobacter, anthracoides and yeasts.

The results revealed the fact that during the oestrous phase, the percentage of bacteria isolated was much lower than that obtained during dioestrus in buffaloes anc cattle. It was also found that animals suffering from inactive ovaries showed high incidence of bacteria than those affected with follicular cyst.

INTRODUCTION

It was believed that the healthy uterus of bovines is generally germ free (CARPENTER, 1920; KILMMER et al., 1929 and HAUPT, 1930). But many authors indicated that the uterus, even when healthy, has its own flora (DAWSON, 1950 and GEISSLER, 1954).

Recently many species of bacteria were isolated from apparently healthy non-pregnant genitalia. The most important isolated bacteria were: E. coli, M. albus, streptococci, C. pyogenes, M. aureus, proteus, B. subtilis, B. aerogenes, M. citreus, B. liqueficans and yeasts. (DAWSON, 1950; GEISSLER, 1954: DAWSON, 1960; EL-SAWAF et al., 1961; ZAKI et al., 1960 & 1961 and BARAKAT, 1965).

During dioestrus, MULJBERGS (1937) stated that the bacteria florished in the uterus and considered the uterus to be sterile during oestrus. DAWSON (1959) found that rythmic alteration between oestrous and dioestrous phases had a powerful effect in eliminating pathogenic bacteria from the uterus. The low incidence of bacteria during oestrus may be attributed to the effect of oestrogen (SHARAF et al., 1963 and ROBERTS, 1971).

In certain ovarian dysfunctions, VICEK (1960) reported that 26 out of 43 uterine samples from cattle suffering from cystic ovarian degeneration were bacteriologically sterile, from the remainder (17 cases) a variety of organisms was isolated. BARAKAT (1965) examined the bacterial flora of 100 non-pregnant buffalo uteri among which 6 were suffering from inactive ovaries and 2 with follicular cysts, with isolation of coliforms, corynebacterium and streptococci.

The aim of this work was to investigate the bacterial flora of the uterine secretions of the non-pregnant buffaloes and cows during the different phases of the oestrous cycle and in some ovarian dysfunctions.

MATERIALS AND METHODS

The materials used in this work had been obtained from apparently healthy non-pregnant 29 buffaloes and 31 cows (5-9 years old and all of them gave more than one calving).

These animals were presented to the Veterinary Clinic at Batanon, Monefia, Egypt. By rectal and vaginal examination the selected cases were proved to be non-pregnant. These animals were grouped according to the different phases of the oestrous cycle (proestrus, oestrus and dioestrus).

Bacterial samples were collected from each animal by vaginal swabs. Each sample was taken from the external os using a sterile gauze swabs, which were directly placed in 1% glucose broth. The collected samples were taken to the laboratory as soon as possible and were incubated at 37°C. for 24 hrs and then frequently streaked on the following media on plates (DIFCO, 1964):- Nutrient agar, 5% sheep blood agar, NacConkey's agar, Sabaroud's agar and Serum-Liver infusion agar with gentian violet (1/200.000). The inoculated plates were incubated at 37°C for 24 to 72 hrs. at least.

Different colonies were picked and purified by further subculturing and the isolates were identified morphologically and biochemically, and were classified into the following:-

1- Gram-positive cocci: Representing the members of the family Micrococcaceae and were identified according to ABD-ELMALEK and GIBSON (1948), BREED et al.(1957) and WILSON and MILES (1964) while members of genus Streptococcus were identified according to SEELEMAN (1954), BREEK et al. (1957) and MERCHANT and PACKER (1967).

2- Gram-positive non-sporulated rods: The isolates belonging to genus Corynebacterium were identified according to BREED et al. (1957), KIELSTEIN and MOTSCHE (1963) and COWAN and STEEL (1965).

3- Gram-negative rods: Isolates belonged to the family Enterobacteriaceae were identified according to BREED et al.

(L957),COWAN and STEEL(1965) and EDWARDS and E ING(1969).

4- Yeasts: were identified according to cultural character and morphological appearance.

All biochemical reactions applied were recorded finally after five days incubation at 37°C. at least.

RESULTS

According to the findings obtained from the rectal and vaginal examination, the examined animals were classified as shown in table 1.

Bacteriological examination revealed that all the examined samples of cows proved to be bacteriologically positive (100%, while in buffaloes 27 cows were positive (92.1%) and only two cases were negative. The distribution, incidence and frequency of the bacterial species isolated from the examined buffaloes and cows at the different phases of the oestrus cycle are listed in table 2, and that of ovarian dysfunction are tabulated in table 3.

