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DIAGNOSIS OF SUBCLINICAL MASTITIS IN BOVINE USING CONVENTIONAL METHODS AND ELECTRONIC DETECTOR

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ABSTRACT

Sub clinical mastitis is an important disease of dairy cows and buffaloes causing huge economic losses in form of reduced milk production. In the current study, 125 animals (25 buffaloes, 30 crossbred cows, 15 Sahiwal and 55 Achai breed) apparently mastitis free, were selected for the collection of milk samples. These samples were subjected to surf test, NaOH, pH meter and Electronic detector. On the electronic detector 65.2% animals were positive for subclinical mastitis. On Surf and NaOH tests, 56.8% animals were found positive for each test. While On pH meter, 40.8% animals were positive for subclinical mastitis. In case of Sahiwal cattle, prevalence of subclinical mastitis was more in left fore quarter ($P<0.05$). In buffaloes, it was more prevalent in both hind quarters. In Achai and crossbred cows, no significant difference was found in quarter wise prevalence. The results of the current study indicate that electronic detector is more sensitive than surf test, NaOH and pH detector. It gives the results on spot and can also help to detect quarter wise prevalence. So the farmers can easily use this technique to screen out their dairy animals for subclinical mastitis.

Keywords: sub clinical mastitis, bovine, electronic detector, surf test, NaOH, pH meter test, prevalence.

INTRODUCTION

Mastitis is an important disease of dairy animals. It is an inflammation of the mammary gland (udder) that causes physical and chemical changes in milk and leads to pathological condition of the glandular tissue. It is generally associated with poor hygienic and husbandry practices. Bruising of mammary tissue or teats from traumas, nursing, flies bites, or other wounds predisposes the females to mastitis. The infection rate of mastitis in cows with pendulous udder is higher than those having non-pendulous udder (Sori *et al*., 2005). A wide range of pathogens including viruses, bacteria, fungi and their toxins can cause the disease. The primary reservoir of contagious pathogens is the mammary gland itself. Frequency of contagious pathogens among mastitis cases is greater (Sori *et al*., 2005). The infectious agent enters through the milk canal, interacts with the mammary tissue cells and multiplies. The mammary tissue reacts to these toxins and becomes inflamed.

The dairy industry is facing a great set back due to high prevalence and incidence of mastitis in milch animals. Subclinical mastitis affects milk quality and quantity causing great economic loss for producers (Swinkels *et al*., 2005; Halasa *et al*., 2007). Annual losses in the dairy industry due to mastitis was approximately 2 billion dollars in USA and 526 million dollars in India, in which subclinical mastitis are responsible for approximately 70% of these losses (Varshney and Naresh, 2004). Apart from causing huge economic losses, this disease also posses the risk for the transmission of zoonotic diseases like tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to human beings (Radostits *et al*., 2000).

The pH of normal cow's milk lies in the range from 6.5 to 6.8 but may exceed 7.0 in milks with high cell counts (Marschke and Kitchen, 1985). The pH of normal buffalo's milk ranges from 6.6 to 6.9 which indicate that it is slightly acidic. The acidity of buffalo's milk is due to the presence of phosphates, proteins and to some extent CO_2 and citrates. The fresh milk contains no lactic acid (Bilal and Ahmad, 2004). In a previous study, prevalence of clinical and sub clinical mastitis was higher in hindquarters than forequarters and among hindquarters; left hindquarters were more susceptible than the right (Khan and Muhammad, 2005). In cross bred cows, incident of mastitis is higher due to rapid removal of large amount of milk which causes injury to teats and predisposing to infection. In Pakistan, due to lack of good husbandry practices like teat dipping and dry period antibiotic therapy, mastitis is highly prevalent and causes high economic losses (Arshad, 1999). Environmental pathogens are most often responsible for the disease. Dry period antibiotic therapy can eliminate 70% of environmental streptococcal infections (Jones, 2006). Sub clinical mastitis is more common as compared to clinical mastitis and causes great economic losses in the dairy herds (Jasper *et al*., 1982).

Concentration of Na and Cl ions is increased in mastitis which leads to increase electric conductivity of milk from the infected quarter (Kitchen, 1981). From the mid-eighties, work has been carried out in order to automate the detection of mastitis by means of sensors (Hogeveen *et al*., 2010) and interest in the application of

sensors to detect mastitis and abnormal milk has been increasing considerably in recent years (Brandt *et al*., 2010). The current study was conducted to evaluate the prevalence of subclinical mastitis in and around the Surezai Station, Peshawar, Khyber Pukhtunkhwa through surf test, electronic detector, pH meter and NaOH test in buffalo, local and cross bred cows.

