Dept. of Buffalo Diseases,
Animal Health Research Institute, Dokki, Egypt.
Head of Dept. Prof. Dr. M.B. Morcos.

STUDIES ON MASTITIS IN COWS AND BUFFALOES WITH REFERENCE TO MYCOTIC INFECTIONS OF UDDER

(With 6 Tables)

By

M.B. Morcos; S. Sh.E. SOLIMAN; A.H. ELYAS;*
SEHAM M. ALI** and A.A. AMER***

(Received at 15/5/1989)

دراسة عن التهاب الضرع فى الأبقار والجاموس
مع الإشارة إلى العدوى بالفطريات

مرقص بولس ، شاكر سليمان ، عادل حلمى ، سهام محمد على ، أحمد عامر

أجريت دراسة على المسببات البكتريولوجية والفطرية لإلتهاب الضرع من قطيع من الأبقار الفريزيان والجاموس . وأجرى الفحص المذكور فى ضروع الحيوانات السليمة إكلينيكياً كما تم دراسة الإرتباط بين عزل هذه المسببات وأرباع الضرع المختلفة وموسم الحليب وكذلك مدى تواجد الفطريات المعزولة من الحالات المرضية ومثيلتها فى علائق هذه الحيوانات . وقد أحدث التهاب الضرع تجريبياً فى الأبقار والجاموس عن طريق حقن إفرازات من ضروع الحيوانات المريضة ومن مستنبت الجلوكوز الفطرى كما وحقن مستنبت الفطر المعزول من علائق هذه الحيوانات بتركيزات مختلفة فى وريد الأرانب ودرس تأثيره عليها . كما إستخلص ـ تبعاً لذلك ـ أن التلوث الفطرى لعلائق هذه الحيوانات ربما كان سبب التهاب الضرع .

SUMMARY

The following fungi were isolated from: udders of cows and buffaloes Aspergillus fumigatus (37.6%, 30%), A. niger (14.6%, 26.4%), A. flavus (11%, 17%), A. minor (11%, 13.4%), Candida albicans (40.2%, 52.8%), Candida tropicalis (19.5%, 24.5%), Candida vrusei (14.6%, 3.8%), Mucor spp. (33%, 28.6%), Rhizopus spp. (4.9%, 5.7%) in buffaloes and cows respectively. Penicillium. Clandosporium and Cephalosporium spp. were at lower percentages. The incidence of mycotic infection was assessed in both apparently healthy quarters and in milk samples from clinical cases.

In addition to mycotic flora there was an associated contamination of udders with gram positive and gram negative organisms in 21 buffaloes and 13 cows. the isolated bacteria included: Escherichia coli (20.7%,

*: Dept. of Med. & Infectious Deseases, Assiut University, Assiut Egypt.
**: Animal Health Research Institute, Assiut.
***: Dept. of Vet. Med., Assiut University.

20.7%), Staph. pyogenes (15.8%, 18.9%), Beta-hemolytic streptococci (11.0%, 17.0%), Pseudomonas pyocyanea (7.3%, 13.2%), Paracolon spp. (4.9%, 13.2%) and Corynebacterium spp. (7.3%, 3.7%) in buffaloes and cows respectively.

There was predominance of Gram negative over Gram positive organisms (1.0 : 0.9). The source of bacterial contamination was not determined and it might have occurred with opportunistic bacteria. The results have implications whenever successful treatment in the mixed infections are considered while cases of mycotic infection alone require an antimycotic for optimal results.

Right half and hind quarters were found to be affected with either mycotic or bacterial mastitis at greater frequency than left half and fore quarters. The highest incidence of mastitis was seen during the second stage of lactation (3-5 months).

Study on mycoflora of food grains used for feeding the animals revealed infection with many toxic fungi like: Aspergillus spp. (66.6%), Rhizopus spp. (45%). There were similarities in flora types and colony characters between mycoflora isolated from mouldy food grains and udder secretions.

Mastitis was reproduced experimentally in normal udders of buffaloes and cows with mastitis secretions and with glucose broth cultures of fungi mouldy food grains. Clinical mastitis developed within few days in all infused quarters and mycoflora were recultured from their secretions. Fungal broth cultures of mouldy grains proved toxic to rabbits and death occurred after intravenous injections with various dilutions. According to these observations the mouldy food grains would have acted as a source of mycotic mastitis. This finding would be of value at the present time to those in general practice and to breeders.

