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Epidemiological and Bacteriological Survey of Buffalo Mastitis in Nepal

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ABSTRACT. A total of 355 Murrah cross buffaloes, consisting of 23 subclinical and 332 clinical mastitis cases brought to the Veterinary Teaching Hospital, Chitwan, Nepal from 2002 to 2005, were analyzed to determine the organisms involved, the seasonal occurrence of mastitis, and antibiotic susceptibility of mastitis pathogens. Coagulase negative Staphylococci (CNS) such as *Staphylococcus albus* and *S. epidermidis* were the predominant organisms associated with subclinical cases, and CNS and Coliforms in clinical cases. The maximum number (16%) of clinical cases of mastitis were observed in the month of July, when temperature and humidity are highest. The incidence of clinical mastitis was higher in animals during 1st calving and during the 1st month of parturition. Resistance to antibiotics was determined for 55, 23 and 149 isolates of Staphylococcus spp., Streptococcus spp. and Coliforms, respectively. *In vitro* drug sensitivity testing revealed that enrofloxacin had the highest average sensitivity (91%) for all types of bacteria. The effectiveness of other drugs detected were gentamicin (87%), tetracycline (83%) and chloramphenicol (82%). The antibiogram showed that both gentamicin and enrofloxacin are slowly becoming resistant. Mastitis pathogens have developed resistance to ampicillin and penicillin.

KEY WORDS: Antibiotics, buffalo, mastitis, milk, sensitivity.

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The economic losses from mastitis due to severe drop in milk production, potential health risks for other animals and human beings, increased cost of treatment and culling processes, are tremendous [6, 12]. In addition to economic losses to farmers, effective control of mastitis is also important from the consumer's and processor's point of view, because milk from affected animals may harbor organisms potentially pathogenic to humans and the processing of such milk may result in substandard fermented products [1].

Identification of mastitis causing pathogens and the results of the antibiotic resistance of the isolated bacteria are important prerequisites for implementation of effective control of buffalo mastitis. Such information is needed not only to treat and control mastitis but also to support public health concerns in developing countries. However, epidemiological and bacteriological studies of buffalo mastitis have not been fully conducted in Nepal.

This study was performed to ascertain the epidemiological and bacteriological analysis for the detection of mastitis and antimicrobial resistance of pathogens isolated from buffalo mastitis in Chitwan district of Nepal.

MATERIALS AND METHODS

Buffaloes: Milk samples from 355 Murrah cross buffaloes brought to IAAS Veterinary Teaching Hospital, Rampur, Chitwan district, Nepal were examined. A total of 319 cases recorded at the mastitis laboratory of IAAS were analyzed for the prevalence of clinical mastitis during different seasons. Quarter milk from udders was categorized as subclinical mastitic based on the following criteria: absence of visible abnormalities of milk secretions; electrical conductivity (EC) values > 3.7 mS/cm as measured by a conductivity meter (Milk checker N4 Oriental Instruments Ltd, Tokyo, Japan); and positive results of bacteriological isolates [4, 9, 12]. Clinical mastitic milk was categorized based on the following criteria: presence of clinical signs in the udder and abnormal milk secretions; EC values > 3.7 mS/cm; and positive bacteriological isolates from quarter milk samples.

Milk sampling: The teat end was disinfected with cotton soaked in 70% ethyl alcohol. The first few streams of foremilk were discarded. Samples for microbiological analysis were then collected into sterilized test tubes and were held on ice until delivery to the laboratory within 15 to 30 min of collection. Samples were kept at room temperature before streaking onto culture plates in the laboratory.

Isolation of bacteria and bacterial count: Bacteria were isolated and identified using biochemical tests, as shown by Cowan and Steel [2], as well as API system.

