Journal of Animal and Veterinary Advances 10 (1): 68-72, 2011
ISSN: 1680-5593

Bovine Mastitis in Subtropical Dairy Farms, 2005-2009

[1,2]Feng-Li Yang, [1]Xiao-Shan Li, [1,2]Bao-Xiang He, [1]Yu-Lan Du, [1,2]Gong-He Li,
[1]Bin-Bin Yang and [1,2]Huang Qin-Hua
[1]Animal Science and Technology College, Guangxi University,
Nanning, 530005 Guangxi, P.R. China
[2]Guangxi Key Laboratory for Subtropical Bio-Resource Conservation and
Utilization, Guangxi University, Nanning, 530005 Guangxi, P.R. China

Abstract: The purpose of this study was to investigate the incidence of Clinical Mastitis (CM) and Subclinical Mastitis (SCM), isolation and identification of the major pathogens and test the antimicrobial resistance of milk bacterial isolates in subtropical dairy farms in Guangxi region (south of China) between 2005 and 2009. The average percentages of blind quarter(s) at cow and quarter level were 11.5 and 3.7%, respectively. The incidence of CM at cow and quarter level were 8.7 and 3.7%, respectively while that of SCM at cow and quarter level were 48.8 and 19%, respectively. A total of 105 and 56 microorganisms were isolated from the 109 CM and 67 SCM samples, respectively. The most common bacterial isolates from CM cases were *S. aureus* (29.5%), *E. coli* (25.7%) and *C. neoformans* (16.2%), however in SCM they were *S. aureus* (32.1%), CNS (19.7%) and *St. agalactiae* (17.9%). The antimicrobial sensitivity test indicated that all the antimicrobial agents (except for ampicillin) showed lower proportion of resistant isolates of all the isolated bacteria (except for *C. neoformans*), among the employed antimicrobials, Ruyanxiao showed the lowest proportion of resistant isolates.

Key words: Mastitis, subtropical, incidence, antibiotic resistance, proportion, bacterial isolates

INTRODUCTION

Mastitis an inflammatory reaction of the mammary gland that is usually caused by a microbial infection is recognized as the most costly disease in dairy cattle despite of the progress in improving general udder health in recent years. When a modern dairy farm in the tropics was first adopted, mastitis was predicted to be an important disease in dairy cattle (Mungube *et al*., 2004). In the efforts to avoid mastitis, some vaccines that can reduce the severity of this illness were generated. However, these vaccines still do not control efficiently the development of mastitis (Leitner *et al*., 2003). On the other hand, it has been verified that the indiscriminate treatment with antibiotics, without either a technical prescription or identification tests of the pathogen can contribute to an increased resistance of these micro-organisms, making the cure of mastitis still more difficult (Gruet *et al*., 2001).

It is very limited that published articles on the incidence of dairy Clinical Mastitis (CM) and Subclinical Mastitis (SCM), major pathogens and their antimicrobial resistance in subtropics of China. To fill the vacancy of current research on the incidence of bovine mastitis and antibiotic resistance patterns in subtropical dairy farms of China, this study was designed to investigate the incidence of dairy CM and SCM, isolate the major microorganisms responsible for CM and SCM and test their antimicrobial sensitivity between 2005 and 2009.

MATERIALS AND METHODS

Study area and animals: The study was conducted at two Holstein dairy farms from 2005-2009 in Guangxi region, where is locates in the area of subtropical monsoon climate. The annual average milk production is 4813 kg per cow in 2008. Milking was done three times per day and all lactation cows except CM cows were machine-milking. They are treated with antibiotics at the time of drying off. The animals are allowed feeding for 7 h indoors every day and outdoors for the rest of time.

Clinical examination and California Mastitis Test (CMT): All the lactating cows of the two dairy farms were clinically examined for the blind quarters and diagnosis of CM and SCM in the spring every year during the study period. CM was diagnosed on the basis of visible signs of inflammation if a quarter or the milk was shown abnormal and inflammation response will be considered the cow had CM.

