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A Preliminary Report on Bacteria isolated from the Female Genital Tract of Sudanese Sheep and Goats

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ملخص البحث

أجريت هذه الدراسة لمعرفة أنواع الباكتريا المختلفة التي تتواجد في الجهاز التناسلي لإناث الضأن والماعز ومقارنة ذلك بمشاكل عدم الإنجاب . جمعت العينات لهذه الدراسة من مواقع مختلفة في السودان حيث تربى قطعان الضأن والماعز . باستخدام طرق الاستنبات الهوائي للبكتريا استنبتت مسحات من المهبل وعنق الرحم و عينات من غسيل الرحم. وجدت البكتريا فى مهبل و عنق الرحم و رحم الماعز بنسبة 21.6% ، 16.7% ، 30.8% على التوالي وفى الضأن بنسبة 14.7% ، 26.9 % ، 33.3% فى المهبل وعنق الرحم والرحم على التوالي أيضا . بعض أنواع الباكتريا المعزولة معروفة بأنها ممرضة مقارنة بأنواع أخرى التي تتواجد بصورة طبيعية في الجهاز التناسلي لإناث الضأن والماعز . أجريت مقارنة بين سجلات الخصوبة بمحطة ام بنين وبعض البكتريا التي تم عزلها من أغنام تلك المحطة. المعلومات التي جمعت في هذه الدراسة أسست للعلاقات بين وجود الباكتريا الممرضة وبين مشاكل الخصوبة والولدات القادمة خاصة في أنثى الضأن حيث وجود بعض أنواع الباكتريا لا يؤثر على خصوبة الحيوان .

Summary

The present study was carried out to isolate and identify bacteria inhabiting the female reproductive tract of goats and sheep with the purpose of correlating their presence with problems of infertility. Specimens were collected from goats and sheep reared at different localities of the Sudan. Swabs were used for collecting vaginal and cervical specimens while uterine specimens were collected by washing. Using aerobic cultural methods, various bacterial species were isolated from the regions of the reproductive tract of the two animal species. The incidence of bacteria was 21.6%, 16.7% and 30.8% for the vaginal, cervical and uterine specimens of goats respectively, while in sheep the incidence was 14.7%, 26.9% and 33.3% for the vaginal, cervical and uterine specimens, respectively. Some of the bacterial isolates are known to be potential pathogens, while others are considered as normal microflora.

Breeding records of ewes sampled at um Benien Dairy farm showed an association between isolation of coagulase-positive *Staphylococcus aurous, Streptococcus* species and *Listeria* species and cases of abortion and/or failure of conception. The data collected established a relationship between the presence of certain species of pathogenic bacteria in the reproductive tract and subsequent infertility problems, particularly in ewes, whereas, the presence of normal bacterial flora appeared not to influence the reproductive performance of the animals.

Introduction

Fertility and normal reproductive performances are the most important determinants of successful sheep and goats farming. There are many different causes of infertility in farm animals, among which infections play important role (Elsayed *et al*., 1994). Organisms gaining access to the female reproductive tract, as a result of improper manipulation during parturition or when dealing with retained placentae, could cause infection and subsequent infertility problems (Arthur and Pearson, 1982). Moreover, contamination of the female genital tract by faecal flora could lead to colonization of the vagina and/or abortion of pregnant animals (Levinson and Jawetz, 1994).

Primarily, Zaki and Saber (1962) studied the bacterial species Inhabiting the female reproductive tract of sheep and goats. Danis (1974) studied the prenatal lamb mortality, congenital infection and neonatal infection and he concluded that, non-specific bacterial infections may cause abortion and lamb mortality. Intra-uterine infection could also affect the length of the oestrus cycle of goats and may induce abortion in pregnant animals (Shallali, 1986). Certain species of Gram-negative bacilli were identified as causative agents of sporadic cases of abortion in ewes following their isolation from the reproductive tract (Anon, 1992). Recently, the obligatory intracellular bacterium *Chlamydia psittaci*, which is very often excreted in the faeces of healthy sheep and goats, was tentatively considered as one of the major causes of abortion and reproductive wastage in sheep (Souriau *et al*., 1993).

In the Sudan, neither the incidence of bacteria in the female reproductive tract nor problems of infectious infertility have been thoroughly investigated in sheep and goats (Shallali, 1986). The purpose of conducting this study was to isolate and identify aerobic bacterial species resident at different levels of the female genital tract of sheep and goats, to estimate their prevalence and to correlate it with problems of infertility.

Materials and Methods

Livestock:

Sheep and goats raised at Kassala, Sennar and Soba areas and Um Benein Dairy Farm were sampled (Table 1). The latter site was selected to represent an organized farm with records. All animals

sampled were at the age of breeding, apparently healthy and showing clinically normal genital tracts.

