

## MICROFLORA OF THE CERVICO-VAGINAL MUCUS OF BUFFALOES

BAHAMAN, A.R., ZAKARIA, M. and JAINUDEEN, M.R.

*Faculty of Veterinary Medicine and Animal Science  
Universiti Pertanian Malaysia  
43400 Serdang, Selangor, Malaysia*

**SUMMARY:** One hundred and fifteen bacterial and 16 fungal isolates were obtained from 46 cervico-vaginal mucus samples of buffaloes. Majority (85.1%) of the mucus harboured one to three species of bacteria which represented the normal bacterial flora in the urogenital tract. The bacterial genera identified from the mucus, in order of importance, were *Bacillus*, *Staphylococcus*, *Escherichia*, *Streptococcus*, *Micrococcus*, *Pseudomonas* and *Corynebacterium*. No difference was observed in the distribution of the bacterial species amongst the different reproductive groups of buffaloes. No evidence of pathogenic bacteria was detected in any of the mucus samples.

**Key words:** microflora, cervico-vaginal mucus, buffalo

### INTRODUCTION

Buffaloes are potential animals for large scale production of meat in the tropics. They breed well in tropical Australia and form a part of the export animals and meat industry in the Northern Territory (Anon., 1981). Although large scale buffalo farming has not been developed in Malaysia, such plans are being considered. Infectious diseases like brucellosis, campylobacteriosis, leptospirosis and trichomoniasis as well as non-specific bacterial infections are known to interfere with the reproductive performance of cattle but little is known on the prevalence of these diseases in buffaloes. Economic losses in potential calf, milk production and unproductive buffalo cows due to these diseases have not been assessed. This preliminary study examined the microflora of cervico-vaginal mucus of buffaloes at the Universiti Pertanian Malaysia Buffalo Unit and assessed their significance.

### MATERIALS AND METHODS

#### *Test Animals*

This study was carried out from June to November 1983. Buffaloes of different ages and reproductive status from the Universiti Pertanian Malaysia Buffalo Farm were examined. Animals were randomly sampled on three occasions during routine pregnancy testing. Altogether 47 cervico-vaginal mucus samples from 11 pregnant, five anoestrus, two repeat breeding and 29 post-partum buffaloes were obtained. The reproductive status of these animals was determined from farm records and by rectal examination.

*Sample collecting device*

A cotton swab on a flexible stick (48 cm long and about 0.3 cm diameter) was placed in a polyvinyl-chloride tube (40 cm long and 2 cm diameter) and a cotton stopper was inserted in the tube to stabilise the swab during sampling. The swab was sealed in the tube by closing both ends of the tube with cotton stoppers to prevent contamination. Finally, the device was sterilised in a hot air oven at 170°C for six hours.

TABLE 1  
Media and cultural requirements for primary isolation of microorganisms  
from cervico-vaginal mucus of buffaloes

Media	Purpose	Environment	Incubation Time
Blood agar	Aerobes and facultative anaerobes	Microaerophilic (candle jar) 37°C	24–48 hrs
MacConkey agar	Coliforms and related organisms	Aerobic 37°C	24–48 hrs
Sabourauds dextrose agar	Yeasts and fungi	Aerobic (room temperature)	24 hrs – 14 days
Vibrio agar	<i>Campylobacter</i> spp.	Microaerophilic 10% CO <sub>2</sub> 37°C	> 4 days
Serum dextrose agar	<i>Brucella</i>	Microaerophilic 10% CO <sub>2</sub> 37°C	> 4 days
<i>Mycoplasma</i> broth & agar	<i>Mycoplasma</i>	Aerobic	> 4 days
		Microaerophilic 37°C	> 7 days

*Collection of the cervico-vaginal mucus samples*

The vulva of the animal was washed clean of faeces with warm water and the inner lips wiped dry before the tube containing the swab was introduced. The anterior end of the tube was lubricated with obstetrical cream and introduced into the vagina until it came in contact with the cervix as indicated by rectal palpation. The cervico-vaginal mucus was quickly but gently sampled by pushing out and rotating the cotton swab from inside the tube. The swab was pulled back into the tube and withdrawn together with the tube from the vagina. The swab was placed in a bottle of Stuarts transport medium.

*Laboratory culture and identification*

## a. Culture media

The media and their purpose, the environment and time of incubation for primary isolation of the microflora were listed in Table 1.

## b. Laboratory procedure

The cervico-vaginal mucus was directly streaked onto the media. Direct smears were made from the swabs and stained with appropriate stains for direct microscopic examination. The plates were incubated at the required temperature, environment and time period. Any colonies seen were subcultured for maintenance culture and for identification purposes. The isolates were identified on the basis of their colonial morphology, biochemical reactions and serological responses of the isolates as outlined by Cowan and Steele's Manual for the identification of medical bacteria (Cowan, 1974).

