Journal of Animal and Veterinary Advances 14 (8): 237-243, 2015
ISSN: 1680-5593

Investigation of Bovine Mastitis Pathogens in Two Northwestern Provinces of China from 2012-2014

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Abstract: The objective of this study was to determine the prevalence of mastitis, identify the frequency alteration of predominant pathogens related to clinical and subclinical mastitis in Gansu and Ningxia Province of China. The 29 dairy farms comprising 2000 lactating cows enrolled from April 2012 to November 2014 of which 43.0% (860/2000) were found positive for mastitis by physical examination and Lanzhou Mastitis Test (LMT), the prevalence of Clinical Mastitis (CM) and Subclinical Mastitis (SCM) at cow level were 5.10 and 37.90%, respectively. The proportion of samples contained major pathogens were 97.60% and 871 isolates obtained from 860 milk samples. The common isolates from CM were *E. coli* (26.89%), *S. agalactiae* (21.01%) and *S. aureus* (19.33%) while from SCM were *S. agalactiae*, *S. dysgalactiae* and *S. aureu*, accounted for 30.45, 18.62 and 14.89%, respectively. The contagious pathogens had showed to predominate over the environmental pathogens and the *Streptococcus* sp. was found the predominant species which remains a significant cause of mastitis in local cattle herds.

Key words: Bovine mastitis, prevalence, predominant pathogen, dairy cow, CM

INTRODUCTION

Bovine mastitis is an inflammation of the mammary gland and is still the most prevalent diseases for high yielding cows. The major economic losses caused by mastitis refer to the negative effects on the milk production, milk protein quality, reproduction (Wilson *et al*., 1996; Schrick *et al*., 2001) which lead to increased treatment costs, discarded milk, increased in culling and associated dairy cow replacement rates (De Graves and Fetrow, 1993; Petrovski *et al*., 2006; Piepers *et al*., 2009). In view of cow mastitis which had complex etiology, variable clinical performance and various drugs used in clinical treatment, the frequency of isolated pathogens causing mastitis changed over time and region (Atyabi *et al*., 2006;Waller *et al.,* 2009). The annual incidence of Clinical Mastitis (CM) in large-scale dairy farms in China ranged from 9.7-55.6 cases per 100 cows while for the Subclinical Mastitis (SCM) it ranged from 61.03-79.62% based on the isolation of pathogens and Lanzhou Mastitis Test (LMT) which reported by Pan *et al*. (1996). The most common cause of clinical and subclinical mastitis is intra-mammary infection from several predominant bacterial species, *Streptococcus*, *Staphylococcus* and *Coliform* sp. were among the most common etiological agents. Since, the pathogens was close related to environment and management factors in dairy farms, the incidence rate among dairy herds was differ from farm to farm and place to place obviously, the contribution of various strains of bacteria as a cause of mastitis might vary over time or region (Tenhagen *et al*., 2006; Riekerink *et al*., 2008).

Gansu and Ningxia are major milk source regions in China and has >600 thousands dairy cows which located in Northwest China with a temperate continental climate and plentiful sunshine. Most areas are dry and the temperature varies greatly from day to night. Therefore, they were fit for the development of the dairy industry. The mastitis control program and intensive dairy production system are implemented much better in large scale state-owned dairy farms than some of smallholder dairy farms which were almost with hand milking. However, in some well-managed modern dairy farms mastitis is still a major serious problem for local

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dairy industry. Most studies on bovine mastitis were conducted limited in local dairy herds, no updated data are available for the causative predominant pathogens based on a large-scale research for the two provinces in the Northwest China. By means of microbiological culture of milk samples remains the most effective strategy to identify causative pathogens and to develop an effective mastitis control program (Berry and Hillerton, 2001; Ruegg, 2003) as well as standard mastitis control practices could substantially reduced the prevalence of major contagious pathogens such as *S. agalactiae* (Holgan and Smith, 2003; Pitkala *et al*., 2004). The objective of the current study was to have a further research on the predominant pathogens related to the incidence of bovine mastitis in dairy herds and to describe the prevalence and frequency change of predominant pathogens in the milk samples collected from dairy farms in Gansu and Ningxia provinces from 2012-2014. Furthermore, to present information and recommend practices to local farm managers which would be important and helpful to design a more effective extension programs to reduce the contagious pathogens substantially and produce high quality milk for local dairy farms.