One hundred μl of milk from each quarter was streaked onto MacConkey and 5% ovine blood agar plate for bacterial culture and isolation. The colonies were counted after 24 and 48 hr of incubation at 37°C. Bacterial numbers > 25 cfu /100 μl were the standard for the presence of mastitis infection. Pure colonies from the respective plates were identified on the basis of Gram stain, morphological findings, colony characteristics and biochemical tests (API System, Bio Merieux Marcy l'Etoile, France).

Analysis of rainfall and temperature records: Temperature and rainfall records from 2002 to 2005 were collected from Bharatpur Municipality of the Chitwan district, Nepal

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[5]. The maximum and minimum temperature of different months was recorded and the mean temperature for all the months of the 4 years was calculated. The maximum rainfall was noted for each month of every year. Then the mean maximum rainfall for particular months was calculated from the values of the same month over 4 years. Temperature was measured in °C and rainfall was measured in millimeters (mm).

In vitro antibiotic sensitivity test: The different strains of isolated organisms from clinical cases of mastitis were streaked onto Mueller Hinton agar plates and 7 antibiotic discs (gentamicin, chloramphenicol, enrofloxacine, penicillin, ampicillin, streptomycin and tetracycline, Hi Media company, India) of various concentrations were diffused on them to observe the zone of inhibition. Nearly 5 colonies were transferred into trypticase soya broth and incubated for 2 to 4 hr at 37°C so as to obtain turbidity. After the disc had been applied, the plates were kept at 10°C for 3 to 4 hr to allow pre-diffusion of the antibiotics. The plates were then incubated at 37°C for 24 hr and observed for sensitivity by measuring the zone of inhibition. Results were recorded as sensitive, intermediate sensitive and resistant according to the manufacture's instruction.

Statistical analysis: The cumulative data was entered in Microsoft Excel for analysis. The student's *t*-test was used for statistical analysis of the data, and $P<0.05$ was regarded as significant.

RESULTS

The occurrence of clinical mastitis in buffaloes during different months was recorded (Table 1). The maximum number of cases of clinical mastitis was observed in the month of July (16%) followed by June (12.2%) and August (13.5%). Results show that 37.3% of buffaloes had clinical mastitis during the summer season followed by the autumn season (31.7%). The months from June to August have the highest rainfall in Chitwan district, when the average temperature was 30°C (Fig. 1).

The highest incidence of clinical mastitis (43.6%) was also observed during the first month of parturition. Following parturition, there was a decreasing trend of mastitis cases in buffaloes (Fig. 2). A greater (51.6%) incidence of clinical mastitis was found in animals during their 1st calving, with subsequent reduction in the 2nd calving. The occurrence of mastitis decreased as the number of calvings increased (Fig. 3).

Coagulase negative Staphylococci (CNS), such as *S. albus* (33.3%) and *S. epidermidis* (11.1%), followed by Micrococcus (19.3%), were the most common isolates found in milk with subclinical infections(Fig. 4). Bacillus spp., Streptococcus spp. and Clostridium spp. were other environmental pathogens isolated from quarters with subclinical mastitis. In clinical mastitis, CNS (35.7%) and Coliforms (10.7%) were the most commonly isolated pathogens (Fig. 5).

The results of in vitro drug sensitivity of the isolates from clinical mastitis against 7 different antibacterial agents are presented in Table 2. Coliform bacteria were most sensitive to enrofloxacin (96%) followed by gentamicin (90%) in the year 2002. However, the sensitivity decreased to 92 and 84.8% in 2005, respectively. Coliform bacteria showed higher resistance towards streptomycin and chloramphenicol.

Staphylococcus spp. were most sensitive to enrofloxacin (93%) followed by gentamicin (92.5%) in the year 2002. The sensitivity of these bacteria towards enrofloxacin and gentamicin also decreased in the year 2005, to 80 and 71.4% respectively. Ampicillin was the least effective antibiotic.