Colonies on the Sabourauds dextrose agar were examined for yeasts and moulds. Smears made were stained with Gram's stain as well as by lactophenol cotton blue. The yeasts and fungi were identified according to the Manual of Clinical Microbiology (Lennette *et al*, 1976).

## RESULTS

One hundred and fifteen bacterial and 16 fungal isolates were obtained from the 46 cervico-vaginal mucus samples. Majority (85.1%) of the samples harboured one to three species of bacteria (Table 2). The predominant bacterial genera seen in the samples, in order of importance, were *Bacillus*, *Staphylococcus*, *Escherichia*, *Streptococcus*, *Micrococcus*, *Pseudomonas* and *Corynebacterium* (Table 3). The distribution of the various bacterial and fungal species was not confined to any particular group of buffaloes or the reproductive status of the animals. No evidence of *Brucella*, *Campylobacter* or *Mycoplasma* was seen in any of the cervico-vaginal mucus samples. Gross examination revealed no apparent pathological lesions or abnormal discharge from the urogenital tract of the animals examined. The frequency of occurrence of different bacterial species amongst the various groups of buffaloes is shown in Table 4.

TABLE 2  
Distribution of bacterial isolates from cervico-mucus samples of 47 buffaloes

Reproductive status	No. of animals examined	No. of bacterial species seen						
		Sterile	One	Two	Three	Four	Five	Six
Pregnant	11	0	2	3	4	2	0	0
Post-partum	29	1	7	12	7	1	1	0
Repeat breeder	2	0	1	1	0	0	0	0
Anoestrus	5	0	2	1	0	1	0	1
Total	47	1	12	17	11	4	1	1
Percentage		2.1	25.5	36.2	23.4	8.5	2.1	2.1

TABLE 3  
Bacterial and fungal isolates from the cervico-vaginal mucus of buffaloes

Organisms	No. Isolates	Percentage (%)
1. BACTERIA	115	
i. Gram positive bacteria	58	50.4
Bacillus spp.	19	16.5
Staphylococcus spp.	11	9.6
<i>S. epidermidis</i> (10)		
<i>S. pyogenes</i> (1)		
Streptococcus spp.	11	9.6
<i>S. bovis</i> (5)		
<i>S. pyogenes</i> (3)		
<i>S. faecalis</i> (3)		
Micrococcus spp.	9	7.8
Corynebacterium bovis	8	7.0
ii. Gram negative bacteria	57	49.6
<i>E. coli</i>	12	10.4
Pseudomonas spp.	9	7.8
Aeromonas spp.	7	6.1
Pasteurella spp.	7	6.1
<i>P. multocida</i> (6)		
<i>P. ureae</i> (1)		
Enterobacter spp.	6	5.2
<i>E. aerogenes</i> (2)		
Other spp. (4)		
Klebsiella spp.	5	4.3
Acinetobacter spp.	4	3.5
Citrobacter spp.	3	2.6
Neisseria spp.	2	1.7
Moraxella spp.	1	0.9
Proteus spp.	1	0.9
2. FUNGI	16	
i. Moulds	9	56.3
Mucor spp.	5	31.3
Aspergillus spp.	2	12.5
Culvaria spp.	2	12.5
ii. Yeasts	7	43.8
Candida spp.	7	43.8
<i>C. krusei</i> (4)		
Other spp. (3)		

TABLE 4  
Prevalence of bacterial genera in the cervico-vaginal mucus of buffaloes

Buffalo group	Prevalence of important bacterial genera*
Pregnant (11)	Bacillus, Aeromonas, Micrococcus, E. coli, Streptococcus
Post-partum (29)	Bacillus, Staphylococcus, E. coli, Corynebacterium, Streptococcus
Repeat breeders (2)	Bacillus, Corynebacterium
Anoestrus (5)	Bacillus, Aeromonas, E. coli, Klebsiella, Pasteurella, Streptococcus

( ) Number of animals

\* Genera of low prevalence not shown

## DISCUSSION

Non-specific bacterial infections were responsible for many cases of infertility in cattle. Studies on the microflora of cervico-vaginal mucus, the cervix and anterior vagina of normal and repeat breeding cows have shown wide spectra of microflora. Anoestrus and repeat breeding cause economic losses through loss of potential calf and reduced milk production. The losses incurred by culling and selling non-producing cows are no less negligible (Panangala and Barnum, 1979). There was no difference in the frequency of occurrence of different bacterial species amongst the various groups of buffaloes in this study (Table 4). Similarly, it was noted that there was no difference in prevalence of the various species of bacteria mentioned earlier. This indicated that a normal bacterial flora is prevailing in the cervico-vaginal mucus of buffaloes. The vagina serves as a habitat for many types of saprophytic organisms, some of which are well known opportunists which under favourable circumstances may impair fertility (Vigue *et al.*, 1959). This normal flora may also play an important part in the defense system of the animal by preventing the colonisation of pathogenic bacteria.

