DETECTION OF PH INDICATOR PAPER OF BOVINE MASTITIS IN COMPARISON WITH CALIFORNIA MASTITIS

M.H. TABIDI[1], H.H. MUSA[2], M.A. MUKHTAR[1]

[1]Department of Animal Production, College of Agricultural Studies, Sudan University of Science and Technology
[2]Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan
E-mail: mtabidi@hotmail.com

Summary

In the present study bovine mastitis was investigated using California mastitis Test (CMT) and pH indicator paper bromothymol blue in Khartoum State, Sudan. Ten dairy farms were selected randomly, from each farm 10 cows were selected among the total of hundred cows. Six farms suited in Khartoum North, two farms in each Khartoum and Omdurman locations. A simple bromothymol blue indicator was evaluated for farms diagnosis of bowline mastitis. The test required highly absorbent bolting paper impregnated with four sports of bromothymol blue indicator color scores 1 to 4 for quarter foremilk's increased with somatic cell count and pH, although variability within each color score was large. Sensitivity of the bromothymol blue test ranged from 51 to 56% and specificity from 89 to 90% for most reference criteria used to classify normal and abnormal milk. In this study the results revealed that pH indicator bromothymol blue was more accurate to detect subclinical mastitis than California mastitis Test. Results of previous study showed that 73% the general infected farms which considered very huge in dairies farms that due of lack of biosecurity and prevention to control the disease. Examine of bacteriology was performed to samples. The sensitiveness of bacteria to antibiotics was found that most common bacteria *Staphylococcus*, *Streptococcus* and *E. coli* were sensitive to erythromycin, and penicillin cephoxitin.

Key words: BMT, CMT, Zoonotic disease, Bromothymol blue

Bovine mastitis is the most costly disease to the dairy industry worldwide, with losses estimated at 1.4 billion dollars per year in United State (15). This disease characterized by an in-crease somatic cells especially leukocytes in the milk and by pathological changes in the mammary gland tissue (13) causes economic losses, but also hold the risk for the transmission of zoonotic disease like tuberculosis, brucellosis leptospirosis and streptococcal sore throat to human beings (2). Several methods were applied to detect bovine mastitis from which California Mastitis test (CMT) was used for a quality measurement of somatic cells counting milk, a screening test for subclinical mastitis that can be used easily at cow-side (9). The use of CMT to identify infected quarter has been extensively validated in cows that were not in early lactation. The California is rapid accurate cow-side to help determine somatic cell count in specific cow.

A simple bromothymol blue indicator test was evaluated for farm diagnosis of mastitis, the test required highly absorbent paper impregnated with four sport of

reagent of bromothymol blue. Indicator color scores (1 to 4) for quarter for milk increased with somatic cell count and pH although variability within each color score. PH-indicator technique in positive reaction the spot was charge from yellow to green or bluish green. PH indicator paper can detect rapidly streptococcus mastitis (11). The milk of healthy cows changes the indicator color to yellowish green. The test can be used by dairy producers to screen herds with relatively high incidence of mastitis or used in combination with cow cell counts to local abnormal quarters. However, the disease can be control by pasteurization technique, but a variety of bacteria still contribute illness and disease outbreak (6). The milk from an infected animal is the main source of pathogenic bacteria (6).

Bovine mastitis is caused mainly by certain *Staphylococcus* and *Streptococcus* species include *Staphylococcus aurous*, staph, chromo genes *staph-epidemidis*, *staph scinaltans* and *Streptococcus agalictiae sprepto dysgaloctiae* and *Streptococcus bevies* (5). One of the common types of chronic mastitis is caused by the bacteria *Staphylococcus aurous* which is often significant, subclinical and extremely difficult to control by treatment alone (12). The most commonly used antibiotics on conventional dairy farms were penicillin, cephalosporin and tetracyclines. For mastitis penicillin, ampicilin and tetracycline are suitable for treatment of Bovine mastitis. The main purpose of this study was to find the efficiently of California mastitis test to detected subclinical mastitis n comparison with pH indicator paper, and to define the incidence of mastitis in Khartoum state. Confirm the etiological agent of bovine mastitis and sensitive test of bacteria to antibiotic.

