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Observations on Sub-Clinical Mastitis in Buffalo-Cows with Emphasis on Measuring of Milk Electrical Resistance For its Early Detection

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Abstract: Buffaloes are the main dairy animals in some developing countries worldwide. Mastitis is one of the most economically important diseases of dairy animals. It causes great economic losses and affects the quality and quantity of milk. So, this work was design to investigate this problem with emphasis on its early detection and to correlate between bacteria isolates from sub clinically mastitic gland and vaginal isolates. Milk samples and vaginal swabs were collected from apparently healthy lactating buffalo-cows reared at Al-Sherkia governorate during the period from 2004-2007. The electric conductivity of milk was measured using a manual meter which offers the potential of a simple, rapid test that allows objective interpretation of results. Standard laboratory methods were used for the culture and identification of bacteria. Results revealed that 9.69% of the examined buffalo cows suffered from sub clinical mastitis, 76.93% of these animals showed the affection during the 4 – 6th weeks post partum. High incidence of bacterial isolates (*E. coli, S. epidermidis, C. bovis, Klebsiella* spp., *S. uberi, S. aureus* and *S. agalactiae*) was recorded for milk samples collected from sub clinical mastitic group as compared to healthy animals. A tight relationship was found between the incidence of major pathogenic bacteria (*E. coli, Klebsiella* spp., *S. aureus* and *E. faecalis*) isolated from the vagina and those isolated from the udder in sub clinical mastitic group. Resistivity and electrical resistance values were significantly($P<0.01$) higher, while pH values was not significantly changed in milk samples collected from sub clinical mastitic group as compared to the healthy group. It was concluded that lactating buffalo-cows should be periodically checked for sub clinical mastitis and a suitable method such as electric conductivity should be used for early diagnosis of mastitis to decrease the possible economic losses. A Tight relationship was found between bacteria isolated from the udder and the vagina of affected buffalo-cows.

Key words: Buffalo · mastitis · vagina · bacteria · electrical conductivity

INTRODUCTION

Buffaloes are the main dairy animals in some developing countries worldwide despite this species tends to have relatively slow rate of reproduction and more reproductive problems such as inactive ovaries, long calving interval and mastitis [1-5].

Mastitis is one of the most economically important diseases of dairy animals. It causes changes in glandular tissues as well as it affects the quality and quantity of milk. The incidence of clinical mastitis in buffalo ranges from 8 to 40%. Subclinical mastitis cause reduction in milk quality and its market value as well as it is responsible for up to 70% of the losses in mastitis [6]. Moreover, quarter-wise prevalence of intramammary infection in buffalo was 66%, especially during the periparturient period, whereas the incidence is highest during the 30 days after calving [7].

Staphylococcus aureus is the cause of a widespread spectrum of infections in humans and different animal species. It has been isolated frequently from bovine mastitis [8, 9].

Early diagnosis of mastitis is important for reduction of production losses and for enhancing the prospects of recovery. Also, the identification of sub clinically infected gland is urgently required for successful control of mastitis in dairy animals. A number of diagnostic systems for detection of subclinical mastitis were used including

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bacteriological examination of milk and assessment of udder inflammation using somatic cells count (SCC) which reflects the disease-combating response of the animal to pathogen [10].

Recently, electrical conductivity is used for detection of mastitis in dairy cattle before the appearance of visible signs and it was proved to be more sensitive, specific and timeless than conventional diagnosis [11]. Moreover, it was found that determination of resistivity (the reciprocal of electrical conductivity) of milk offers the potential, simple and rapid test for diagnosis of subclinical mastitis [12].

The current investigation was designed to:

- Monitor the incidence of sub clinical mastitis in lactating buffalo-cows
- Measure of the electrical resistance of milk samples from healthy and mastitic mammary glands for the feasible application of this simple mean for early detection of mastitis, before the emergence of clinical signs to make treatment more reasonable.
- Correlate between bacteria isolates from sub clinically mastitic gland and vaginal isolates.

MATERIALS AND METHODS

The current work was carried out on buffalo-cows reared at villages of Al-Sherkia governorate, Egypt during the period from November 2004 to December 2007 during regular field trips carried by members of National Research Center project No. 7120106 to improve buffalo productivity at smallholder farms.

Samples collection: A total of 813 milk samples were collected from the mammary glands of apparently healthy lactating buffalo-cows. Foremilk samples (up to 10 ml) were collected aseptically from individual quarters from all buffalo-cows for bacteriological culture, using routine techniques for aseptic collection of milk. The udder was palpated for signs of infection. Samples were examined for bacteria and the Somatic Cells Count was measured [13].