The bacterial isolates obtained were mainly non-specific bacteria which were usually found on the animal and/or its environment. Some of these commensals have been reported as the cause of non-specific bacterial infections of the genital tract. Hardenbrook (1958) reported that *Micrococcus*, *Streptococcus*, *Corynebacterium*, *Proteus* and *E. coli* were the cause of non-specific infections of the reproductive tract. Dawson (1960) on the other hand, reported cases of endometritis due to *Staphylococcus*, *Streptococcus* and *E. coli*. It has been shown that microorganisms were amongst the important causes of infertility (Shah and Dholakia, 1983) and were probably the cause of the high prevalence of abortions and infertility amongst Indian buffaloes (Prasad and Malik, 1966).

Sixteen bacterial and four fungal species were identified in this study. No difference between the prevalence of Gram positive and Gram negative bacteria was observed. *Bacillus* spp. were the most prevalent group of bacteria in the cervico-vaginal mucus. This group of bacteria or anthracoids is commonly found in the environment and could easily gain access into the urogenital tract of the buffaloes, particularly during wallowing. Next in importance was *E. coli*. Like the *Bacillus*, this species is closely associated with ani-

mals and their environment. Being excreted with the faeces, *E. coli* has easy access to the urogenital tract due to the proximity of the urogenital tract to the anus. Another interesting finding from this study was the presence of *Pasteurella multocida* in the mucus. The normal habitat for this species is the upper respiratory tract and this finding suggested the possibility of another habitat for this species. Should this be so, the pathogenesis and epidemiology of haemorrhagic septicaemia may have to be reconsidered.

This preliminary study indicated the presence of a normal microflora of bacteria and yeasts in the cervico-vaginal mucus of buffaloes. In cases of abortions and infertility, it is important to differentiate this microflora from the actual pathogen causing the problem. Further research is warranted to determine whether these non-specific bacteria and fungi isolated could cause infertility in affected buffaloes.

## REFERENCES

- ANON. (1981). Northern Territory cattle, buffaloes and fishery statistics: Jan–Dec 1981. Technical Bulletin, Dept. of Primary Production, Northern Territory, Australia (1983). No. 63: p. 20.
- COWAN, S.T. (1974). Cowan and Steele's Manual for the Identification of Medical Bacteria. 2nd ed., Cambridge University Press, London.
- DAWSON, F.L.M. (1960). Bovine endometritis: A review. *Br. Vet. J.* 116: 448–466.
- HARDENBROOK, H. (1958). The diagnosis and treatment of non-specific infection of the bovine uterus and cervix. *J. Am. Vet. Med. Ass.* 132: 459.
- LENNETTE, E.H., SPAULDING, E.H. and TRUANT, J.P. (1976). Manual of Clinical Microbiology. 2nd ed. American Society for Microbiology, Washington D.C.
- PANANGALA, V.S. and BARNUM, D.A. (1978). Microflora of the cervico-vaginal mucus of repeat breeder cows. *Can. Vet. J.* 19: 83–89.
- PRASAD, C.B. and MALIK, B.S. (1966). Study of the microflora from the female genital tract of buffaloes. *Indian Vet. J.* 43: 1043–1051.
- SHAH, N.M. and DHOLAKIA, P.M. (1981). Microflora of the cervico-genital mucus of Surti buffaloes and their drug resistance pattern. *Indian J. Anim. Sci.* 53: 147–150.
- VIGUE, R.F., FITZGERALD, W.H. and CASTRUCCI, R.F. (1959). Management of bovine genital infections. I. Intravaginal therapy. *J. Am. Vet. Med. Ass.* 134: 308–311.

## RINGKASAN

### MIKROFLORA MUKUS SERVIKO-VAGINA KERBAU

Satu ratus dan lima belas isolat bakteri dan 16 isolat kulat telah dapat diasing dari 46 contoh mukus serviko-vagina kerbau. Kebanyakan (85.1%) contoh mukus tersebut mengandung antara satu hingga tiga spesies bakteri yang mewakili flora bakteri yang biasa terdapat dalam trak urogenital. Genera bakteri yang telah dikenalpasti dari mukus mengikut turutan kepentingan adalah *Basilus*, *Stafilokokus*, *Escherisia*, *Streptokokus*, *Mikrokokus*, *Pseudomonas* dan *Korinebakterium*. Tidak ada perbezaan didapati dalam pertaburan spesies bakteri antara kumpulan-kumpulan reproduktif kerbau. Tidak terdapat bukti adanya bakteri patogenik diantara contoh mukus yang dikaji.