Materials and methods

The study was conducted in Khartoum State included three provinces Khartoum, Khartoum North and Omdurman. Ten Farms were randomly selected, and ten cows were selected from each farm. Experimental animals were cross breed Friesian, local breed Butana, and Kenana breeds. The test applied to detected bovine mastitis California mastitis test (CMT), which required a plastic paddle having reagent was dispensed in plastic bottle provided with a fine nozzle. Equal volume milk and reagent was put in each cup and grittily rotated by movement of the paddle in horizontal plane, the reaction was observed immediately. The California mastitis test (CMT) with rapid mastitis test reagent was done on 2 ml of test milk mixed with 2 ml of reagent. Results of California mastitis test (CMT) were scored on scale 1 to 4, corresponding to increasing viscosity of the milk reagent mixture. Score was assessed as a normal quarter and scores 2, 3 and 4 as abnormal quarters.

pH indicator paper bromothymol blue was made to determine mastitis (Mana fractured by Kruse company in Denmark). The test was applied by adding one drop of milk on yellow sport and to observe the change in color, within 1 to 2 min of sampling the bromothymol test the color of each indicator sport was scored

on a scale 1 to 4 according to color standards. Score 1 (pale green) was assessed as a normal quarter and scores 3.3 and 4 (increasing from moderate green to dark blue green) were assessed as abnormal quarters (3).

Milk samples were collected under strict aseptic condition as stated by Barrow and Felltham (3). Samples of milk were immediately frozen in ice and simplified for bacteriological examination within 24 hours. Tool of bacteria logical exam in examination sterile Bijou bottles after cleaning the outer surface of the adder and teal with potassium permanganate and with cotton wool soaked in 70% alcohol. The love milk was stripped off and about 5 ml of milk were drawn in sterile Bijou bottle. All samples collected were immediately placed on ice in thermo flask after collection. The two media used in culture were blood agar and Macconkey agar. After culturing the plates were incubated at 37^{o}C purification was achieved by further sub culturing on nutrient agar incubated at 37^{o}C for 24 hours. After purification the plates were examined for cultural characteristic and biochemical reactions according to standard key (3). After isolation and identification of bacteria the sensitivity test was applied by multi discs of different antibiotics, put the culture incubation for 24-48 hours at 37^{o}C aerobically. The effectiveness read by diameter of growth inhibition around different antibiotics multi discs.

Results and discussions

The California mastitis test was applied to detected mastitis in ten farms in Khartoum State. Result obtain for this test showed that the lower farms were infected by mastitis reputed at Sudan University farm (Kuku). Percentage of injection was 3% which can be easily controlled. The private sector ① farm and University of Khartoum the moderate percentage of infected farm by mastitis recorded 10-16%.The highly infected farm by mastitis was reported in Kafoury farm the precipitate record at 48%, that due to bad management and lack of biosecurity to prevention and control. The paper bromothymol blue was tested in ten farms the results revealed that the lower infected farm by mastitis registered in Sudan university Kuku the percentage recorded 23%, the moderate infected farm by mastitis was reported in Judiciary and private section the percentage 66-60%. Kafoury farm also held the highly infected mastitis which reported at 88-87 which consider bad indicator for biosecurity in this farm and Khartoum University farm the problem due lack of udder hygiene and efficient isolation infected cows form others and not applicable the treatment in early stage. Incidence of mastitis in Khartoum university farm reasons of the disease which presents sub clinical mastitis this study consist with Batavani et al. (3). Accordingly, from results obtain Sudan University farms Kuku, Shambat recorded lower occurrences of bovine mastitis in California mastitis test and ph indicator paper that due of respect the biosecurity program. The pH indicator paper characterized than other test of mastitis can easy detect sub clinical mastitis. Technique is more accurate sensitive, easy and rapid. It

can be done in the field at the time of the milking collection which consistent with the results of the previous work and reports (8, 15).

California Mastitis Test (CMT) is easily carried and considered as known and largely used to detect mastitis. The result of the test is not affected by external factors. The last diagnostic test takes about 15 seconds which is longer than pH indicator paper test. This test needs skilled personnel to perform it. Laboratory examination of pathogenic slides revealed that the pathogens were *Streptococcus*, *Staphylococcus* and *E. coli* and they are more sensitive antibiotics like erythromycin, penicillin and cephoxitin. The results are in agreement with the findings of (10).The study revealed that the farms with cross breeds cows showed the highest incidence of mastitis. It seems inevitable to rise cross breed cows for high milk production. It was observed that in farms of manual milking the spread of mastitis was higher.