MATERIALS AND METHODS

Sample collection

A total of 125 animals (25 buffaloes, 30 crossbred cows, 15 Sahiwal and 55 Achai breed), apparently mastitis free, were selected for milk samples. The samples were brought to dairy technology center of Livestock Research and Development Station, Surezai, to investigate the incidence of subclinical mastitis.

Surf field mastitis test

The samples were subjected to surf test. For this purpose, 3% Surf solution was prepared by addition of three grams of commonly used detergent powder (Surf Excel, Unilever, Pakistan) in 100 ml of water. Milk samples and surf solution were then mixed in equal quantities in Petri dishes. The formation of gel depicted the positive samples (Muhammad *et al*., 1995).

NaOH test

The test is based on the increase in number of leukocytes in mastitis milk. The original test was described by Whiteside (1939) and was modified by Schalm *et al.*, (1971). For this purpose, 4% NaOH solution was prepared taking 4ml NaOH mixed with 96ml distilled water. Three ml of milk sample was mixed with 3ml of NaOH solution. The gel formation indicated a positive result.

pH detector

As described by the manufacturer, pH reading was recorded by pH meter (Hanna Instruments, ISO-9001) for checking the alkalinity of milk sample. The pH level more then 6.8 indicated the incidence of sub clinical mastitis.

Electronic detector

Electronic detector (Drasminski Mastitis Detector) was used according to the manufacturer's instructions. The Draminski Mastitis Detector is electronic device which can also be used for testing the milk from strings of cows and buffaloes or the bulk tank for somatic cell levels, checking the recovery status of treated quarters, checking cows at time of dry-off for udder infection status or to periodically check the milk of every cow in the herd.

Statistical analysis

The data was analyzed by one-way ANOVA using SAS software program. The results were declared significant if $P < 0.05$ and declared non significant if $P > 0.05$.

RESULTS

The present study was conducted to probe the quarter wise prevalence of sub clinical mastitis in different breeds of bovine at Surezai Station and to compare the results of Surf test, NaOH test, pH meter and electronic detector. For this purpose, 125 animals (25 buffaloes, 30 crossbred cows, 15 Sahiwal and 55 Achai breed) apparently mastitis free, were selected for the collection of milk samples. The samples were brought to dairy technology center of Livestock Research and Development Station, Surezai. Quarter wise detection of mastitis in different breeds was also studied through electronic detector. The details of the results are given in Tables 1 and 2.

Comparison of the results showed an increased sensitivity of the electronic detector ($P<0.05$) in all the bovine species under study. Surf field mastitis test and NaOH test depicted the same results. The pH meter test was least sensitive. The detail of the statistical analysis is given in Table-3. In case of Sahiwal cattle, prevalence of sub clinical mastitis was more in left fore quarter ($P<0.05$) as compared to other quarters. In buffaloes, sub clinical mastitis was more prevalent in both hind quarters. In Achai and crossbred cows, no significant difference was found in quarter wise prevalence of sub clinical mastitis (Table-4).

Table-1. Showing results for the total number of animals positive for sub clinical mastitis.

Tests	**Cross bred cows** (30)	**Achai** (55)	**Sahiwal** (15)	**Buffalo** (25)	**Total** (125)
Electronic detector	25(83.3%)	30(54.5%)	9(60%)	20(80%)	84(67.2%)
Surf test	21(70%)	26(47%)	6(40%)	18(72%)	71(56.8%)
NaOH test	21(70%)	26(47%)	6(40%)	18(72%)	71(56.8%)
pH meter	15(50%)	18(32%)	4(26.6%)	13(52%)	51(40.8%)

Table-2. Showing quarter-wise prevalence of sub clinical mastitis.

Number of animals	Right fore quarter	Left fore quarter	Right hind quarter	Left hind quarter
Cross bred cows 25(83.3%)	8(32%)	7(28%)	4(16%)	6(24%)
Achai 30(54.5%)	9(30%)	10(33%)	3(10%)	8(26%)
Sahiwal 9(60%)	1(11%)	5(55.5%)	1(11%)	2(22%)
Buffalo 20(80%)	3(15%)	2(10%)	8(40%)	7(35%)

Table-3. Showing statistical analysis of different diagnostic tests.

Breeds	**Name of the test** (mean percentage)*			
	Surf test	**NaOH test**	**pH meter**	**Electronic detector**
Cross bred cow	70^{ab}	70 ^{ab}	50 ^{b}	83 ^{a}
Achai	46 ^{a}	46 ^{a}	33 ^{a}	53 ^{a}
Sahiwal	40 ^{ab}	40 ^{ab}	26 ^{b}	60 ^{a}
Buffalo	73 ^{ab}	73 ^{ab}	50 ^{b}	80 ^{a}

*Means with different superscripts are different ($P < 0.05$)

Table-4. Showing quarter wise statistical analysis of the study.