Table 1. Prevalence of clinical mastitis in buffaloes at different seasons

Season	Months	Samples	Percent	
Summer	May	29	9.09	
	June	39	12.23	37.31
	July	51	15.99	
Autumn	August	43	13.48	
	September	32	10.03	31.66
	October	26	8.15	
Winter	November	24	7.52	
	December	25	7.84	23.2
	January	25	7.84	
Spring	February	10	3.13	
	March	10	3.13	7.83
	April	5	1.57	

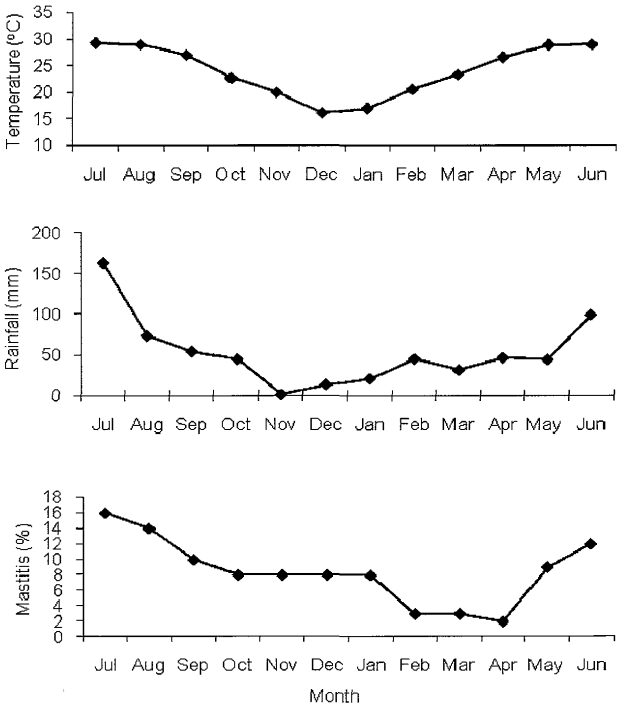

Fig. 1. Comparison of clinical mastitis cases with mean temperature and rainfall for 4 years. Data for temperature and rainfall was from Bharatpur Municipality, Chitwan District, Nepal [5].

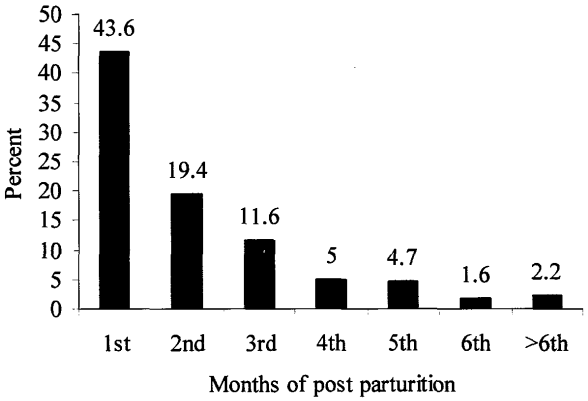

Fig. 2. Occurrence of clinical mastitis in buffaloes according to stage of lactation.

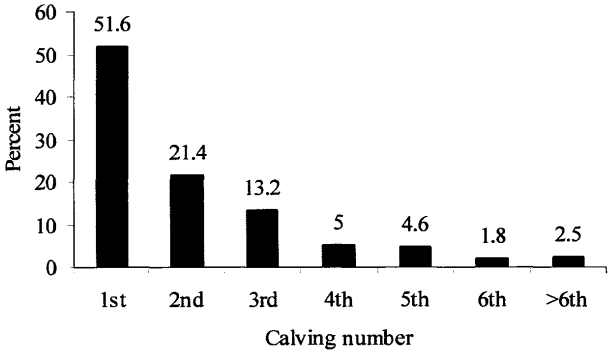

Fig. 3. Occurrence of clinical mastitis in buffaloes according to number of calving.