Corresponding Author: Bao-Xiang He, Animal Science and Technology College, Guangxi University, Nanning, 530005 Guangxi, P.R. China

Somatic Cell Count (SCC) has been accepted as the best index in both evaluating milk quality and predicting udder infection in cow (Pyorala, 2003). Under field conditions, determination of SCC in cow's milk is usually performed by CMT. If a cow was considered for CM, the cow was didn't test SCC. The test result was interpreted based on the thickness of the gel formed by CMT reagent and milk mixture and scored as 0 (negative), T (trace), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Quarters with CMT score of 1 or above were judged as positive for SCM; otherwise negative. When more than one quarter turned out to be positive for CMT, cows were considered positive for CMT.

Collection of milk samples: Milk was sampled from CM and SCM quarters of the cows in accordance with standard milk sampling techniques (Sears *et al*., 1993; Getahun *et al*., 2008).

Bacteriological examination of milk samples: Milk samples were examined following the standard procedures (Sears *et al*., 1993; Getahun *et al*., 2008). Isolation of two or more types of colonies from a sample was considered as mixed growth.

Antibacterial sensitivity test: Kirby-Bauer disk diffusion method was employed to test *in vitro* antibiotic sensitivity (Bauer *et al*., 1966; Carter and Chengappa, 1991) and the antibacterial activity of Ru Yanxiao (Chinese medicine) (Song *et al*., 2009). The following seven antimicrobial drugs were used: Carbenicillin (CAR) (100 μg), Cephazolin (CEP) (30 μg), Kanamycin (KAN) (30 μg), Chloramphenical (CHL) (30 μg), Erythromycin (ERY) (15 μg), Ciprofloxacin (CIP) (5 μg) and Ruyanxiao (RYX). The cut off values for the evaluation of the susceptibility of isolates were according to Getahun *et al*. (2008) for antibiotics and Song *et al*. (2009) for Ruyanxiao.

Statistical analysis: CM incidence was calculated based on clinical examination and SCM prevalence rate was calculated as defined by the CMT score. In addition, on the analysis of pathogen, a ratio between contagious and environmental pathogens was calculated to summarize the contribution of contagious and environmental pathogens to the infection pattern. Contagious pathogens in this calculation were *S. aureus*, *St. agalactiae* and Coagulase-Negative Staphylococci (CNS). Environmental pathogens were *E. coli*, *St. uberis*, *St. dysgalctiae* and *C. neoformans*.

RESULTS AND DISCUSSION

The average of lactating cows which had blind quarter(s) from 2005-2009 is shown in Table 1. The details of CM incidences at cow and quarter levels of the lactating cows in the period between 2005 and 2009 are shown in Table 2. The details of SCM incidences at cow and quarter levels of the lactating cows in the period between 2005 and 2009 are shown in Table 3. Distributions of different CMT scores of the milk from 2005-2009 are shown in Table 4.

A total of 109 clinical mastitic milk samples and 67 subclinical mastitic milk samples were examined in the

Table 1: The average lactating cows had blind quarter(s) of the animals from 2005-2009

Parameters	2005	2006	2007	2008	2009	Mean
NC	973.0	1032.0	1105.0	1151.0	1184.0	1089.0
NCBQ (%)	111 (11.4)	113 (10.9)	135 (12.2)	128 (11.1)	139 (11.7)	125 (11.5)
NBQ (%)	143 (3.7)	152 (3.7)	170 (3.8)	169 (3.7)	183 (3.9)	163 (3.7)
NCBQ1 (%)	79 (8.1)	74 (7.2)	100 (9.0)	87 (7.6)	95 (8.0)	87 (8.0)
NCBQ 2 (%)	32 (3.3)	39 (3.8)	35 (3.2)	41 (3.6)	44 (3.7)	38 (3.5)

NC = Number of lactating Cows; NBQ = Number of Blind Quarters; NCBQ = Number of Cows had Blind Quarter(s); NCBQ1 = Number of Cows had one Blind Quarter; NCBQ2 = Number of Cows had two Blind Quarters

Table 2: The incidence of CM at cow and quarter levels of the animals from 2005-2009