INTRODUCTION

Mycotic mastitis in cattle is relatively common and is rarely accurately diagnosed before laboratory diagnosis. Its low frequency of diagnosis is attributable to its bizarre clinical presentation and uncharacteristic findings. Hence it is often forgotten in the differential diagnosis of bovine mastitis.

The etiology of mycotic mastitis has been urged even since the first was described by ROLLE in 1934. MURPHY and DRAKE reported in 1947 on the infections of the bovine udder with yeast-like fungi identified as a species of Trichosporon. Candida has also been reported frequently in conjunction with mastitis in cattle (YEO and CHOI, 1982; SIMARIA, et al., 1936 and NATALIA, et al., 1985). Recently the disease draws the attention of many workers (MISRA and PANDA,1986; WEIGHT and AHLERS,1982; RAHMAN and BAXI, 1983; SHARMA, 1983; KIRK et al., 1986) and others. Since review

of literature revealed also an unclear presentation on mycotic mastitis in Egyptian cattle, the disease must run the risk of exposure of investigation to get appropriate data on incidence and causes.

In view of the fact that food grains are subjected to spoilage under field storage conditions resulting in contamination of the farm with the possibility of infections of the udder, the problem of mycotic mastitis, hence, has become more acute. SINHA and WELLACE (1965) reported fungus infections of wheat grains in India and QASEM and CHRISTENSEN (1958) reported Aspergillus flavus infection in maize corn.

We report mycotic mastitis in two dairy farms, which to the best of our knowledge, is the first to explain the source of infection to draw attention to the disease.

MATERIAL AND METHODS

In 1987, a dairy herd of 210 Friesian cows and a second one of 182 buffaloes were subjected routinely to mastitis examination. Mycotic infection of the udder in these animals was assessed in apparently healthy quarters of lactating buffaloes and cows and in milk samples from clinical cases. There were 42 buffaloes with apparently healthy quarters and 23 with mastitis, and the Friesian herd presented 22 cows with apparently healthy quarters and 13 clinical cases. Thus, the overall buffaloes was 65 and cows 35. There were 64 animals with apparently normal udders and 36 with clinical mastitis.

The two herds were about 4 kilometers apart and all management and feeding methods were similar. The ages of animals ranged between 3 and 7 years (for cows and buffaloes). Animals were kept out at grass during the day but in barns during the night. Almost all the animals were in good condition. The food used for both farms was received from the same grain depot and method of storage was similar. Food composed of hay, concentrated cakes, rice-straw and mineral supplement. The cakes were composed of: ground maize corn, barn and molassess.

Relative data regarding previous history of mastitis, stage of lactation and the lactation number were recorded. Animals with previous mastitis or those recently treated were not included in the study.

The overal milk samples from buffaloes were 82 & 53 from cows. As a control group, milk samples from 10 buffaloes and 10 cows, proved free from udder infection, were also studied.

1- Milk examination :

With strict aseptic precautions, and after discarding the fore milk 135 samples (82 from buffaloes and 53 from cows) from apparently healthy and clinically infected quarters were collected into separate.

A primary culture was made on blood agar, MacConkey's agar media and nutrient broth by standard techniques. Surface colonies were used for typing and their characteristics were determined.

Secondary culture were made on Sabouraud's medium. Two plates of Sabouraud's dextrose agar medium were used: one contained penicillin and streptomycin and the second chloramphenicol and cyclohexamide. The plates were incubated at 35°C for 4-6 days. Identification of fungi was based on rapidity of growth, colony morphological characters, pigment production, microscopic identification and biological characters. When large quantities of spores were produced, concentrated suspension of growth was prepared in saline. Individual fungal isolation was made by steak method. Cultural characters of the growth were recorded by examining the growth of Sabouraud's glucose agar medium. Biological characters were noted after preparing the broth culture and inoculating them into experimental animals intravenously or into teats (AINSWORTH and AUSTWICK, 1973).

2- Food grains examination :

Isolation of fungi from food grains were made by culture on Sabouroud glucose agar medium. The method used was as mentioned before.