MATERIALS AND METHODS

Ethics statement: This project was approved by the ethics Committee of Animal Experiments of Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou, China. Before carrying out this research, we contacted the owners of all dairy farms involved in the study and obtained their permission for collection of milk samples and the protocol was permitted by the owners of the dairy farms under investigation. Milk samples were obtained with consent from animals with clinical and subclinical mastitis under the approval granted by the Lanzhou institute of animal science and veterinary pharmaceutics, Chinese Academy of Agricultural Sciences (CAAS). All efforts were made to minimize animal suffering.

Herds and study area: The study was carried out from April 2012 to November 2014 and 29 randomly selected dairy herds (4 large state-owned farms, 10 medium-size and 15 smallholder farms) at 8 regions in Gansu and Ningxia provinces of China. The herd structure varied from 20-1200 milk cows per herd and Chinese Holstein breed comprised the majority of milk cows. In this study 2000 randomly selected Chinese Holstein breed dairy cows were in first to ninth parity and in third to eighth age. Only lactating cows with macroscopically altered milk in one or more quarters, namely clinical mastitis and diagnosed positive for subclinical mastitis by Lanzhou Mastitis Test (quarters with LMT scores of 2, 3 or 4 were considered as positive) were selected to collect milk samples aseptically for bacteriological culture and identification.

The study was conducted on every farm with the help of dairyman and owner. The farms were visited at an interval of 6 months from April 2012 to November 2014 to examine cows and collect milk samples. A visit involved evaluation of milking practices, herd size, housing conditions, hygiene of cows, contemporary mastitis management and collection of data on the incidence of mastitis.

Clinical examination, Lanzhou Mastitis Test (LMT) and milk sample collection: Clinical examination including the udder's size, sensitivity, local temperature, skin condition, hardness, milk color, milk yield and if have milk pieces by visual inspection and palpation. Clinical mastitis was defined as an inflammation of the udder leading to occurrence of flakes, clots or other gross alterations in milk. Farmers were instructed to examine the udder and observe for changes in milk two times a day and record it for the duration of the study. Dairy cows without clinical signs of mastitis (udders without clinical abnormalities and giving apparently normal milk) were subjected to further subclinical mastitis examination using Lanzhou Mastitis Test (LMT, a modified California mastitis test) for screening. The LMT diagnostic solution was manufactured by Lanzhou Institute of Animal Science and Veterinary Pharmaceutics, Chinese Academy of Agricultural Sciences (CAAS), the production number was No. 20120226, the test was carried out following manufacturer's instructions. Sample with LMT scores of 0 or 1 were considered as negative, while those with LMT scores of 2, 3 and 4 were considered as positive for SCM and subjected to culture. A cow would be considered as positive when it had at least one of quarters with positive result of LMT score.

Participating farmers were asked to collect quarter milk samples aseptically from cows with CM prior to antibiotic treatment as well as cows diagnosed positive for SCM by LMT test (quarters with LMT scores of 2, 3 or 4 were considered as positive). Milk sample were collected aseptically by the following routine procedures, wash the ill udder with warm water, 0.2% Benzalkonium bromide and wipe out it, then disinfect the teat with 75% alcoholic tampon, milked surplus milk (initial 3 milk streams) from the ill udder in hand to eliminate microbial contamination, approximately 20 mL of milk was collected in sterilized polypropylene transport tube with screw cap and labeled, then kept at 4°C in ice box and transported to microbiological laboratory for bacterial culture.

Bacterial culture: Milk samples were examined following the standard procedures (Sears *et al*., 1993; Quinn *et al*.,

1994). For the primary culture, 10 μL of each milk sample was streaked onto 7% sheep blood agar plate in triplicates (SBA, Hangzhou microbial regent Co., LTD., Hangzhou, China), 10 μL of each milk sample on to MacConkey's agar plate (MCA, Hangzhou microbial regent Co., Ltd, Hangzhou, China) and Edward's modified Medium (EDM; Oxoid LT, Basingstoke, Hampshire, UK), respectively. The plates were incubated at 37°C for up to 48 h under aerobic condition and examined for bacterial growth, morphology and hemolytic features at 24 and 48 h after inoculation.