Breeds	**Quarters** (mean percentage)*			
	Right fore	**Left fore**	**Left hind**	**Right hind**
Cross bred cow	32^{a}	28 ^{a}	24 ^{a}	16^{a}
Achai	32 ^{a}	32^{a}	24 ^{a}	12^{a}
Sahiwal	12 ^{b}	56^{a}	24 ^{b}	08^{b}
Buffalo	16 ^{ab}	08 ^{b}	36 ^{a}	40 ^{a}

* Means with different superscripts are different ($P < 0.05$)

DISCUSSIONS

Successful breeding from the health as well as economic point of view is highly dependent on the healthy udder of dairy animals. The affected cows and buffaloes produce less milk in lactation and in the entire life. Due to permanent damage of one or more quarters of the udder, the market value of the animal is highly reduced or the animal may be isolated from production. Different tests have been used for early diagnosis of the disease like Surf field mastitis test, California mastitis test (CMT) and somatic cell count (SCC). Measurement of electric conductivity of milk for diagnosis of subclinical mastitis is a new technique and is not frequently being used by the farmers in Pakistan. In the current study, the results of the four tests including, Surf field mastitis test, NaOH test, pH meter test and electronic detector were compared. Quarter wise prevalence of subclinical mastitis among different bovine species was also studied.

On the electronic detector, 65.2% animals were positive for sub clinical mastitis. On Surf and NaOH tests, 56.8% animals were found positive. While on pH meter, 40.8% animals were positive for subclinical mastitis. These results showed that the electronic detector technique is more sensitive than other tests.

There was higher incidence of sub clinical mastitis in hindquarter in buffaloes than cross bred and local cows. Among hindquarters, right hindquarter was more susceptible than the left hindquarter. In case of forequarters, there were high incidences in cross bred and local cows then the buffaloes. Among fore quarters; right forequarter were found to be more prone to infection in cross bred cows and left fore quarter in local cows. In the previous study, Khan and Muhammad (2005) reported that the total number of quarters affected with subclinical mastitis were 54(27%) out of 200 in buffaloes. Among these 8(14.8%) were right fore, 16(29.6%) right hind, 10(18.5%) left fore and 20(37%) left hind quarters. In cows (36%) 72/200 quarters were

infected in which 14/72 (19.4%) right fore, 20/72 (27.8%) right hind, 13/72(18.1%) left fore and 25/72(34.7%) left hind were infected. So there was high incidence rate of subclinical mastitis in hind quarter in buffaloes than the cross bred cows. In hindquarter; left hind quarter were found to be more susceptible as reported by Saini *et al.*, (1994). Quarter wise prevalence of sub-clinical mastitis in Pakistan has been reported 37.75% (Sharif and Ahmad, 2007). The difference in quarter wise prevalence of subclinical mastitis observed in current study and previous studies may be due to difference in breeds of animals, immune status and manage mental practices. As dung and urine should be removed immediately as these are constant source of infections at farm. Any bad odor within the animal shed indicates the infection. Clean and dry farm premises ensure risk free environment for animal health (Sharif et al., 2009). Due to mammary gland infection, increased in somatic cell count was reported by Schalm *et al.*, (1971) and Eberhart *et al.*, (1979). In another study reported by Bachaya *et al.*, (2005), the quarter wise prevalence was found to be 58.75% while animal wise prevalence was recorded as 77.98%. Fazal-ur-Rehman (1995) reported quarter wise and animal wise prevalence of mastitis in buffaloes as 64 and 30.5%, respectively. Anwar and Chaudhry (1983) investigated that the prevalence in buffaloes were 47.5% after using Strip cup test, pH test and White side test. There is gel formation when subclinically infected milk is mixed with detergent solution as surf solution (Schalm *et al*., 1971). Inflammation of mammary gland, increased cells count can be detected easily on electronic detector that is more sensitive then other tests like surf test, NaOH test and pH meter. Electrical conductivity of milk has been used for diagnosis of mastitis. The specific conductance of milk reflects the concentration and activity of sodium and chloride ions. Higher values of these ions represent mastitis. This change in the concentration of sodium and chloride is detected by digital detector.

By using electronic devices timely useful information can be obtained for making timely management decisions. It can be an effective management tool for helping producers to get more profit from their dairy business. The producers should be encouraged to discuss early mastitis monitoring methods with their veterinarian, extension agent, milk handler, field representative, equipment supplier or dairy consultant. Farmers can easily screen out their animals for subclinical mastitis by using electronic detector and can be able to locate the abnormal quarters. This technique gives the results on the spot and can help to know the stage of infection.

REFERENCES

Anwar M. and Chaudhry A. Q. 1983. Subclinical mastitis in buffaloes around Lahore. Pak. Vet. J. 3: 142.