	2005		2006		2007		2008		2009		Mean	
Observation level	TN	PN (%)	TN	PN (%)	TN	PN (%)	TN	PN (%)	TN	PN (%)	TN	PN (%)
Cow level	973	75 (7.7)	1032	84 (8.1)	1105	106 (9.6)	1151	103 (8.9)	1184	107 (9.0)	1089	95 (8.7)
Quarter level	3749	127 (3.4)	3976	141 (3.5)	4250	165 (3.9)	4435	167 (3.8)	4553	173 (3.8)	4193	155 (3.7)

TN: Total Number; PN: Positive Number

Table 3: The incidence of SCM at cow and quarter levels of the animals from 2005-2009

	2005		2006		2007		2008		2009		Mean	
TN Observation level	TN	PN (%)	TN	PN (%)	TN	PN (%)	TN	PN (%)	TN	PN (%)	TN	PN (%)
Cow level	898	405 (45.1)	948	439 (46.3)	999	547 (54.8)	1048	506 (48.3)	1077	528 (49.0)	994	485 (48.8)
Quarter level	3466	621 (17.9)	3659	664 (18.1)	3859	827 (21.4)	4046	739 (18.3)	4159	806 (19.4)	3838	731 (19.0)

TN: Total Number; PN: Positive Number

Table 4: The distributions of CMT scores of the milk from 2005-2009

CMT score	2005	2006	2007	2008	2009	Mean
0 + T	2845 (82.1)	2995 (81.8)	3032 (78.6)	3307 (81.8)	3353 (80.6)	3106 (81.0)
1	320 (9.2)	339 (9.3)	386 (10.0)	356 (8.8)	386 (9.3)	357 (9.3)
2	167 (4.8)	171 (4.7)	235 (6.1)	205 (5.0)	227 (5.5)	201 (5.2)
3	134 (3.9)	154 (4.2)	206 (5.3)	178 (4.4)	193 (4.6)	173 (4.5)
Total	3466 (100.0)	3659 (100.0)	3859 (100.0)	4046 (100.0)	4159 (100.0)	3838 (100.0)

Table 5: Number and proportion of microorganism isolates from clinical and subclinical mastitic milk samples

Type of bacterial isolates	CM No. (%)	SCM No. (%)	Total No. (%)
S. aureus	31 (29.5)	18 (32.1)	49 (30.4)
E. coli	27 (25.7)	4 (7.1)	31 (19.3)
C. neoformans	17 (16.2)	5 (8.9)	22 (13.7)
St. agalactiae	8 (7.6)	10 (17.9)	18 (11.2)
St. uberis	5 (4.7)	5 (8.9)	10 (6.2)
St. dysgalctiae	3 (2.9)	2 (3.6)	5 (3.1)
CNS	5 (4.7)	11 (19.7)	16 (9.9)
Mixed growths			
S. aureus + C. neoformans	3 (2.9)	1 (1.8)	4 (2.5)
E. coli + C. neoformans	3 (2.9)	-	3 (1.9)
S. aureus + E. coli	2 (1.9)	-	2 (1.2)
S. aureus + St. agalactiae	1 (1.0)	-	1 (0.6)
Total isolates	105 (100.0)	56 (100.0)	161 (100.0)

No = Number of milk samples positive for the specific bacterial isolate; % = proportion from the total of the same column

Table 6: *In-vitro* antimicrobial susceptibility test results of bacterial isolates recovered from mastitis milk samples

		AMP (%)		CEP (%)		KAN (%)		CHL (%)		ERY (%)		CIP (%)		RYX (%)	
Isolates	No.	Sus	Int	Sus	Int	Sus	Int	Sus	Int	Sus	Int	Sus	Int	Sus	Int
S. aureus	49	8.2	22.4	55.1	38.8	22.5	36.7	24.5	46.9	18.4	49.0	26.5	49.0	55.1	38.8
E. coli	29	6.9	13.8	55.2	44.8	34.5	37.9	37.9	41.4	27.6	20.7	48.3	51.7	37.9	55.2
C. neoformans	22	0.0	0.0	0.0	9.1	0.0	4.5	0.0	4.5	0.0	22.7	0.0	27.3	36.4	50.0
St. agalactiae	18	5.6	22.2	38.9	55.5	22.2	50.0	22.2	55.6	22.2	38.9	27.8	50.0	44.4	50.0
St. uberis	9	0.0	44.4	44.4	55.6	11.1	33.3	33.3	44.5	11.1	44.4	22.2	44.5	22.2	77.8
St. dysgalactiae	4	0.0	25.0	75.0	25.0	0.0	50.0	50.0	50.0	25.0	75.0	0.0	50.0	25.0	75.0
CNS	15	0.0	40.0	73.3	20.0	13.3	53.4	26.7	40.0	6.7	40.0	40.0	46.7	40.0	60.0