Plates were considered culture-negative if no growth occurred within 48 h in the primary culture and then the same refrigerated milk sample was subcultured into 5 mL nutrient broth (with 2% serum) at 37°C aerobically for 24 h for enrichment. In the same way, the enriched sample was streaked onto SBA, MCA and EDM culture plates, respectively, incubated aerobically under the identical condition and examined for growth at 24 and 48 h and determined using identical standard as above.

Bacterial examination and identification: Bacteria on culture-positive plates were identified preliminarily according to the colony morphology and the presence of haemolysis using laboratory procedures as defined by the National Mastitis Council guidelines. *Coliforms* sp. was identified by their growth on SBA and MCA, *Streptococcus* sp. by growth on SBA and EDM and *Staphylococcus* sp. by growth on SBA alone. Bacterial isolates were further characterized by Gram-staining and biochemical tests (Quinn *et al*., 1994). Growth on MCA or EDM, Catalase test (H_2O_2 test), Oxidase test, O-F test, Triple Sugar Iron Agar test (TSI test) and Motility test were used for primary identification. And then Methylene Red test (MR test), Voges-Proskauer test (V-P test), Indole test, H_2S test, Urease test, Aesculin Hydrolysis test, Sodium Hippurate Hydrolysis test, Tube Coagulase test and Munch-Petersen test (CAMP test) were used as secondary biochemical tests (Sears *et al*., 1993; Quinn *et al*., 1994). For Gram-positive cocci, catalase tests were performed to distinguish *Staphylococcus* sp. from *Streptococcus* sp. and the Tube Coagulase test (using fresh rabbit plasma) was used to identify *Staphylococcus aureus* and other coagulase-positive Staphylococci from Coagulase-Negative Staphylococci (CNS).

Streptococcus sp. was identified by their growth on SBA and EDM with α, β or γ-hemolysis on SBA plate with Gram-positive staining result and negative result of Catalase test and Munch-Petersen (CAMP) test was needed for further identification. *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* were identified according to their ability to split aesculin and sodium hippurate. *Streptococcus agalactiae* differed from other Streptococci by its positive result of CAMP test, both the result of aesculin hydrolysis test and Sodium Hippurate Hydrolysis test were negative. While for *Streptococcus dysgalactiae* the result of CAMP test was negative, the results of aesculin hydrolysis test and the Sodium Hippurate Hydrolysis test were negative. *Streptococcus uberis* had a negative result of CAMP test, however the results of aesculin hydrolysis test and sodium hippurate hydrolysis test were positive. *Staphylococcus aureus* was identified by their growth on SBA alone, forming round and golden-yellow bacterial colony with β-hemolysis on plate with Gram-positive staining result, the results of Catalase test, Coagulase test and Triple Sugar Iron Agar test were positive and a positive CAMP (Munch-Petersen test) reaction was presumed to it. *Escherichia coli* was identified by their growth on SBA and MCA and forming a typical appearance of a pink bacterial colony on MCA with Gram-negative staining result, growth in triple sugar iron agar and identified further with negative result of oxidase test, H_2S test, V-P test and urease test and positive result of Indole test and MR test.

Isolation with >3 difference bacteria colonies from a sample was considered contamination and the result was considered non-diagnostic.

RESULTS

Prevalence of mastitis and microbiological cultures: Between April 2012 and November 2014, in the 29 dairy farms at 8 regions in Gansu and Ningxia Province of China, 860 dairy cows showed positive result for mastitis at cow level, accounted for 43.00% (860/2000) of total Chinese Holstein dairy cows examined, among which 102 (5.10%) were CM cases and 758 (37.90%) were SCM cases in the current study. Most of cows with the positive result of SCM and had LMT score of 2, followed by score 3 and 4 in descending order. The proportion of milk samples cultured positive were 97.56% (839/860) after primary and enrichment culture while 21 samples (2.44%) yielded no bacterial growth in this study. Sample yielding 2 different colonies (mixed culture) accounted for 7.67% (66/860) of total milk samples. The 34 samples yielding three or more different colonies were classified as contaminated which accounted for 3.95% (34/860) of total milk samples (the result was no available). In this study, the mixed infection including the results of mixed culture (2 colonies) and contamination (≥3 colonies) which accounted for 11.63% (100/860) of total milk samples. A total of 871 isolates were obtained from 739 milk samples cultured with single colony morphology and 66 milk samples yielded two colony types (Table 1).