Immediately after collection of milk samples the conductivity of milk for each quarter was measured using a manual meter (Milk Checker; Eisai, Tokyo, Japan).

2- A total number of 240 vaginal swabs were collected under aseptic condition as outlined by [14] and the reproductive status was recorded.

Bacterial isolates and culture methods: All milk samples were collected for bacteriological examination. Vaginal swabs were inoculated into tryptic soy broth and incubated at 37°C for 24 hours. Standard laboratory methods were used for the culture, isolation and identification of bacteria [15].

Some biophysical analysis:

- The electrical conductivity of strict foremilk was measured with a hand-held digital conductivity meter and resistivity was calculated as the reciprocal of electrical conductivity and it was related to the infection status [12].
- The pH was assessed using a microprocessor precision digital pH/mv meter model pH-539 [16].

Statistical analysis: Data were statistically computed and analyzed according to [17].

RESULTS

Bacteriological examination of milk samples from 813 apparently healthy, lactating buffalo-cows indicated that 78 animals (9.69%) suffered from sub clinical mastitis with no respective observable clinical symptoms on animals or palpable abnormalities in udders. Owners of some cases complained from a decrease in milk production with no detectable changes in its physical characteristics. Interestingly, 60 cases (76.93%) of these buffalo-cows showing the affection during the 4-6th weeks post partum.

Regarding milk samples, *E. coli*, *S. epidermidis*, *C. bovis*, *Klebsiella* spp., *S. uberi*,, *S. aureus* and *S. agalactiae* were the most predominant isolates and were isolated with a higher rate from buffalo- cows with sub-clinical mastitis than from healthy animals, while, *S. pyogenes*, *P. multocida*, *M. haemolytica* and *A.pyogenes* were only isolated from sub clinical mastitic group (Table 1).

Of the examined vaginal swabs, the most predominant isolates from buffalo- cows suffering from sub-clinical mastitis were *E. coli*, *Micrococcus* spp., *Y. enterocolitica*, *E. faecalis*, *Klebsiella* spp., *C. diversus*, *S. aureus* and *C. bovis* On the other hand, *S. pyogenes* were only isolated from sub clinical mastitis group (Table 1).

It is clear from Table 1 that a tight relationship was found between the incidence of isolation of major pathogenic bacteria including *E. coli*, *Klebsiella* spp.,

Table 1: Bacteria isolated from milk samples and vaginal swabs of lactating buffalo-cows (%)

	Milk samples		Vaginal swabs	
Isolated strains	Healthy group[1]	Subclinical Mastitic group[2]	Healthy group[1]	Subclinical Mastitic group[2]
Y.enterocolitica	00.0	00.00	37.88	53.33
E. coli	75.76	94.99	71.21	93.33
C. diversus	00.00	00.00	25.75	26.67
Klebsiella spp.	34.85	51.67	7.58	35.00
M. haemolytica	00.00	5.00	00.00	00.0
P. multocida	00.00	8.33	9.09	13.33
P.mirabilis	00.00	00.00	4.55	00.00
E. aerogenes	6.06	15.00	00.00	00.00
S. aureus	16.67	33.33	3.03	26.67
S. agalactiae	3.03	31.67	00.00	00.00
S. dysagalactiae	6.06	23.33	00.00	00.00
S. uberis	13.64	46.67	00.00	00.00
A.pyogenes	00.00	3.33	00.00	00.00
S. pyogenes	00.00	8.33	00.00	24.99
E. faecalis	7.58	24.00	39.39	38.33
C. bovis	45.45	55.00	4.55	23.33
S. epidermidis	59.10	78.33	7.58	13.33
Micrococcus spp.	00.00	00.00	40.91	56.67
Bacillus spp.	00.00	00.00	18.18	16.67

1- N0 = 66. 2- No= 60

Table 2: Effect of sub clinical mastitis on some biophysical parameters in milk of buffalo-cows (Mean±SE).

Group	Resistivity Cm/ms	Electrical resistance (units)	pH
Healthy animals	0.2020±0.084	288.0±24.0	6.53±0.16
Sub clinical mastitis	0.1798±0.067**	254.0±49.0**	6.52±0.19

** Significantly differ at P<0.01

S. aureus and *E. faecalis* from the vagina and those isolated from the udder in sub clinical mastitic group.

Table 2 reveals that the resistivity and the electrical resistance values were significantly (P<0.001) lower while pH values was not significantly changed for milk samples collected from sub clinical mastitis group as compared to healthy animals.