No.: Number of observations; AMP: Ampicillin; CEP: Cephazolin; KAN: Kanamycin; CHL: Chloramphenicol; ERY: Erythromycin; CIP: Ciprofloxacin; RYX: Ruyanxiao

period between 2008 and 2009. The number and proportions of microorganism isolated from clinical and subclinical mastitic milk samples are shown in Table 5. From clinical and subclinical mastitic milk samples, 4 (3.7%) and 11 (16.4%) showed negative, 96 (88.1%) and 55 (82.1%) had single growth and 9 (8.2%) and 1 (1.5%) had mixed growths, respectively. The common isolated microorganisms from the clinical mastitic milk samples were *S. aureus* (29.5%, n = 31), *E. coli* (25.7%, n = 27) and *C. neoformans* (16.2%, n = 17) while from subclinical mastitic milk samples were *S. aureus* (32.1%, n = 18), CNS (19.7%, n = 11) and *St. agalactiae* (17.9%, n = 10). Other isolates include *St. uberis* and *St. dysgalctiae*.

The results of antimicrobial sensitivity test for the different isolates are shown in Table 6. This study was employed 7 antimicrobial agents, all the antimicrobial agents (except for ampicillin) showed lower proportion of resistant isolates of all the isolated bacteria (except for *C. neoformans*).

In this study, the average of lactating cows which had blind quarter(s) at quarter level is 3.7% which is in agreement with the reports by Mungube *et al*. (2004) (3.7%) and Almaw *et al*. (2008) (3.8%), it is higher than the reports by Kivaria *et al*. (2007) (2.1%) and Getahun *et al*. (2008) (2.3%), while at cow level (11.5%), it is higher than the reports by Kivaria *et al*. (2004) (5%). The blind quarters observed in this study might be an indication of a serious mastitis problem on the farms such as in 2007.

The average incidence of CM at cow and quarter levels in the study period (8.7 and 3.7%) are higher than the reports by Mungube *et al*. (2004) (6.6 and 2.8%), Kivaria *et al*. (2004) (3.8 and 2.1%) and Getahun *et al*. (2008) (1.8 and 0.51%). The average prevalence rate of SCM (based on CMT) at cow level (48.8%) is in agreement with the reports by Mungube *et al*. (2004) (46.6%) and Gianneechini *et al*. (2002) (52.4%). It is higher than the report by Getahun *et al*. (2008) (22.3%). However, the study is by far lower than the reports of Dego and Tareke (2003) (62.9%) and Kivaria *et al*. (2004) (90.3%). The average incidence of SCM at quarter level in this study (19%) is higher than that of Getahun *et al*. (2008) (10.1%) and is lower than that of Mungube *et al*. (2004) (27.8%) and Gianneechini *et al*. (2002) (27.6%). The climatic differences of the farms may also have been a factor for

CM and SCM. As in the area of subtropical monsoon climate, the higher humidity and temperature conditions at the two farms may enhance the survival of bacteria in the environment, where they remain as sources of infection.

Although calving is not strictly seasonal in the investigated area, a relatively higher calving rate in the wet season than in the dry season may contribute to the high prevalence CM and SCM during the wet season, because of birth-related problems (Dego and Tareke, 2003). High serum selenium concentrations were associated with reduced rates of mastitis and lower bulk-tank somatic cell counts in Ohio dairy herds (Spears and Weiss, 2008) while it is significance higher selenium level in the soil of Guangxi than the average of China and USA (Ban and Ding, 1992), so it is relatively high in serum selenium concentration of cows in this area and this is might be a factor that the incidence of CM and SCM in this study is lower than some areas.

The average distributions of 1, 2 and 3 CMT score of SCM quarters is 9.3, 5.2 and 4.5% (Table 4), respectively. The result is lower than reported by Contreras *et al*. (1996) (27.6, 20.9 and 4.6%, respectively) and Sung *et al*. (1999) for goat. The animals had the best score in 2005 and the worst in 2007.