Previous studies showed that heredity has influence on mastitis incidence in special in Holstein Friesian. The mastitis incidence varied between 9-22% which is great if compared with other reproduction characteristics. The studies also showed that the correlation between heredity and environmental interaction in the highest mastitis incidence. The environmental impact varies from difference environments in the research conducted by (1).

Table 1

California mastitis test for the infected Animals

Name of Farms	No. of cows tested	No. of teats test		Total no. of teats	Morbidity rate %
		Infected	Non infected		
Private sect ①	10	5	33	38	13%
Private sect ②	10	5	35	40	13%
Sudan university Kuku	10	1	3	40	3%
Judiciary Farm	10	8	31	39	21%
Sudan university Shambat	10	1	38	39	3%
University of Khartoum	10	4	35	39	10%
Private sect③	10	6	32	38	16%
Private sect④	10	4	36	40	10%
Kafoury farm	10	19	21	40	48%
Private sect⑤	10	8	32	40	20%

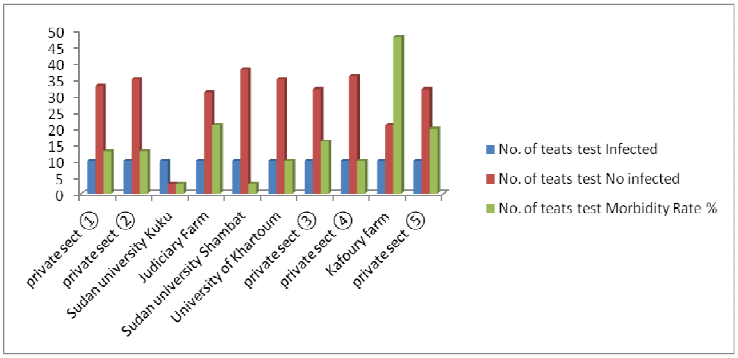

Fig.1. California mastitis test for the infected animals

Table 2

pH indicator paper test for the infected animals

Name of Farms	No. of cows test	No. of teats test		Total No. of Teats	Morbidity rate %
		Infected	No infected		
Private sect ①	10	25	13	38	66%
Private sect ②	10	28	12	40	70%
Sudan university Kuku	10	9	31	40	23%
Judiciary Farm	10	24	15	39	62%
Sudan university Shambat	10	10	29	39	26%
University of Khartoum	10	34	5	39	87%
Private sect ③	10	21	17	38	56%
Private sect ④	10	24	16	40	60%
Kafoury farm	10	35	5	40	88%
Private sect ⑤	10	26	14	40	65%

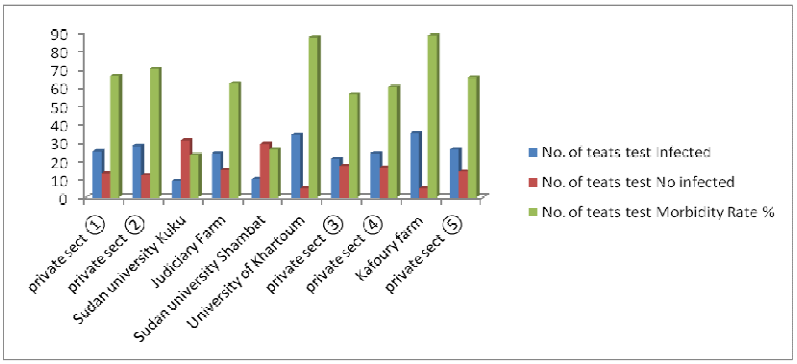

Fig. 2. pH indicator paper test for the infected animals

Table 3

Degree of sensitive types of bacteria to various types of antibiotics

Type of antibiotics	Types of bacteria	
	Staphylococcus	Streptococcus
Erythromycin (ERY)	Sensitive	More sensitive
Penicillin (Pen)	Less sensitive	Sensitive
Kanamycin (KAN)	Sensitive	Resistance
Furazoludone (FUR)	Sensitive	Resistance
Cephoxitin (CEP)	Sensitive	Resistance
Oxacillin (OXA)	More sensitive	Resistance
Tetracyclin (TET)	Sensitive	Sensitive
Nalidix Acid (NAL)	Less sensitive	Resistance