It was observed that the high incidence of isolates from buffaloes was in dioestrus (43.7%) followed by oestrus (9.4%) than proestrus (6.3%). It was possible to define 64 isolates from 27 buffaloes. The most predominent isolats belonging to family Micrococcaceae (19 isolates), where 10 strains were recovered from animals in oestrus (15.7%),6 strains from inactive ovaries and 3 isolates (4.7%) from buffaloes suffering from follicular cysts, while there was no micrococci in proestrus and oestrus. From the 19 micrococci isolated, 9 were Staph. epidermidis, 4 as Staph. aureus, 3 Sarcina lutea, 2 M. caseolyticus and one strain from M. colpogenes, M. varians and M. flava.

Twelve streptococcus species were recovered (18.9). from which 6 isolates from buffaloes suffering from inactive ovaries (9.4%), which were differentiated into 3 Str. bovis, 2 alpha-haemolytic streptococci and one Str.Zymogenes. Four strains of Str.bovis were recovered from buffaloes during dioestrus, while in oestrus 2 streptococci were revealed(Str.pyogenes and Str.faecalis).Streptococcus species could not be isolated from buffaloes either in proestrus or buffaloes with cystic ovaries.

Twelve isolates of anthracoides were recovered, 5 isolants from buffaloes in dioestrus, 4 strains from inactive ovaries, 2 from oestrous phase and one strain from a sample with cystic ovary. No anthracoides isolated from buffaloes in proestrus.

Eleven strains of Gram-negative rods from family Enterobacteriaces were identified (17.4%), 9 isolates were E.coli one Pr.rettgeri and other citrobacter. A marked increase in Gram-negative bacteria was demonstrated from dioestrous buffaloes (9.5%), followed by inactive ovaries (4.7%), while a marked drop in proestrous cases (3.2%) and complete absence in oestrous phase.

Regarding Corynebacterium, 8 isolates were revealed (12.8%), 2 isolates were recovered from buffaloes in proestrus, oestrus, dioestrus, and those suffering from inactive ovaries. The 8 corynebacteria isolates were identified as 5 C.bovis, one as C.haemolyticum and 2 unclassified corynebacterium. Only 2 strains of yeasts (3.2%) were isolated , one from a case of cystic ovary and the other from inactive ovary.

In case of cows, all the 31 samples were positive, from which 75 isolants were identified. From table 2,it was clear

that the high incidence of isolates was from cows in the dioestrous status (49.4%), then followed by samples obtained from oestrous and proestrous cows (10.7% each).

The dioestrous cows characterized by harbouring more microorganisms (37 isolants) than other oestrous cycle phases. The most pronounced isolates were micrococci and Gram-negative bacilli (7 strains of each) then followed by anthracoides and yeasts (6 of each). In addition, 3 isolates of each of Str.bovis and C.haemolyticum, while only one strain from alpha- haemolytic streptococci, Str.faecalis, C.equi and unclassified corynebacterium.

In samples obtained from buffaloes and cows showing dysfunctions of ovaries specially follicular cyst and inactive ovaries, the results are tabulated in table 3, where 26 isolates were recovered from buffaloes (40.6%) and 22 strains from cows (29.1%).

In cases of follicular cyst in buffaloes, 5 isolates from Sarcina lutea, anthracoides and yeast. While in cows, 6 strains were recorded (7.9%), 2 of anthracoides and one isolate from Staph.epidermidis, Str.bovis, unclassified corynebacterium and yeasts. In contrast, it was noticed in cases of inactive ovaries that the incidence in buffaloes (32.8%) was higher than that in cows (21.3%) as indicated in table 3.

DISCUSSION

Vaginal swabs were collected from apparently normal non-pregnant bovines for bacteriological examination. The present results revealed that 27 cases were positive (92.1%) and two cases were negative (6.9%) in buffaloes, while all the 31 cases of cows were positive (100%). This result is considered higher than that presented by BARAKAT (1965) in buffaloes (62%).

The present results of identification and the species of the isolates were agreed with DAWSON (1960), VICEK (1960) ZAKI et al. (1960), EL-SAWAF et al. (1961), SHARAF et al. (1963), BARAKAT (1965) and ZAKI and MOUSA (1965).

In this investigation, cyclic variations regarding the presence of bacteria in the genitalia of buffaloes and cows could be demonstrated. In the follicular phase the percentage of the bacteria isolated was much higher than that obtained during the luteal phase. The bacteria isolated during proestrous and the oestrous phases showed a marked drop in their incidence. This result coincides with the finding of MULJBERGS (1937) who recorded that bacteria increased during dioestrous phase and RAWSON et al. (1953) who added that all samples collected during the luteal phase were bacteria positive.