Table 1: Culture result and isolates from milk samples after primary and enrichment culture

Samples	Separated culture (single colony)	Mixed culture (2 colony)	Contamination (≥3 colony)	Culture-negative (no growth)	Total
Primary culture result (No. of samples)	709	53	26	72	860
	75 (CM)	21 (CM)	2 (CM)	4 (CM)	102 (CM)
	634 (SCM)	32 (SCM)	24 (SCM)	68 (SCM)	758 (SCM)
Enrichment culture results (No. of samples)	30	13	8	21	72
	0 (CM)	1 (CM)	0 (CM)	3 (CM)	4 (CM)
	30 (SCM)	12 (SCM)	8 (SCM)	18 (SCM)	68 (SCM)
No of isolates	739 (1 colony)	66×2 (2 colony)	34 (No available)	0	871
	75 (CM)	22×2 (CM)		0	119 (CM)
	664 (SCM)	44×2 (SCM)		0	752 (SCM)

CM: Clinical Mastitis; SCM: Subclinical Mastitis

Table 2: Frequency of mastitis predominant pathogens and distribution in CM and SCM cases

Species	No. of isolates (% of total isolates)	Types	No. of isolates (% of total isolates)	Clinical (CM) (% of total CM)	Subclinical (SCM) (% of total SCM)
Streptococcus sp.	523(60.05%)	*S. agalactiae*	254 (29.16)	25 (21.01)	229 (30.45)
		S. dysgalactiae	152 (17.45)	12 (10.08)	140(18.62)
		S. uberis	80 (9.18)	7 (5.88)	73 (9.71)
		Other Streptococci	37 (4.25)	8 (6.72)	29 (3.86)
Staphylococcu sp.	218(25.03%)	*S. aureus*	135 (15.50)	23 (19.33)	112 (14.89)
		CNS	83 (9.53)	7 (5.88)	76 (10.11)
Coliform sp.	106 (12.17%)	*Escherichia coli*	69 (7.92)	32 (26.89)	37 (4.92)
		Other	37 (4.25)	5 (4.20)	32 (4.26)
Minor pathogens	24 (2.76%)	Yeast	13 (1.49)	0	13 (1.73)
		Fungus	11(1.26)	0	11(1.46)
Total isolates	871		871	119	752

CM: Clinical Mastitis, SCM: Subclinical Mastitis, CNS: Coagulase-Negative Staphylococcus, Other: Growth of unidentified bacterial species

Predominant pathogens identified from CM and SCM cases: The frequency of major pathogens caused bovine mastitis in this study showed in Table 2. *Streptococcus* and *Staphylococcus* Sp. were the predominant species. The most frequently isolated pathogens were *S. agalactiae*, followed by *S. dysgalactiae*, *S. aureus* and CNS. Among the major contagious pathogens *S. agalactiae* was the predominant agent rather than *S. aureus*. While *S. dysgalactiae*, CNS, *S. uberis* and *E. coli* were the vast majority of the environmental pathogens, accounted for 44.08% of the total isolates. Minor pathogens accounted for 2.76% of total isolates, the most commonly identified being yeast and fungus.

E. coli, *S. agalactiae* and *S. aureus* occupied prime position with 67.23% of the total isolates from the CM milk samples. While *S. agalactiae*, *S. dysgalactiae* and *S. aureus* were the predominant pathogens accounted for 63.96% of the total isolates from the SCM milk samples.