Conclusions

Continuous monitoring of mastitis and its management is essential for the well-being dairy herd. This can be achieved through the detection of inflammation at its early stages and, subsequently, the detection and treatment of the mastitis infection. Traditional and well-established tests include SCCs and culture based methods. We recommend the development of novel analytical platforms incorporating enzymatic assays, immunoassays, biosensors and nucleic acid tests are progressively replacing the more conventional methods. Also, with advances in proteomics and genomics, new biomarkers are being discovered, allowing the disease to be detected at earlier stages. This will lead to assays with higher sensitivity, which can provide additional quantitative information on the level of inflammation `on-site' and 'on-line' and which are also faster and less expensive. Furthermore, recent advances in microfluidics will facilitate the development of improved technologies that could subsequently be incorporated into automatic monitoring systems and portable assays for sensitive and rapid detection of mastitis.

References

1. **Amin, A.A., Gere T.**, Genetic parameters of udder mastitis and milk traits in two different climatic areas using animal model analysis, Gzech. J. Anim. Sc., 2000, 45, 193-199.
2. **Bachaya, H.A., Raza, M.A., Murtaza, S., Akbar, I.U.R.**, Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan), J. Anim. Plant Sci., 2011, 21(1), 16-19.
3. **Barrow, G.I., Feltham, R.K.A.,** Cowan and Steel's manual for identification of medical bacteria, 3rd edition, Cambridge Press, 2003.
4. **Batavani, R.A., Mortaz, E., Falahian, K., Dawoodi, M.A.,** Study on frequency, etiology and some enzymatic activities of subclinical ovine mastitis in Urmia, Iran. Small Ruminant Res., 2003, 50, 45-50.
5. **Forsman, P., Tilsala-Timisjarvi, A., Alatossava, T.,** Identification of staphylococcal and streptococcal causes of bovine mastitis using 16S-23S RNA spacer regions, Microbiology, 1997, 143, 3491-3500.
6. **Gilmour, A., Harvey, J.,** Society for Applied Bacteriology Symposium Series, 1990, 19, 147S-166S.
7. **Junaidu, A.U., Salihu, M.D., Tambuwala, F.M., Magaji, A.A., Jaafaru, S.,** Advances in applied science research, 2011, 2(2), 290-294.
8. **Kitchen, B.,** Review of the progress of dairy science: Bovine mastitis: Milk compositional changes and related diagnostic tests, J. Dairy Res., 1981, 48, 167-188.
9. **Leslie, K.E., Dingwell, R.T.,** Mastitis control: where are we and where are we going? In: Andrews, AH (Ed.), The health of dairy cattle, (1 st. Edn.), Malden, Blackwell Series, 2000, 370-381.
10. **Leslie, K.E., Jansen, J.T., Lim, G.H.,** Opportunities and implications for improved on-farm cow side diagnostics, Proc. De Laval Hygiene Symp., 2002, 147-160.
11. **Marschke, R.J., Kitchen, B.J.,** Detection of bovine mastitis by bromothymol blue pH indicator test, J Dairy Sci., 1985, 68(5), 1263-1269.
12. **Radostits, O.M., Gay, C.C., Blood, D.C., Hinch Cliff, K.W.,** Mastitis V, In: Veterinary Medicine, 9th ed., W. B. Saunders, London, 690-720.
13. **Ranjan, R., Gupta, M.K., Singh, S., Kumar, S.,** Current trend of drug sensitivity in bovine mastitis, Vet. World, 2010, 3(1), 17-20.
14. **Rasmussen, M.D., Bjerring, M., Skjoth, F.,** Visual appearance and CMT score of foremilk of Individual quarters in relation to cell count automatically milked, J. Dairy Res., 2005, 72, 49-56.
15. **Sahoo, N.R., Kumar, P., Bhusan, B., Bhattacharya, T.K., Dayal, S., Sahoo, M.,** Lysozym in livestock: A guide to selection for disease resistance: a review, J. Anim. Sci. Adv., 2012, 2(4), 347-360.