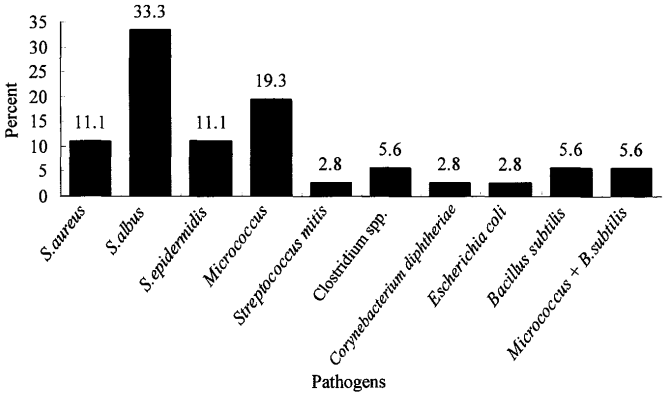

Fig. 4. Frequency of organisms in 36 isolates from the quarter milk of subclinical mastitic buffaloes.

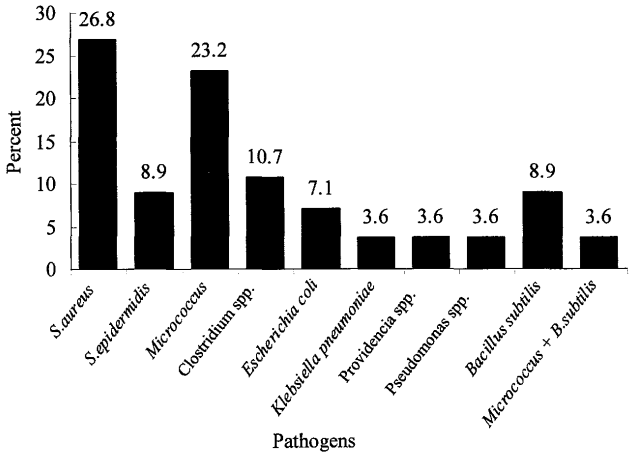

Fig. 5. Frequency of organisms in 56 isolates from the quarter milk of buffaloes with clinical mastitis.

In 2002, its sensitivity was 70.7% and decreased to 53.3% in 2005.

Streptococcus spp. showed 100% sensitivity towards enrofloxacin in the year 2002, which dropped to 83% in the year 2005. Sensitivity of Streptococcus spp. to gentamicin dropped from 87.5% to 83.3% from 2002 to 2005, respectively. Ampicillin was the least effective (66.7%) against Streptococcus spp.

DISCUSSION

The highest incidence of clinical mastitis was found in the summer season in Chitwan district, Nepal, and the most frequently isolated bacteria were CNS and Coliforms. This finding was consistent with that of Moroni *et al.* [9] who reported that CNS were the most common pathogen (66% of the positive samples) isolated in dairy buffalo milk in northern Italy. The greatest number of clinical cases of mastitis was observed in the month of July. The reason for the high incidence of clinical mastitis during this month may be associated with not only heat stress and poor hygienic conditions found in buffaloes during high temperature and humidity in this season.

In this study, 62% of buffalo affected by subclinical mastitis and 64% of buffaloes with clinical mastitis had an infection in a single quarter. The greatest number of buffaloes were affected with clinical mastitis at their 1st calving followed by 2nd and 3rd calving. Joshi and Shrestha [8] reported a 17.6% prevalence of clinical mastitis during 1st calving. However, Pal and Verma [11] reported the highest incidence at 3rd calving. The difference found among reports may be due to various factors such as breed, season and husbandry system (9).

The incidence of clinical mastitis was found to be higher within the 1st month after parturition. This finding was consistent with the results of Yas *et al.* [15] who found that the incidence of mastitis was higher during the first 2 months of lactation and declined in subsequent months. The reason for this remains to be elucidated: it may be related to the characteristics of decreased host defense capability during the post-parturient period in buffaloes (unpublished observation), as observed widely in dairy cows [10].