It was claimed that the low bacterial content of the non-pregnant uterus during the follicular phase can be mainly attributed to the effect of oestrogens. This was demonstrated experimentally by FAULKNER (1943) who found that diethylstilbestrol was bactericidal and in lesser concentrations bacteriostatic to the Gram-positive cocci, C. diphtheria and Neisseria catarrhalis. In addition MOURSI (1961) observed that stilbestrol possessed a very powerful antibacterial action in vitro on Micrococcus pyogenes. In agreement with the previous authors, SHARAF et al. (1963) proved that the injection of stilbestrol had an inhibitory effect on the bacterial flora of the external os and uteri in cattle in Egypt. The inhibitory effect of oestrogens on bacterial growth was claimed also by RUSS and COLLINS (1940), RAWSON et al. (1953) HEIM (1954), MILACOVIC (1960) and CAUTCO (1962).

In the present work, it was clear that the highest incidence of bacteria was during the luteal phase both in buff-

aloes and cows. These findings coincide with those of MULJBERGS (1937) and RAWSON et al. (1953). The increased incidence during this phase can be explained by the fact that progestrone had a slight inhibitory effect on the bacterial flora of the genitalia.This conclusion was previously reported by RAWSON et al. (1953), MOURSI (1961) and SHARAF et al. (1963). On the other hand, KOCH (1947) stated that negative cultures were associated with the luteal phase of the menstrual cycle when progestrone activity and acidity of the mucous were at their height.

In cases of ovarian dysfunctions, it was found that the incidence of bacteria isolated from buffaloes and cows suffering from inactive ovaries were higher than that obtained from animals with follicular cysts. These results can be interprated when we consider the absence of considerable level of oestrogen in cases with inactive ovaries,in contrast with the continuous output of these hormones in these with follicular cysts (ROBERTS, 1971 and HAFEZ, 1974).

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Table (1)

Number of animals examined as regards the reproductive status.

Animal species	No. examined	Reproductive status				
		proestrus	Oestrus	Dioestrus	Foll.cyst	Inactive ovary
Buffaloes	29	3	3	12	2	9
Cows	31	3	4	14	3	7

Foll. = Follicular

Table (2)

Correlation between the bacterial flora of non-pregnant bufaloes and cows with different phases of oestrous cycle.

Types and Species of isolatets	Pro Reproductive status					
	Proestrus		Oestrus		Dioestrus	
	Buff.§	Cows§§	Buff.	Cows	Buff.	Cows
Staph.aureus	-	-	-	-	2	1
Staph.epidermidis	-	-	-	-	5	2
Staph.citreus	-	-	-	-	-	2
M.colpogenes	-	-	-	-	-	1
M.caseolyticus	-	-	-	-	2	-
M.varians	-	-	-	1	-	-
Sarcina lutea	-	-	-	1	2	1
Str.bovis	-	-	-	1	4	3
Str.pyogenes	-	-	1	-	-	-
Str.faecalis	-	-	1	-	-	-
Str.Alpha-H.	-	-	-	-	-	1
C.bovis	1	1	1	1	2	1
C.haemolyticus	-	-	-	-	-	3
C.equi	-	-	-	-	-	1
Coryne uncl.	1	1	1	-	-	1
Anthracoides	-	3	2	2	5	6
Citrobacter	1	-	-	-	-	1
Aerobacter	-	1	-	-	-	4
Yeasts	-	1	-	2	-	6
T o t a l	4	8	6	8	28	37
Incidence	6.3%	10.7%	9.4%	10.7%	43.7%	49.4%

Buff.♂ = Buffaloes.

§ = each isolant from buffaloes represented an incidence of 1.6%.

§§ = each isolate from cows represented 1.3%.

Str.alpha-H.= Streptococcus of alpha haemolysis.

Coryne uncl.= Unclassified corynebacterium strains.

Table (3)

Corelation between the bacterial flora of buffaloes and cows with some ovarian dysfunction.

Species of isolates	Follicular cyst		Inactive ovaries	
	Buff.§	Cows§§	Buff.§	Cows§§
Staph. aureus	2	-	1	-
Staph epidermidis	-	1	1	-
Staph. citreus	-	-	1	-
M. varians	-	-	1	-
M. flava	-	-	1	-
Sarcina lutea	1	-	-	-
Gaffkya tetragena	-	-	-	1
Str. bovis	-	1	3	1
Str. zymogenes	-	-	1	1
Str. alpha-H.	-	-	2	-
C. bovis	-	-	1	-
C. equia	-	-	-	1
C.haemolyticum	-	-	1	-
Coryne. uncl.	-	1	-	2
Anthracoides	1	2	4	2
Yeasts	1	1	1	2
T o t a l	5	6	21	16
Incidence	7.8%	7.9%	32.8%	21.3%

Buff. = Buffaloes

§ = each isolants from buffaloes represented an incidece of 1.6%.

§§ = each isolat from cows represented 1.3%.

Str.alpha-H.= Streptococcus of alpha haemolysis.

Coryne. uncl.= Unclassified corynebacterium strains.