DISCUSSION

Mastitis is an important mammary gland affection that is usually caused by bacterial infection. If untreated, it constitutes a serious problem in dairy herds with considerable economic losses, mainly due to poor milk quantity and quality [18] as well as treatment cost [19]. So, the present study was designed to investigate sub clinical mastitis in the main Egyptian dairy animals which is buffalo that produce 65% of dairy product with special emphasis to find a practical marker for its early diagnosis.

Results revealed that 9.69% of the examined buffalo- cows suffered from sub clinical mastitis depending upon bacterial isolates and SCC in milk samples. such results were in harmony with [5].

In the current study, 76.93% of buffalo-cows showed the affection during the 4 – 6th weeks post partum. This result correlates well with the period of the peak milk production, In this respect, it is recorded that the maximum milk yields in buffalo is obtained during 4 – 6th postpartum weeks, whereas the animal is under the metabolic stress of production as well as the physical stress of the manual milking process.

High incidence of bacteria was isolated from the milk samples of buffalo-cows suffered from sub clinical mastitis, the most prevalent isolates were *E. coli, S. epidermidis, C. bovis, Klebsiella* spp., *S. uberi,, S. aureus* and *S. agalactiae*. Several bacterial pathogens were reported to cause mastitis such as *Staphylococcus aureus* which was recorded as one of the most important etiologic agents in mastitis of dairy animals [20], *S. aureus, Micrococcus* spp., *Bacillus* spp., *S. dysgalactiae* and *E. coli*. [21] added that *S. aureus*, but not *E. coli* pathogens frequently cause subclinical, chronic infections of the mammary gland. Infections with live *E. coli*, but not *S. aureus* pathogens induce strongly IL-8 and TNFalpha gene expression in the udders. Barkema *et al.* [22] recovered *S. aureus* from 65.3% of clinically affected mammary glands, coagulase-negative staphylococci from 2.9%, enterobacteria, mainly *E. coli*, from 7.3%, Streptococcus spp. from 4.6%, *Mannheimia haemolytica* from 1.8% and various other bacteria from 4.9%, while no bacteria were cultured from 13.2%

In the current investigation, a tight relationship was evident between the major pathogenic types of bacteria isolated from the vagina and the udder with higher rate of isolation in subclinical mastitis group in comparison with the healthy group. This condition was previously attributed to the relatively suppressed immune response during the postpartum period, whereas estrogen level is low [23]. Also, the condition was attributed to the high level of plasma corticosteroids during such stressful condition. Moreover, it was recorded that the functional capacity of neutrophils is reduced after parturition in cattle [24] and buffaloes [25] and predispose to the establishment of infection. Moreover, it was concluded that the phagocytic and bactericidal capacity of bovine

blood leucocytes were lower during the post partum acyclic period compared to the post partum cyclic period whereas estrogen level was high [26].

Early diagnosis of mastitis is a must for reduction of production losses and for enhancing the prospects of recovery. Also, the identification of sub clinically infected gland is urgently required for successful control of mastitis in dairy animals. A number of diagnostic systems for detection of subclinical mastitis were used including determination of resistivity (the reciprocal of electrical conductivity). Whereas, it was found in the present investigation that the electrical resistance of milk samples from sub clinical mastitic group was significantly ($P<0.001$) lower as compared to healthy animals. To the best of our knowledge, no data were traced in the available literature about this topic in buffaloes. However in Australian dairy cows, [12] stated that the mean electrical resistance of infected is significantly lower than that of uninfected quarters and it offers potential, simple and rapid test for diagnosis of subclinical mastitis, so using of hand-held electronic detectors has been promoted earlier detection which permits earlier treatment.

In conclusion, sub clinical mastitis was reported in a considerable high percent of lactating buffalo-cows. A tight relationship was evident between the types of bacteria isolated from the vagina and those isolated from the udder in animals showing sub clinical mastitis. These animals should be periodically checked using a suitable and objective method such as conductivity measuring system for its early diagnosis and treatment to decrease the possible economic losses.