Collection of specimens:

The external genitalia of each animal were washed with soap and water prior to sampling. Three types of samples were collected as follows:

(a) Vaginal and cervical secretions:

By the aid of a vaginoscope, sterile swabs were introduced into the vaginal and cervical regions. The swabs were rubbed gently on the mucosae of the two regions so as to collect the largest amount of secretions, then taken out and kept in sterile glass test tubes. Each swab was cultured immediately or stored in a transport medium (Difco) until cultured.

(b) Uterine washings:

Sterile 10% peptone water (PW) or sterile phosphate buffer saline (PBS pH 7.2) were used for washing the urteri. By the aid of a vaginascope, a long sterile plastic catheter was introduced into the uterus, followed by infusing 10ml of sterile PW or PBS into the uterus. The washing procedure was repeated 3-5 times for each animal and an inoculum of about 4-5 ml was withdrawn from the uterus, kept in a sterile MacCarteny bottle and stored frozen at –20 ^{o}C until cultured.

3) Isolation and identification of bacteria:

Culture media used for isolation and purification of bacteria included: blood agar, MacConkey agar, nutrient agar and 10% salt agar (Oxoid). Inoculated media were incubated aerobically at 37 ºC for 24 hours. Bacteria isolated from specimens were then identified following the methods of Cowan (1974). *Staphylococcus* coagulase test was performed using the tube method of Cowan (1974).

Results

Various species of bacteria were present at the different regions of the reproductive tract of some of the goats and sheep investigated. Bacterial isolates from the vaginal swabs, cervical swabs and uterine washings of goats and sheep are shown in Tables 2 and 3, respectively.

Table 4 shows the prevalence of bacterial isolates from ewes at Um Benien farm. In goats the prevalence was 30.8% for uterine washings while vaginal and cervical swabs showed prevalence of 21.6% and 16.7%, respectively. In sheep the prevalence was 14.7%,

26.6% and 33.3% for vaginal, cervical and uterine specimens, respectively. The uterine washings of both animal species showed the highest bacterial prevalence. The results, however, indicated that the three regions of the reproductive tract were free from bacteria in the majority of animals examined. Some of the isolated bacterial species were potential pathogens. They were more frequently isolated from cervical and vaginal swabs rather than uterine washings (table 2 and 3). They included coagulase-positive *Staphylococcu aureus, Listeria* species, *Corynebacterium pseudotuberculosis* and *Strepococcus* species. Other bacterial isolates are not known to be pathogenic in normal aniamls and may be considered to be normal microflora. Those frequently isolated included *Staph. epidermidis, Lactobacilli, Propionibacterium* species, *Pseudomonas* species and *Pediococcus* species.

Specimens from Um Benein Dairy Farm were collected only from ewes (Table 1). Bacteria isolated from cervical swabs of these animals are shown in Table 4. Referring to the history and breeding records of the animals, the results showed that the isolation of coagulase-positive *S. aureus, Listeria* species and *Streptococcus* species was associated with cases of abortion and failure of conception which continued for one year or more. Ewes bearing other bacterial species had no apparent clinical signs of cervical or uterine infection and were recorded as having normal reproductive performance (Table 5). On one occasion, a *Corynebacterium* species mixed with *Ureaplasma urealyticum* were isolated from a cervical swab obtained from ewe no. 928, which was a normal breeder.

Table 1: Specimens collected from goats and sheep for aerobic bacterial isolation.

Area	Animal species involved	Vaginal swabs	cervical swabs	Uterine washings
Sennar	Goat	82	82	25
	Sheep	101	101	25
Kassala	Goat	15	40	40
	Sheep	15	15	10
Soba	Goat	-	40	-
Khartoum				
Um Benein	Sheep	40	40	10
Total		**253**	**318**	**110**

Table 2: Bacteria isolated from the reproductive tract of female goats*

Organism	***Cervical swabs***	***Vaginal swabs***	***uterine washing***
Staph. Aureus	9	5	2
Staph. Epidermidis	4	1	7
Streptococcus species	-		-
Proteus species	2	1	-
Pseudomonas speices	-	-	1
Lactobacillus jensenii	-	-	1
Lactobacillus casei	-	-	1
Lactobacillus plantarum	-	-	1
Lactobacillus brevis	2	-	1
Corynebacterium pseudotuberculosis	2	-	1
corynebacterium murinm	1	-	1
Listeria grayi	1	-	-
Pasteurella species	1	-	-
Micrococcus luteus	-	-	1
Pediococcus	1	1	-
Total No. of isolates	**23**	**18**	**17**

* Identification was performed according to Cowan (1974).