3- Experimental infection :

The pathogenicity of fungal cultures obtained from mastitic milk (field cases) and from food grains was tested in rabbits by I. V. injection of $10X10^6$ fungal spores or 10^6 yeast organisms.

Secretions from four field cases (clinical mastitis) were infused into two quarters of each of four normal cows. Observation continued for 10 days after experimental inoculation and trails were made for reculture.

Various dilutions of culture in glucose broth were infused into quartes of two cows and two bufaloes.

Two normal cows and two normal buffaloes were also used to test the pathogenicity of fungal cultures from mouldy grains. The cultures were also tested intravenously in rabbits and trails were adopted to reisolate yeasts and fungi.

RESULTS

Eighty two quarters of 65 buffaloes and fifty three quarters of thirty five cows developed mastitis between acute and subclinical forms. Acute mastitis was characterized by severe induration of the udder, a marked drop in milk yield and a febrile reaction of about 2°C. The udder secretion contained yellowish-grey clots in a supernatant fluid. On general, the mastitis lasted about 3-8 days without treatment before time of present investigation.

Milk examination :

Primary cultures revealed a pattern of bacteria isolated from milk samples. Twenty one bufaloes and thirteen cows had the following isolates:-

MASTITIS, MYCOTIC INFECTIONS

This table (1) it is indicated that the most predominant organisms are: Escherichia coli, Staphylococcus pyogenes, Beta haemolytic strept. Pseudomonas pyocyanea, Paracolon spp. and Corynebacterium spp. with the percentage incidences of 20.7, 15.8, 11.0., 7.3, 4.9 and 7.3 in buffaloes and 20.7, 18.9, 17.0, 13.2, 13.2 and 3.7 in cows respectively. The overall incidence of Gram positive to Gram negative organisms isoalted from 135 mastitic quarters was 0.9 : 1.

The incidence of mycotic infection of the udder was as follows: out of 135 milk samples from bufaloes and cows, fungi and yeasts were isoalted as presented in table (2).

The table shows that Candida spp. were isoalted from 61 (74%) of 82 quarters (65 buffaloes) and 43 (81%) of 53 quarters (35 cows). The isolates were similar in both clinical and apparently normal quarters. These included Candida albicans, Candida tropicalis Candida crusei and Phodotorula glutinis. Gram positive organisms were isoalted with the yeasts in approximatlly 20.7% of total samples. Most of these isolates were from buffaloes and cows with clinical mastitis.

Aspergillus spp. associated with mastitis were: Asp. fumigatus, 39, 16; Asp. niger 12, 14; Asp. flavus 9, 9 and Asp. minor 9, 11 in buffaloes and cows respectively.

Incidence of Aspergillus spp. were single isolates in only 8 buffaloes and 9 cows with apparently healthy quarters. Aspergillus isolates in other cases were in addition to Candida spp. Staphylococcus pyogenes, Pseudomonas aeroginosa and Streptococci spp.

The above data shows that the incidence of Aspergillus spp. and Candida spp. form the highest number of isoaltes from milk samples. These fungus isolates produced undesirable efects in the udder of buffaloes and cows.

Frequency susceptibility of quarters to mastitis is recorded in table (3).

It indicates that the hind quarter infection was greate than fore quarters.

The effect of the stage of lactation on the incidence of mycotic mastitis is shown in table (4). The incidence is high during 3 to 6 months after calving.

From tables (4 & 5) it appears that the high incidence of quarter infection appears to take place in the second lactation stage but decreased in the following ones.

Food grains examination :

The following fungi were isoalted from the food grains: Aspergillus spp., Mucor spp., Rhizopus spp., Helminthcsporum spp. and Penicillium spp.

It is found from the above table that the incidence of Mucor was 91.6% i.e. the fungus isoalte infecting the highest number of the grain samples. Incidence of Aspergillus niger was about 66.6% and of Rhizopus 45.8%. There were mixed infection in many samples.

There were similarities in flora types and colony characters between mycoflora isolated from udder secretions and mouldy food grains. In both cases for example, Asp. flavus was characterized as follows: white mycilium tufts changing yellow to dark brown, head globosa with rdiating candida, growth between temp. 21°C - 37°C. Optimum temp. 32°C. Colony growth at 3.7 pH and colony size about 14 mm., at pH 9.3 of the media, colony size about 40 mm.

