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RESEARCH ARTICLE

Prevalence of Sub-Clinical Mastitis among Dairy Cattle in Bhaktapur District, Nepal

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ABSTRACT

The study was conducted to estimate the prevalence of subclinical mastitis (SCM) in dairy cows in the Bhaktapur District of Nepal. A total of 200 milk samples were collected from 50 dairy cows. The California mastitis test (CMT) was carried out on quarter milk samples to determine the prevalence of subclinical mastitis. California Mastitis Test (CMT) of these samples revealed that 52 % of animal suffered from SCM. The samples positive to CMT were cultured in Nutrient, MacConkey and blood agar. The bacteria isolated were *Staphylococcus* spp. (50 %), *Streptococcus* spp. (27 %), *Escherichia coli* (10 %), *Corynebacterium* (8 %), *Salmonella* (4 %) and *Enterobacter* spp. (1 %).

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INTRODUCTION

Mastitis is a common disease entity of dairy cows, accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). The disease is usually classified as subclinical, acute, sub acute and chronic based on etio-pathological findings and observations (Triptahi and Chaltopadhyay, 1993). Sub-clinical mastitis causes significant and appreciable losses through decreased milk production, treatment cost and replacement of dead or unproductive individual. About 150 species of micro-organisms were found as the etiological agent of mastitis (Ebrahimi and Nikookhah, 2005). In Asia, major mastitis causing organisms are *Staphylococcus aureus*, *Streptococci*, *E. coli*, *Corynebacterium* spp. and *Klebsella* spp., recent reports indicating the changing trends from *Staphylococcus aureus* to Coagulase Negative Staphylococci (CNS) as major mastitis causing organism (Sharma *et al*., 2012). Subclinical mastitis (SCM) may be of public health significance as SCM causing organisms have been isolated in several milk borne diseases in human beings (Verma *et al*., 1978). It is now a well known fact that the SCM is more serious and is responsible for much greater loss to the dairy industry (Kader *et al*., 2002). Dhakal and Thapa (2003) estimated the losses of Rs.4287 or $63 per buffaloes per lactation in the Nepalese context. Decreased milk production accounts for about 70% of the total cost of mastitis (Sapkota, 2012). About 10-26 % of total milk loss occurs in quarters with subclinical intra-mammary infection (Dhakal, 2007). If an animal can be screened for SCM and treated, it will prevent economic loss resulting from disease state (Bansel and Singh, 2004). The California mastitis test (CMT) is a commonly used rapid test for sub-clinical mastitis that detects somatic cell nuclear material, relying on a threshold of 300,000 SCC per milliliter (Radostits *et al*., 2000). It is an inexpensive and relatively easy test recommended for detecting subclinical mastitis. Therefore, the present research work was undertaken with the aim to observe the prevalence and etiological agents of subclinical mastitis (SCM) among dairy cows in Bhaktapur District.

MATERIALS AND METHODS

A total of 200 quarter milk samples were collected from 50 dairy cows. Samples were collected from dairy pocket areas of Bhaktapur District. Clinically healthy cows with history of unsatisfactory milk production were used as samples for this study. Simple random sampling was done. Milk samples were not collected from cows, which were treated with antibiotics by any route, till 96 hours of treatment. The apices of the teats was mopped with sterile gauge several times, finally with gauge soaked in 70 % alcohol and allowed to dry. A few streams of fore-milk were discarded then 5-10 ml of milk sample from each quarter was collected aseptically in four separate, sterilized screw-capped test tubes. They were marked as right front (RF), right hind (RH), left front (LF)

and left hind (LH) and transported in ice-packed containers to the Laboratory of the Himalayan College of Agricultural Sciences and Technology (HICAST).

Detection of sub-clinical mastitis

The samples collected in the sterile test tubes were brought to the HICAST. Subclinical mastitis was determined using the CMT reagent. Three milliliters of the milk sample from each quarter and equal amount of the CMT reagent was placed in each cup of CMT paddle and the content was mixed by gentle circular motion of the paddle in a horizontal plane. Based on the thickness of the gel formed by CMT reagent-milk mixture, test results were scored as 0 (negative / trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive) within 10 seconds of gentle swirling of the reagent paddle. In this study, milk samples with test results of negative / trace were assessed as having originated from cows free of subclinical mastitis while CMT results of +1, +2 and +3 were classified as evidence of subclinical mastitis.

Bacterial Culture

A loopful of sub-samples of each CMT positive milk sample was cultured in the Nutrient agar, Mac-Conkey agar and Blood agar. Bacterial growth was identified and recorded after 24 h and 48 h of incubation at 37 °C. The plates were examined for the growth. Bacterial isolates were characterized by macro- and micro-morphology, culture characteristics, gram's staining and bio-chemical tests with different types of sugars. Other tests include motility, coagulase, catalase, indole, methyl red, Voges-Proskauer and oxidase reactions so that the different bacteria isolated could be identified. Special test for suspected organism, such as methylene blue staining for *Corynebacterium* spp. was done.