REFERENCES

1. Singh, J., A.S. Nanda and G.P. Adams, 2000. The reproductive pattern and efficiency of female buffaloes. Animal Reproduction Science, 60-61: 593-604.
2. Ahmed, W.M., H.A. Sabra, E.M. Hanafi and S.I.A. Shalaby, 2002. The present situation of ovarian inactivity of cows and buffaloes in Egypt. Beni-Suef Veterinary Medicine Journal, 12: 13-46.
3. Hussein, F.M., 2002. Reproduction in the buffalo-cow – An overview. Proceeding of the Annual 14th Congress of Egyptian Society of Animal Reproduction and Fertility, Giza, pp: 5.
4. Ahmed, W.M., A.R. Abd El-Hameed and F.M. El Moghazy, 2008. Some reproductive and health aspects of female buffaloes in relation to blood lead concentration. International Journal of Dairy Science, 3: 63-70.
5. Piccinini, R., M. Miarelli, B. Ferri, C. Tripaldi, M. Belotti, V. Daprà, S. Orlandini and A. Zecconi, 2006. Relationship between cellular and whey components in buffalo milk. Journal of Dairy Research, 73:129-133.
6. Kirk, J. and P. Bartlett, 1988. Economic impact of mastitis in Michigan Holstein herds using a computer records programme. Agri-Practice, 9: 3-6.
7. Moroni, P., C. Sgoifo, G. Pisoni, V. Bronzo, B. Castiglioni and P.J. Boettcher, 2006. Relationships between somatic cell count and intramammary infection in buffaloes. Journal of Dairy Science, 89: 998-10038-Barkema et al., 2006.
9. Watts, J.L., 1988. Etiological agents of bovine mastitis, Veterinary Microbiology, 6: 41-66.
10. Schalm, O.W., 1968. The leukocytes: Origin and function in mastitis. Journal of the American Veterinary Medicine Association, 153: 1688.
11. Milner, P., K.L. Page, A.W. Walton and J.E. Hillerton, 1996. Detection of clinical mastitis by changes in electrical conductivity of foremilk before visible changes in milk.Journal of Dairy Science, 79: 83-86.
12. Seguya, A.G. and P.D. Mansel, 2000. An evaluation of a hand-held electrical resistance meter for the diagnosis of bovine subclinical mastitis in late lactation under Australian condition. Australian Veterinary Journal, 78: 21-27.
13. Hillerton, J. and A. Walton, 1991. Identification of subclinical mastitis with a hand –held electrical conductivity meter. Veterinary Record, 128: 513-515.
14. El Jakee, J.K., W.M. Ahmed, F.R. El Seedy and S.I. Abd El Moez, 2008. Bacterial profile of the genital tract in female-buffalo during the different reproductive stages. Global Veterinaria, 1: (in press).
15. Finegold, S.M. and E.J. Baron, 1986. "Diagnostic Microbiology" 7th Edition. The C.V. Mosby Co. St. Louis, London.
16. Bansal, B.K., J. Hamann, N.T. Grabowskit and K.B. Singh, 2005. Variation in the composition of selected milk fraction samples from healthy and mastitic quarters and its significance for mastitis diagnosis. Journal of Dairy Research, 72: 144-152.
17. Snedecor, G.R. and R.G. Cochran, 1980. Statistical Methods. 7th ed. Iwoa State Univ. Press, USA.
18. Seegers, H., C. Fourichon and F. Beaudeau. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Veterinary Research, 34: 475-491.
19. Beck, H.S., W.S. Wise. and F.H. Dodd, 1992. Cost benefit analysis of bovine mastitis in the U.K. Journal of Dairy Research, 59: 449.

20. Mørk, T., T. Tollersrud, B. Kvitle, H.J. Jørgensen and S. Waage, 2005. Comparison of *Staphylococcus aureus* genotypes recovered from cases of bovine, ovine and caprine mastitis. Journal of Clinical Microbiology, 43: 3979-3984.
21. Guliye, A.Y., C. Van Creveld and R. Yagil, 2002. Detection of subclinical mastitis in dromedary camels (*Camelus dromedarius*) using somatic cell counts and the N-acetyl-beta-D-glucosaminidase test. Tropical Animal Health and Production, 34: 95-104.
22. Barkema, H.W., Y.H. Schukken and R.N. Zadoks, 2006. Invited Review: The role of cow, pathogen and treatment regimen in the therapeutic success of bovine Staphylococcus aureus mastitis. Journal of Dairy Science, 89: 1877-1895.
23. Ahmed, W.M., 2008. Overview on some factors negatively affecting ovarian activity in large farm animals. Global Veterinaria, 1: (in press).
24. Zerbe, H., C. Ossadnik, W. Leibold and H.J. Schuberth, 2001. Influence of *Escherichia coli* and *Arcanobacterium pyogenes* isolated from bovine puerperal uteri on phenotypic and functional properties of neutrophils. Veterinary Microbiology, 79: 351-365.
25. Ahmed, W.M., A.R. Nada and S.T.A. Shalaby, 1993. Uterine, hormonal and cellular immune response in some cases of genital disorders in buffaloes. Reproduction in Domastic Animals, 28: 298-301.
26. Ramadan, A.A., G.L. Johnson and G.S. Lewis, 1997. Regulation of uterine immune function during the estrous cycle and in response to infectious bacteria in sheep. Journal of Animal Science, 75: 1621-1632.

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