Experimental studies :

1- With mastitis secretions :

Four secretions from field cases were infused into 4 quarters of 3 cows. After 24 hours mastitis similar to that described among field cases was produced. Yeasts were isolated from these quarters in pure culture.

2- With cultures :

Viable organisms were obtained in glucose broth 8 hours after a heavy inoculation. Three quarters of two cows and two quarters of two buffaloes were infused with doses ranging from 1 ml. of 1 in 1000 dilution up to 10 ml. of 1 in 50 diluted culture. Clinical mastitis resulted in all cows and buffaloes.

3- With cultures of mouldy grains :

Fungus isolates obtained in glucose broth was infused into three quarters of two cows and three quarters of two buffaloes. The dose ranged from 1 ml. of 1 in 1000 dilution up to 10 ml. of 1 : 50 diluted culture. Clinical matitis resulted in all experimental animals within 3-11 days, and symptoms were similar to field cases.

4- The pathogenicity of 8 fungal cultures was tested in rabbits. Intravenous injection of 10^6 yeast organisms or $10X10^6$ fungal spores caused 60-100 death with Candida tropicalis and other Candida isolates, Aspergillus spp. and Penicillium spp. Culture were reisolated from these rabbits succumbing to infection.

DISCUSSION

Many workers estimated the importance and magnitude of mastitis in our country in addition to unfitness for human consumption. The complex factors contributing for this disease are the varied aetiological agents incriminated for the prevalence of bovine mastitis, and the inability of the dairy farmers to recognize the disease in its early stages. The incidence of mycotic infection of the udder was not sufficiently assessed in lactating cattle, although it is becoming prevalent. As a result of the present study, mycotic mastitis prevails in cows and buffaloes in diary husbandary system and the following fungi were isolated: Aspergillus spp., Candida spp., Mucor spp., Rhizopus spp., Penicillium spp., Clandosporium and Cephalosporium spp.

The results have implications for the isolation of bacteria, including Staph. aureus, Strept., Pseudomonas aeroginosa in mixed infections with cases yielding fungi. Identical isolations were reported neither in buffaloes nor in cows previously, but it is still

unknown which infection (bacterial or fungal) took place first. Because of this observation, attention should be given to preventive and therapeutic measures for mastitis. AMEMIYA et al. showed recently (1985) that P.aeroginosa and Staph. aureus, decreased the counts of A.fumigatus, but the addition of oxytetracycline at 1.5 mg/ml. to milk containing Staph. aureus increased counts of A.fumigatus compared with milk containing Staph. aureus without antibiotic, but counts remained lower than in control milk without bacteria. Their results also showed that mastitis pathogens (e.g. P.aeruginosa and Staph. aureus) and L.acidophilus (part of th natural bacterial flora of the udder) can inhibit the budding of Candida and the hyphal growth of A.fumigatus isolated from cases of fungal mastitis. MISRA and PANDA (1986) had obtained a mixture of fungi, yeasts and bacteria in 28 cows with mycotic mastitis.

In 1983, SHARMA showed that the pervalence of mycotic mastitis in cows and buffaloes was 8-9% and isolated organisms were Candida spp., Aspergillus spp., Trichosporon cutaneum, Penicillium spp. and Rhizopus spp.

The occurrence and the effect of udders infection depend basically on the microorganisms, the host and the environment. Though virtually any infection may be acquired by animal, there are pathogenic organisms which are particularly associated with lactation stage. Since cattle in lactation have diminished resistance to disease, organisms which are relatively harmless to healthy animals may cause this illness. Local poor resistance (udder) may also develop because of dead necrotic tissue where bacteria can multiply. The highest infection rate was found in second lactation stage and as defined in this study between the 3rd and 6th months. The stage of lactation seems to reduce the resistance activity of the udder tissue to infection. The present observation reviews that the susceptibility of dairy cattle for udder infection with fungi or bacteria becomes great in this stage.