DISCUSSION

Bovine mastitis remains one of the most causes and expensive diseases in dairy industry worldwide despite decades of research and the widespread implementation of mastitis control strategies (Bhutto *et al*., 2012; Sarker *et al*., 2013). Considering that producing high quality milk requires an effective preventive measures and udder health at a herd level there is an increasing focus on the milk quality and hygiene in the dairy industry of China. In this study, the prevalence of bovine mastitis at cow level had decreased considerably over the last 20 years and a total of 860 milk samples were collected from cows with CM and SCM, respectively for bacteriological culture and identification. The overall incidence of CM at cow level declined from 17.07% reported by Yuan and 33.41% reported by Pan *et al*. (1996) to 5.1% in this study and the incidence of SCM declined from 77.71% and 73.91% to 37.90%, the reason is mainly related to the implement of mastitis control practices in dairy herds in China. It showed that SCM was the main performance of the disease which accounted for the majority of bovine mastitis cases in dairy herds it was 7 times more prevalence than CM in this study these result were in agreement with reports in other country (Karimuribo *et al*., 2006; Sharma *et al*., 2010a). Considering the frozen milk samples stored for 4-6 weeks could reduce the sensitivity of milk culture on bacterial isolation, so enrichment cultures used in this study in order to improve the detection limit of culture technique. And generally the milk samples collected in this study were stored in freezer for 3 weeks prior to culture and it was also expected to enhance the detection of *Coliform* sp. in particular, so this method and procedure could be used to facilitate sample collection from distance dairy farms. Furthermore, the LMT used for diagnosis of SCM positive cows in this study provided a real-time result and not expensive, it was considered as a useful predictor of intra-mammary infection in cows.

Findings in the current study showed that the *Streptococcus* and *Staphylococcus* sp. were the most frequently isolated pathogens which could be described as predominant mastitis-causing agents and most of dairy farms showed a similar mastitis pathogen profile. The commonly isolated pathogens from CM cases were *E. coli*, *S. agalactiae* and *S. aureus*, respectively while from SCM cases *S. agalactiae*, *S. dysgalactiae* and *S. aureu* were the predominant pathogens, the result was in agreement with most of previous studies (Pan *et al*., 1996; Hillerton and Berry, 2003; Ferguson *et al*., 2007). Furthermore, the contagious pathogens had showed to predominate over the environmental pathogens which was in accordance with the findings in other developing country (Omore *et al*., 1996; Sharma *et al*., 2010b). The common and most important contagious pathogens found in this study were *S. agalactiae* and *S. aureus* which accounted for 44.66% of total isolates. The frequency of *S. agalactiae* (29.16%) was found decreased obviously compared with the result by Pan *et al*. 1996 (45.48%) this can most probably be attributed to the systematic control measures implemented in the local herds which had previously been effective in preventing *S. agalactiae* mastitis (Hogan and Smith, 2003; Pitkala *et al*., 2004). However for *S. aureus* (15.50%) it had not altered significantly which was similar to the results reported by Pan *et al*. (1996) (17.35%) and Xiao (17.94%). Furthermore, the prevalence of *S. aureus* was greater than that reported by Wilson *et al*. (1996) (9.10%), Makovec and Ruegg (2003) (9.70%) and Osteras *et al*. (2006) (8.2%). Considering that the contagious pathogens survive in the udder of the cow, milk from infected cows is the main source of bacteria for uninfected cows and new infections occur primarily during milking (Ruegg, 2003), so wide distribution of *S. aureus* in teat canals, lesions and infected udder and also on teat and udder skin may be the main related reasons as well as the contagious pathogens were difficult to eliminate from the mammary gland due to very low rate of self cure and treatment result (Chamings, 1984; McDonald, 1977; Smith and Hogan, 1993). Prevalence of *S. agalactiae* and *S. aureus* in two provinces demonstrates these two pathogens remains a significant cause of mastitis in local cattle herds and more effective management and concerted effort are needed to control these organisms.