The present study showed that environmental bacteria, Staphylococci and Coliforms are the most problematic and

Table 2. Antibiotic sensitivity of the bacteria isolated from clinical mastitis

Year	Percent sensitivity						
	G	Cl	Ex	P	Am	Str	T
Coliform (149)							
2002(60)	90	79	96	ND	ND	50	89
2003(36)	93.8	71	95	ND	ND	87	92
2004(20)	87.2	84	93	ND	ND	79	85
2005(33)	84.8	87	92	ND	ND	84	85
Staphylococcus spp. (55)							
2002(18)	92.5	90	93	80.5	70.7	ND	79
2003(19)	90.6	84	92	75	69.4	ND	83
2004(12)	73.3	64	88	68.4	55.6	ND	69
2005(6)	71.4	57	80	60	53.3	ND	57
Streptococcus spp. (23)							
2002 (4)	87.5	100	100	77.8	66.7	ND	100
2003 (7)	88.9	86	91	90.9	70	ND	91
2004 (6)	100	100	83	100	83.3	ND	100
2005 (6)	83.3	78	83	75	66.7	ND	67

G = Gentamicin, Cl = Chloramphenicol,
Ex = Enrofloxacin, P = Penicillin,
Am = Ampicillin, Str = Streptomycin,
T = Tetracycline , ND = Not determined
Figures in parentheses indicate number of isolates.

significant mastitis pathogens of buffaloes in Chitwan district, Nepal. It is known that Staphylococci typically colonize the broken skin and can enter the udder through abrasions of the teat end [3].

The rates of environmental pathogens found in milk from buffaloes with clinical mastitis were also similar to that reported by Moroni *et al.* [9]. Minor pathogens and Coliforms are often isolated from the skin of the udder due to contamination with soil and feces. The virulence of environmental bacteria isolated from soil and feces to the mammary gland of buffaloes is still unclear. Improvement of the production environment, good milking hygiene and proper handling of buffaloes appeared to be important.

It is generally known that Micrococcus are capable of producing a mild degree of udder irritation, slight leucocytosis and reduce milk yield in buffaloes. However, it has not been reported that their presence in the mammary gland may reduce infection rate of primary pathogen in buffaloes. Further study is required to determine the significance of Micrococci in mastitis control.

The *in vitro* antibiotic sensitivity patterns of isolates from clinical mastitis of buffaloes revealed that of the 227 isolates tested, a higher percentage of sensitivity was found to enrofloxacin, followed by gentamicin, tetracycline and chloramphenicol. All organisms showed decreased sensitivity to enrofloxacin with passage of time.

Staphylococcus and Streptococcus spp. showed decreased sensitivity towards all antibiotics. The resistance of Staphylococcus isolates to enrofloxacin and gentamicin was 20 and 28.6% in 2005, an increase from 7 and 7.5% in the year 2002, respectively. Only 5% of Staphylococcus isolates was resistant to gentamicin in 1994 [7]. The resistance of Streptococcus isolates to enrofloxacin and gentamicin was 17 and 16.7% in 2005, an increase from 0 and 12.5% in 2002, respectively. No resistance of Streptococcus isolates to gentamicin was detected in 1994 [7].

The resistance of Coliform isolates to enrofloxacin and gentamicin was 8 and 15.2%, respectively, in the year 2005, which was increased from 4 and 10% in the year 2002, respectively. Similar resistance patterns of Coliform isolates to gentamicin were detected in 2001 (12%) [14]. Currently, enrofloxacin is widely used as the antibiotic of first choice to treat mastitis in Nepal. These results indicate that the wide use of antibiotics and their under dosing for treatment of mastitis may cause drug resistance to antibiotics. Drug residue in milk also poses a public health problem. Proper information concerning the use of antibiotics, antibiotic resistance, drug residues, and the protection of public health should be provided to the farmers and related personnel.

The epidemiological and bacteriological findings reported in this study will lead to the development of more effective control strategies for buffalo mastitis and implementation of the essential steps for improvement of milk quality control in Nepal.

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