In attempts to find out the possible source of mycotic mastitis, interest was directed towards mouldy grains in the surrounding enviroment. Surveillance studies were carried out to record any instance of mouldy food grains in the farms. Study on mycoflora of food grains have revealed that large quantities of food grains were infected with many toxic fungi like Aspergillus and Mucor. Toxicity and infectivity of mycoflora of the mouldy food grains was successfully diagnosed by biological tests in normal cows and in rabbits, which was quite satisfactory. The infectivity and toxicity of the fungal isolates from field cases of matitis were also studied by quarter infusion. Individual fungal isolates were reisolated in addition to development of clinical mycotic mastitis. In this way, the scurce of infection in both cows and buffaloes was maintained towards the mouldy food grains. This was further supported by having colony similarities between individual fungi and yeasts isolated from both the infected udders and the food grains.

Previous observations showed that infection of udder was aquired by animals with uterine infections. The magnitude of post-partum metritis and its relation to bacterial mastitis has been studied (MORCOS et al., 1988). However the relation between mycotic mastitis and mycotic uterine infections was not in the scope of this work.

Some authors have indicated that placentitis in cattle was due to fungi, such as Aspergillus fumigatus, A.nidulans and A.terrus (STUKER et al., 1983). KROGH (1985) showed the presence of Aspergillus spp. and Mucor spp. associated with placental material from aborting cattle. MISRA et al. (1984) recovered Aspergillus spp., Candida, Mucor, Rhizopus, Penicillium from uterine swabs from infertile cows and cows which had aborted. When uterine washings were collected and examined, filamentous fungi and yeasts were isoalted (EL AMINE et al., 1984). SUTKA (1983) isolated Candida spp. from organs of culled cows (liver, brain, udder, supramammary lymph node, ovaries and uteruses). The fungi were also demonstrated in milk samples from cows with udder inflammation. With these models, it becomes less difficult to assume a relation between mycotic mastitis and mycotic uterine infections when the the infective uterine discharges can find a way to contaminate the udder. The teat orifice and canal might be a way for infection of udder, and this can explain the higher infection rate of hind quarters.

REFERENCES

Ainsworth, G.C. and Austwick, P.K.C. (1973): Fungal diseases of animals. 2nd ed., Commenwealth Agricultural Bureaux, England.

Amemiya, J.; Yoshimi, R.; Okamoto, K. (1985): Effect of bacteria on the growth of mastitis-causing Candida spp. in milk. Bulletin faculty of Agric. Kagoshima Univ., 35, 119-125.

El-Amine Dafalla; Fadlelmula, A. and El-Sawi, M.O. (1984): The inter-relationship between nonspecific infection and the bovine infertility in Sudan. (1) Fungi isolated from uterine washing of cows. Bulletin of Animal Health & Production in Africa 32(3), 259-263.

Krogh, H.V. (1985): Bovine mycotic abortion in Denmark. Nordisk Vet. Medicine. 37(1): 27-33.

Kirk, J.H.; Bartlett, P.C. and Newman, J.P. (1986): Candida mastitis in a dairy herd. Vet. Bull. (1987) Vol. 57 (7): 572-577.

Misra, P.K.; Mishra, A. and Panda, S.N. (1984): Fungal agents associated with abortion & repeat breeding in cross breed of Orissa. Ind. J. Animal Health. 23(2): 1/5, 119-124.

Misra, P.R. and Panda, S.N. (1986): Some observations on the occurrence of mycotic mastitis in cows. Ind. Vet. J. 63, 11, 386-388.

Morcos, M.B.; Elyas, A.H.; Safwat, E.A. and Amer, A.A. (1988): Bovine mastitis concometant with post-partum metritis. Assiut Vet. Med. J. 19, 38, 47-54.

Murphy, J.M. and Drake, C.H. (1947): Infection of the bovine udder with yeast like fungi. Am. J. Vet. Res. 9, 43-51.

Natalia, L. and Hastiono, S. (1985): Mycotic mastitis due to Candida albicans in cows. Penyakit Hewan, 17(30): 71-74.

Qaem, S.A. and Christensen, C.M. (1958): The growh of Aspergillus flavus on food grains. Phytopathology 48, 544-548.