Arshad G. M. 1999. A population based active disease surveillance and drug trails of mastitis in cattle and buffaloes of District Sargodha. M. Sc Thesis. Department: Vet. Clinical Medicine and Surgery, University Agricultural, Faisalabad, Pakistan.

Brandt M., Haeussermann A. and Hartung E. 2010. Invited review: Technical solutions for analysis of milk constituents and abnormal milk. J. Dairy Res. 93: 427-436.

Bilal M. Q. and Ahmad A. 2004. Dairy Hygiene and Disease Prevention. Usman and Bilal Printing Linkers, Faisalabad, Pakistan.

Bachaya H. A., Iqbal Z., Muhammad G., Yousaf A. and Ali, H. M. 2005. Subclinical Mastitis in Buffaloes in Attock District of Punjab (Pakistan). Pak. Vet. J. 25: 134-136.

Eberhart R. J., Gilmore H., Hutchinson L. J. and Spencer S. B. 1979. Somatic cell count in DHIA samples. Proc. 21st Annual Meeting of the National Mastitis Council, Louisville, Kentucky, USA. pp. 32-40.

Fazal-ur-Rehman. 1995. Studies on I) Evaluation of surf field mastitis test for the detection of sub-clinical mastitis in buffaloes and cattle, II) Antibiotic susceptibility of pathogens. M. Sc Thesis, University Agricultural, Faisalabad, Pakistan.

Hogeveen H., Kamphuis C., Steeneveld W. and Mollenhorst H. 2010. Sensors and Clinical Mastitis-The Quest for the Perfect Alert. Sens. 10: 7991-8009.

Halasa T., Huijps K., Osteras O. and Hogeveen H. 2007. Economic effects of bovine mastitis and mastitis management: A review. Vet. Q. 29: 18-31.

Jasper D. E., Macdonald J. S., Mochrie R. D., Philpato W. A., Farnsworth R. J. and Spender S. B. 1982. Bovine mastitis research: Needs, funding, and sources of support. Proc. 21st Annual Meeting of the National Mastitis Council, Louisville, Kentucky, USA. pp. 184-193.

Jones G. M. 2006. Understanding the Basics of Mastitis. Virginia Cooperative Extension, Publication No. 404-233, Virginia State University, USA. pp. 1-7.

Kitchen B. J. 1981. Review of the progress of dairy science: Bovine mastitis: Milk compositional changes and related diagnostic tests. J. Dair. Res. 48: 167-188.

Khan A. Z. and Muhammad G. 2005. Quarter-Wise Comparative Prevalence of Mastitis in Buffaloes and Crossbred Cows. Pak. Vet. J. 25: 9-12.

Muhammad G., Athar M., Shakoor A., Khan M. Z., Fazal-ur-Rehman and Ahmed M. T. 1995. Surf field mastitis test: An expensive new tool for evaluation of wholesomeness of fresh milk. J. Food. Sci. 5: 91-93.

Marschke R. J. and Kitchen B. J. 1985. Detection of Bovine Mastitis by Bromothymol Blue pH Indicator Test. J. Dairy Sci. 68: 1263-1269.

Radostits O. M., Blood D. C., Gay C. C. Hinchiff K. W. and Handerson J. A. 2000. Mastitis. In: Veterinary Medicine. 9th Ed. WB Saunders Company, London, UK. pp. 603-700.

Saini S. S., Sharma J. K. and Kwatra M. S. 1994. Prevalence and etiology of sub clinical mastitis among crossbred cows and buffaloes in Punjab. Indian J. Dairy Sci. 47: 103-106.

Schalm O. W., Carrol J. E. and Jain N. C. 1971. Bovine Mastitis. 1st Ed. Lea and Febiger, Philadelphia, USA. pp. 132-153.

Sharif A., Umer M. and Muhammad G. 2009. Mastitis control in dairy production. J. Agri. Soc. Sci. 5: 102-105.

Swinkels J. M., Hogeveen H. and Zadoks R. N. 2005. A partial budget model to estimate economic benefits of lactational treatment of subclinical Staphylococcus aureus mastitis. J. Dairy Sci. 88: 4273-4287.

Sharif A. and Ahmad T. 2007. Prevalence of severity of mastitis in buffaloes in district Faisalabad (Pakistan). J. Agri. Soc. Sci. 3: 34-36.

Sori H., Zerihum A. and Abdicho S. 2005. Dairy cattle mastitis in and around Sebeta, Ethiopia. Int. J. Appl. Res. Vet. Med. 3: 332-338.

Varshney J. P. and Naresh R. 2004. Evaluation of homeopathic complex in the clinical management of udder diseases of riverine buffaloes. Homeopath. 93: 17-20.

Whiteside W. H. 1939. Observation on a new test for the presence of mastitis in milk. Canad. Publ. healt. J. 30: 40.