RESULTS AND DISCUSSION

Out of 200 samples examined, 52 % was found positive in California Mastitis Test (Table-I). Similar result was reported by Karimuribo *et al.,* (2006) in which out of 400 randomly selected samples 46.2 % were found to be SCM positive in CMT test. Joshi and Gokhale (2004) reported that incidence of subclinical mastitis varied from 10 to 50 % in cows and 5 to 20 % in buffaloes in improved and peri-urban dairy farms in India. A score of +1, +2 and +3 in CMT was noticed 22.10 %, 57.34 %, 20.56 % in cows affected with subclinical mastitis respectively. Similar high CMT score in animals with subclinical mastitis was reported by Doxey (1983) and Elango *et al*. (2010). The higher prevalence recorded was not surprising in this study since none of the farmers were applying recommended milking and hygiene procedures. Poor hygiene and milking practices are reported to accelerate the disease (Pankey *et al*., 1984) and might account for the high infection rate among the tested cows. The bacteria isolated were *Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Corynebacterium*, *Salmonella* and *Enterobacter* spp.

The percentage of *Staphylococcus* (50 %) was significantly higher in this study (Table II). These findings was similar to the findings made by Sen *et al.* (1981), Singh and Baxi (1980), Mahbub-E-Elahi *et al.* (1996), Shike *et al.* (1998), Kewler *et al.* (1992) and Umakanthan (1998) who reported highest incidence of *Staphylococcus* spp. in collected samples. The isolation of *Staphylococcus* in this work is also in agreement with that of Ankalo and Sterneojo (2006) who isolated 39 (20.4 %) and 123 (15.4 %), respectively from apparently healthy and mastitic cow in Kenya. Misra *et al.* (1973), Rahman and Boro (1990), Jha *et al.* (1993) and Chanda *et al.* (1998) also reported *Staphylococcus* as the major pathogens of mastitis. Staphylococcus is an opportunistic pathogenic bacterium which survives on the skin of the udder and can infect the udder via teat canal or any wound.

Table I: Prevalence rate of mastitis in selected areas of Bhaktapur District (California Mastitis Test)

S. No.	Areas	Total sample	Positive sample
II	Gatthaghar	460	131
III	Balkot	675	239
IIII	Thimi	565	334
Total		1200	5104

Percentage of cows affected with SCM= 104/200×100=52%

Table II: List and number of isolates of different organisms present in samples collected

S. No.	Organism	No. of isolates	Percentage of isolates
II	*Staphylococcus spp*	252	350%
III	*Streptococcus spp*	128	227%
IIII	*E.coli*	810	110%
IIV	*Corynebacterium spp*	18	18%
VV	*Salmonella spp*	14	44%
VVI.	*Enterobacter spp*	12	11%
	Total	7104	1100%

Infections with *Streptococcus* spp. were found to be 27 % which was identified micro-morphologically by Gram staining. Ferguson *et al.* (2007) stated that *Staphylococcal* infections are most often present in lactating animals. To stop and to reduce infections of mammary gland with pathogen bacteria like streptococcus it is necessarily to take care about nutrition, accommodation and bedding programs for mastitis preventions.

The increased incidence of *E. coli* (10 %) recorded in this study was higher in comparison to the earlier finding (6.0 %) by Mahbub-E-Elahi *et al.* (1996). The major- risk factor in housed cattle is contamination of the teat with environmental pathogens such as *E. coli* and *Streptoccocus uberis* (Blowey and Edmondson, 2011). General cleanliness, drainage and manure disposal was poor in farms. Milking practices were also poor, only fewer farmers were practicing of drying the teats before milking. Detergents, disinfectants and teat dips were not used by any farmers. Incomplete milking also found to be prevalent. This may be attributed to the increase incidence of *E. coli* in this case.

The prevalence of Corynebacterium was 8%. Kapronezai *et al*. (2005) reported a 26.77 % rate in positive mammalian quarters by the microbiological exam, in which *Staphylococcus* spp. (38.99 %), *Bacillus* spp. (18.24 %), *E. coli* (18.24 %), *Klebsiella* spp. (11.3 %), *Corynebacterium* spp. (6.29 %) and *Streptococcus* spp. (3.78 %) were isolated. In developing countries where mastitis control is not in place, contagious organism *like S. aureus*, *Str. agalactiae* and

Corynebacterium are the most predominant mastitis pathogens (Ali, 2009).

The prevalence of *Salmonella* was 4 %. Ankalo and Sterneojo (2006) also isolated *Salmonella* in clinically mastitic cows. The presence of this organism may indicate fecal contamination of milk but more importantly an indicator for poor sanitary practice during milking.

The presence of *Enterobacter* species was recorded 1 %. Though this species isolated, their presence in milk sample is less frequent. However, being a coliform they may occur in milk samples probably as contaminants.

Conclusions

The present study showed high prevalence of sub-clinical mastitis in cow in the study area. Lack of maintenance of strict hygiene and good sanitary environment may be a contributory factor in the cause of mastitis in the study area. It is therefore important that farmers should ensure strict personal hygiene and sanitary condition to control the disease in animals. *Staphylococcus* was found to be major causative agent for subclinical mastitis. Further studies are required to identify risk factors for sub-clinical mastitis. Antibiotic sensitivity tests should be focused to devise treatment and control of sub-clinical mastitis in dairy cow in the area.

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