Rahman, H. and Baxi, K.K. (1983): Prevalence of Candida albicans in bovine mastitis. Ind. J. Microbilogy, Immunology & Infectious diseases 4(1): 49-50.

Rolle, M. (1934): Yeast as a cause of udder infection of cows. (Translated) Deutsche Tier. Woschnschrift 42, 385-389.

Sharma, S.D. (1983): Studies on bovine mastitis with special reference to mycotic infections of udder. Vet. Res. J. 6(1-2): 105-106.

Simaria, M.B. and Dholakia, P.M. (1986): Incidence and diagnosis of mycotic mastitis in cattle. Ind. J. of Animal Sc. 56(10): 995-1000.

Sinha, R.H. and Wellace, H.A.H. (1965): Isoaltion fo fungi from wheat grains. Canad. J. Plant. Sci. 6(3): 12-16.

Stuker, G. and Ehrensperger, F. (1983): Significance and diagnosis of fungal abortion in cattle. In proceedings 3rd Int. Symposium of world Ass. of Vet. Lab. Diagnosticians. Sac., Paulo, Brazil.

Sutka, P. (1983): Candida guilliermondii var guilliermondii in cows slaughtered because of infertility and udder inflammation. Acta Vet. Yugoslavia, 33(5/6): 287-297.

Weigh, W. and Ahlers, D. (1982): Aetiology, symptoms & treatment of yeast mastitis in cattle. Deutsche Tierztliche Wohenschift, 89: 234-238.

Yeo, S.G. and Choi, W.P. (1982): Studies on yeast-like fungi associated with bovine mastitis:

1- Epidemiological study.

2- Sensitivity of yeast-like fungi to antifungal agents.

Korean J. Vet. Res., 22, 2, 121-147.

Table (1): Distribution of bacteria from 21 buffaloes and 13 cows.

Organisms	Total samples		No. of isolates		Percentage	
	Buff.	Cows	Buff.	cows	Buff.	Cows
Escherichia coli	82	53	17	11	20.7	20.7
Staph. pyogenes			13	10	15.8	18.9
Beta haemolytic strept.			9	9	11.0	17.0
Ps. pyocyanea			6	7	7.3	13.2
Paracolon spp.			4	7	4.9	13.2
Corynebacterium spp			6	2	7.3	3.7

Table (2): Fungi & Yeast isolates from 135 milk samples (65 Buffaloes & 35 Cow)

Species isolates	Buffaloes		Cows	
	No. of isolates	%	No. of isolates	%
Aspergillus fumigatus	39	47.6	16	30
niger	12	14.6	14	26.4
flavus	9	11	9	17
minor	9	11	11	13.4
Candida albicans	33	40.2	28	52.8
tropicalis	16	19.5	13	24.5
crusei	12	14.6	2	3.8
Mucor spp.	27	33	15	28.3
Rhizopus spp.	4	4.9	3	5.7
Penicillium spp.	6	7.3	2	3.8
Clandosporium	2	2.4	1	1.9
Cephalosporium	1	1.2	1	1.9

Table (3): Frequency susceptibility of quarters to mastitis

Quarter affected	Mycotic mastitis	Bacterial mastitis
Left hind	32	11
Left fore	68 — 18 — 50	26 — 7 — 18
Right hind	36	15
Right fore	39 — 21 — 57	19 — 12 — 27
Multiple infections	27	3
Total	143	48

MASTITIS, MYCOTIC INFECTIONS

Table (4): Stage of lactation in relation to incidence of mycotic mastitis (100 Cows & Buffaloes)

Stage of lactation	NO. of incidence
1 - 3 months	25
3 - 6 months	32
6 - 9 months	16
10 months & above	6
Unknown	21
	100

Table (5): Effect of stage of lactation on the incidence of bacterial mastitis

Stage of lactation	No. of incidence
1 -3 months	11
3 - 6 months	17
6 - 9 months	7
10 months	3
Unknown	1
	39

Table (6): Fungus flora isoalted from the mouldy food grains

No. of samples		Aspergillus			Mucor	Rhizopus	Helminth sporus	Penicillium
		Flavus	Fumig.	Niger				
Farm I	14	3	4	11	13	7	1	1
Farm II	10	7	4	5	9	4	2	0
	24	10	8	16	22	11	3	1