Table 3: Bacteria isolated from the reproductive tract of female sheep*

Organism	***Cervical swabs***	***Vaginal swabs***	***Uterine washing***
S. Aureus	6	5	-
S. Epidermidis	4	3	2
Corynebacterium species	4	2	-
Corynebacterium pseudotuberculosis	-	1	-
Proteus species	-	1	-
Listeria species	7	1	3
Pseudomonas species	2	-	3
Streptococcus species	9	4	3
Gram-negative bacilli	4	2	-
Pasteurella species	-	-	1
Streptococcus mitor	-	1	-
Lactobacillus brevis	-	-	-
Probionibacterium species	2	-	
Total No. of isolates	**38**	**20**	**12**

- Identification was performed according to Cowan (1974).

Table 4: History of abortion, failure of conception and bacteria isolated from cervical swabs from ewes kept at Um Benein Dairy farm.

Animal No.	Bacteria isolated	Breeding history	Remarks
604	*Listria* species	Abortion	Continued for 3 years
432	*Listeria* species	Abortion and infertility	failed to conceive
618	*Nocardia* species	Normal	-
703	*Listeria* species	Normal	-
996	*Staph. Aureus*	Abortion	for one year
502	*Streptococcus* species	Normal	-
928	*Corynebacterium* species	Normal	-
	+ ureaplasma urealyticum		-
34	*Nocardia* species	Normal	-
486	*Staph. Epidermidis*	Normal	-
18	*Probionbact* species	Normal	-
738	*Streptococcus* species	Normal	-
82	*Staph. Aureus*	Abortion	for one year
244	*Streptococcus* species	Abortion	for two year
240	*Streptococcus* species	failed to conceive	Two year
410	*Staph. Aureus*	Abortion	for two year
48	*Corynebacterium* species	Normal	-
548	*Corynebacterium* species	Normal	-
28	*Gram-negative bacilli*	Normal	-
640	*Steptococcus* species	Abortion	for two years

Table 5: Prevalence of bacteria in vaginal, cervical and uterine specimens of goats and sheep investigated.

	Vaginal swabs		***Cervical swabs***		***Uterine washings***	
	Goats	***Sheep***	***Goats***	***Sheep***	***Goats***	***Sheep***
No. specimens examined	97	156	162	156	65	45
No. specimens positive	**21**	**23**	**27**	**42**	**20**	**15**
prevalence (% positive)	21.6	14.7	16.7	26.9	30.8	33.3

Discussion

The results emphasized the presence of certain bacterial species at the different regions of the female reproductive tract of apparently healthy goats and sheep. This was in agreement with Gardiner (1961), Macleod *et al*. (1974), Danis (1974) and Souriau *et al* (1993). In the present study the highest bacterial prevalence (33.3%) was found in the uterine washings of sheep whilst the lowest (14.7%) in the vaginal swabs of the same animal species. The results showed that the genital tract was free from bacteria in the majority of animals examined. This situation appears to be ideal for maximum reproductive performance (El Sayed *et al*., 1994). Previous reports suggested that non-specific bacterial infections of the reproductive tract may induce abortion (Danis, 1974) and affect the length of the oestrus cycle of goats (Shallali, 1986). However, our findings revealed that only the presence of potentially pathogenic bacteria in the female genital tract might adversely affect its reproductive performance. The presence of other bacterial species did not appear to adversely influence the fertility potential of the carrier animals (Zaki and Saber, 1962). The apparently normal animals seem to benefit from the presence of some microflora in their genital tracts as, for instance, *Lactobacilli* normally secrete lactic acid (Sleigh and Timbury, 1998) and this will help in clearing up other bacterial pathogens from the reproductive tract (Levinson and Jawetz, 1994).

The results cited in this study gave a fairly good picture of the prevalence of bacterial species resident in the female genital tract of goats and sheep as the specimens were collected form animals reared at different regions of the Sudan. Breeding records were available only for ewes sampled at Um Benein Dairy Farm. Coagulase-positive *S. aureus* and *Streptococcus* species were the most frequently isolated bacterial species from goats and sheep specimens while *Listeria species* were isolated more often from cervical swabs of sheep. These species were associated with abortions and/or failure of conception, as shown by the records of Um Benein Dairy Farm. *S. aureus* was previously reported to be the major cause of neonatal death of lambs (Danis, 1974) and influencing the life span of corpora lutea of goats (Shallali, 1986). *Listeria* species were proven to be associated with cases of infectious abortion in sheep (Butchaiah and Khora, 1983). Other bacterial species, like *Chlamydia psittaci* which was generally

incriminated in causation reproductive wastage in sheep (Souriau *et al*., 1993) was not isolated in this study.

It would seem reasonable that bacteria are more readily isolated from genital specimens collected by washing rather than by swabs (Shallali, 1986). Washing was used to collect uterine specimens in the present study. However, more work is needed to develop better procedural methods for collecting specimens for bacteriological examination from the female genital tract of goats and sheep. Further investigations are also required, preferably experimental intervention and examination of specimens collected from other parts of the country, with the ultimate purpose of clearly verifying the role of bacteria involved in infectious infertility in Sudanese small ruminants in order to assess their economic impact conclusively.

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