Cow prevalence of mastitis was more consistently associated with bedding type than with housing and organic bedding is considered as a risk for environmental pathogens (*Coliform* and *Streptococcus* sp.), particularly during the wet and humid condition (Ferguson *et al*., 2007; Smith and Hogan, 1993). In the present study, *S. dysgalactiae*, *S. uberis*, CNS and *E. coli* were found the mainly environmental pathogens which accounted for 44.08% of total isolates. The frequency of *S. dysgalactiae* (17.45%) isolated from milk samples was lower than the results reported by Pan *et al*. (1996) (33.92%) but similar to the findings by Yuan (15.92%). And the proportion of CNS (9.53%) was lower than that reported by Yuan (19.97%). While for the *S. uberis* (9.18%) and *E. coli* (7.92%) which were found increased significantly compared with the result by Yuan (2.27 and 4.03%) but similar to the result by Pan *et al*. (1996) (6.45 and 10.14%), the reason might be associated with poor hygiene, teat injury, bedding materials and management level of dairy farms. Moreover, the mixed infection of bacteria was a common performance and showed an upward trend in this study which accounted for 11.63% of total milk samples this information might be caused by poor sample collection technique or hand-milking such as absence of udder washing and milking with common worker using common udder cloths which could be vectors of spread specially for contagious mastitis. In addition, the frequency of isolated predominant pathogens changed over time was probably related to extensive use of antibiotics and chemical drug. Different treatment on the cows affected mastitis also might lead to the isolated pathogens differed among dairy herds. In this study, *Streptococcus* sp. was found the predominant species in most of dairy farms while in some of herds *S. aureus* and *E. coli* were the major causative pathogens, the reason might be related to the epidemiological difference of predominant bacteria flora among dairy herds. In a way that good mastitis control practice could substantially reduce the contagious pathogens such as *S. agalactiae*, however, it was ineffective against environmental pathogens (Hogan and Smith, 2003; Pitkala *et al*., 2004), the result in this study further confirmed that the mastitis control practice was carried out much better in most of dairy farms in recent years. Meanwhile, it also revealed the lack of dairy knowledge and skills as well as lower management level still existed among farmers, especially in some of the smallholder dairy farms which were almost with hand milking. Therefore, have a good mastitis control practice and develop dairy cow's immunity from diseases were still the most economical and effective supporting measures to control bovine mastitis.

CONCLUSION

In conclusion, mastitis surveillance studies provide information on the current distribution of mastitis pathogens in a country and herd-level sampling for bacteriological culture could be used to define the

prevalence of contagious and environmental pathogens in dairy farms. In this study, we aimed to quantify the prevalence and predominant pathogens related to the incidence of mastitis in dairy herds, the results demonstrated the major pathogens involved in both clinical and subclinical mastitis and the contagious pathogens had showed to predominant over the environmental pathogens, SCM was 7 times more prevalent than CM. This information can be used for advising veterinarians and local farmers and it would helpful to improve the mastitis prevention practices according to the pathogen distribution as well as to develop a more effective mastitis control program. Thus, mastitis surveillance studies should be taken substantially to monitor dairy cow's health in China.

ACKNOWLEDGMENTS

The study was funded by Lanzhou Science and technology development project (No. 2012-2-81), Gansu research and development project in agriculture biotechnology (GNSW-2013-29), the special fundamental research project for central public institutes (BRF 090405). The support and cooperation of Gansu agricultural university and Gansu Holstein dairy cow breeding center are gratefully acknowledged, researchers thank Professor Yong Zhang and Hongsheng Li for excellent technical assistance.

REFERENCES

Atyabi, N., M. Vodjgani, F. Gharazloo and A. Bahonar, 2006. Prevalence of bacterial mastitis in cattle from the Farm around Tehran. Iran. J. Vet. Med., 3: 76-79.

Berry, E. and E. Hillerton, 2001. Monitoring mastitis and maintaining biosecurity. Vet. Rec., 149: 531-532.

Bhutto, A.L., R.D. Murray and Z. Woldehiwet, 2012. California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. Res. Vet. Sci., 92: 13-17.

Chamings, R.J., 1984. The effect of not treating mild cases of clinical mastitis in a dairy herd. Vet. Rec., 115: 499-500.

De Graves, F.J. and J. Fetrow, 1993. Economics of mastitis and mastitis control. Vet. Clin. North. Am. Food Anim. Pract., 9: 421-434.

Ferguson, J.D., G. Azzaro, M. Gambina, G. Licitra, 2007. Prevalence of mastitis pathogen in Ragusa, Sicily, from 2000-2006. J. Dairy Sci., 90: 5798-5813.

Hillerton, J.E. and E.A. Berry, 2003. The management and treatment of environmental streptococcal mastitis. Vet. Clin. N. Am-Food A., 19: 157-169.

Hogan, J.S. and K.L. Smith, 2003. Coliform mastitis. Vet. Res., 34: 507-519.

Karimuribo, E.D., J.L. Fitzpatrick, C.E. Bell, E.S. Swai and D.M. Kambarage *et al*., 2006. Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: Risk, intervention and knowledge transfer. Prev. Vet. Med., 74: 84-98.

Makovec, J.A. and P.L. Ruegg, 2003. Results of milk samples submitted for microbiological examination in Wisconsin rom 1994-2001. J. Dairy Sci., 86: 3466-3472.

McDonald, J.S., 1977. Streptococcal and staphylococcal mastitis. J. Am. Vet. Med. Assoc., 170: 1157-1159.

Omore, A.O., J.J. McDermott, S.M. Armi, M.N. Kyule and D. Duma, 1996. Longitudinal study of milk somatic cell counts and bacterial culture from cows on smallholder dairy farms in Kiambu district, Kenya. Prev. Vet. Med., 29: 77-89.

Osteras, O., L. Solverod and O. Reksen, 2006. Milk culture results in a large norwegian survey-effects of season, parity, days in milk, resistance and clustering. J. Dairy Sci., 89: 1010-1023.

Pan, H., C.C. Liu, L.H. Zhang, Z.C. Zhang and J. Yu *et al*., 1996. Etiology and pathogenesis survey of bovine mastitis in some regions of China. Chin. J. Vet. Sci. Technol., 26: 16-17.

Petrovski, K.R., M. Trajcev and G. Buneski, 2006. A review of the factors affecting the costs of bovine mastitis: Review article. J. S. Afr. Vet. Assoc., 77: 52-60.

Piepers, S., S. de Vliegher, A. de Kruif, G. Opsomer and H.W. Barkema, 2009. Impact of intramammary infections in dairy heifers on future udder health, milk production and culling. Vet. Microbiol., 134: 113-120.

Pitkala, A., M. Haveri, S. Pyorala, V. Myllys and T.H. Buzalski, 2004. Bovine mastitis in finland 2001-prevalence, distribution of bacteria and antimicrobial resistance. J. Dairy Sci., 87: 2433-2441.

Quinn, P.J., M.E. Carter, B. Markey and G.R. Carter, 1994. Clinical Veterinary Microbiology. 1st Edn., Wolfe Publishing Company. Mosby, U.K., London, pp: 497-614.

Riekerink, R.G.M.O., H.W. Barkema, D.F. Kelton and D.T. Scholl, 2008. Incidence rate of clinical mastitis on canadian dairy farms. J. Dairy Sci., 91: 1366-1377.

Ruegg, P.L., 2003. Investigation of mastitis problems on farms. Vet. Clin. N. Am-Food A., 19: 47-73.

Sarker, S.C., M.S. Parvin, A.A. Rahman and M.T. Islam, 2013. Prevalence and risk factors of subclinical mastitis in lactating dairy cows in north and South regions of Bangladesh. Trop. Anim. Health Prod., 45: 1171-1176.

Schrick, F.N., M.E. Hockett, A.M. Saxton, M.J. Lewis, H.H. Dowlen and S.P. Oliver, 2001. Influence of subclinical mastitis during early lactation on reproductive parameters. J. Dairy Sci., 84: 1407-1412.

Sears, P.M., R.N. Gonzalez, D.J. Wilson and H.R. Han, 1993. Procedures for mastitis diagnosis and control. Vet. Clin. North Am. Food Anim. Practice, 9: 445-468.

Sharma, N., V. Pandey and J.S. Joodan, 2010a. Prevalence and associated risk factors of mastitis in lactating dairy cows. Int. J. Infect. Dis., 14: e154-e154.

Sharma, N., V. Pandey and J.S. Soodan, 2010b. Prevalence of mastitis in lactating dairy cows. Indian J. Vet. Med., 30: 102-104.

Smith, K.L. and J.S. Hogan, 1993. Environmental mastitis. Vet. Clinics of North America: Food Anim. Pract., 9: 489-498.

Tenhagen, B.A., G. Koster, J. Wallmann and W. Heuwieser, 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J. Dairy Sci., 89: 2542-2551.

Waller, K.P., B. Bengtsson, A. Lindberg, A. Nyman and H.E. Unnerstad, 2009. Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous cows: Influence of breed and stage of lactation. Vet. Microbiol., 134: 89-94.

Wilson, D.J., R.N. Gonzalez and H.H. Das, 1996. Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects of somatic cell count and milk production. J. Dairy Res., 80: 2592-2598.