In this study, the most frequently bacterial isolates from clinical and subclinical mastitic samples was *S. aureus*, the high prevalence of *S. aureus* mainly attributed to the wide distribution of microorganism inside the mammary gland and on the skin of teat and udder. They usually establish chronic, subclinical infections which serves as a source of infection for other healthy cows during the milking process (Workineh *et al*., 2002). In addition, It is difficult to eliminate the bacteria from the mammary gland due to the very low rate of self cure and a number of factors affect the rate of cure after treatment which is in general low (Getahun *et al*., 2008).

However, mixed growths in CM and SCM are 8.7 and 1.8%, respectively. As far as SCM, it is lower than the report by Almaw *et al*. (2008) (9.9%). Miscellaneous bovine mastitis pathogens such as *S. aureus* + *C. neoformans* are associated with poor and unhygienic housing and milking, unsanitary intramammary infusion practices in discriminate use of antibiotics and non implementation of mastitis control programme.

The most important findings were 16.2 and 8.9% microorganisms isolates from CM and SCM was *C. neoformans*, showing *C. neoformans* constitute a real problem once the therapy against bacterial mastitis is unsuccessful in eliminating the *C. neoformans* present in the quarter. Conditions like the chronic form of mastitis, frequently subjected to prolonged, excessive and repeated antibacterial therapy, help the establishment of *C. neoformans* or mixed infections (bacteria and *C. neoformans*).

The antimicrobial sensitivity test indicated that all the antimicrobial agents were employed (except for ampicillin) showed lower proportion of resistant isolates of all the isolated bacteria (except for *C. neoformans*) in terestingly, among the antimicrobial agents tested, cephazolin and Ruyanxiao showed the least proportion of resistant isolates of all the bacteria tested.

The finding is attributed to the rare use of cephazolin the treatment of bovine CM and Ruyanxiao, whose main components are Tokyo violet herb, honeysuckle flower, Angelica sinesis, Angelica Dahurica and white wax was Chinese medicine. Ruyanxiao had significant antimicrobial effects and was used about 4 years at the farms.

In addition, for the CM cases, the veterinaries usually do intramammary injection of Ruyanxiao accompanying with external used Xiaoyangao which is a compound traditional Chinese herb medicine ointment and its main components are snakegourd root, phellodendri cortex, rhubarb, curcuma and dahuria angelica root. By this method of treatment, the disease was cured in 2 days on average.

CONCLUSION

The important findings in this study were investigated the lactating cows which had blind quarter (s), the incidence of CM and SCM and the distributions of different CMT scores of SCM quarters for the first time in subtropical dairy farms in Guangxi region and the most important findings were 16.2 and 8.9% microorganisms isolates from CM and SCM were *C. neoformans*.

ACKNOWLEDGEMENTS

The researchers would like to acknowledge the cooperating dairy farms. The study was jointly supported by National Natural Science Foundation of China (No. 30960294) and Guangxi Department of Science and Technology (No. 10100014).

REFERENCES

Almaw, G., A. Zerihun and Y. Asfaw, 2008. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. Trop. Anim. Health Prod., 40: 427-432.

Ban, L. and Y.F. Ding, 1992. The soil selenium distribution and characteristic of Guangxi. Environ. Monitor. China, 3: 98-101.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Carter, G.R. and M.M. Chengappa, 1991. Essentials of Veterinary Bacteriology and Mycology. 4th Edn., Lea and Febiger, Philadelphia.

Contreras, A., D. Sierra, J.C. Corrales, A. Sanchez and J. Marco, 1996. Physiological threshold of somatic cell count and California mastitis test for diagnosis of caprine subclinical mastitis. Small Rumin. Res., 21: 259-264.

Dego, O.K. and F. Tareke, 2003. Bovine Mastitis in selected areas of Southern Ethiopia. Trop. Anim. Health Prod., 3: 197-205.

Getahun, K., B. Kelay, M. Bekana and F. Lobago, 2008. Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. Trop. Anim. Health Product., 40: 261-268.

Gianneechini, R., C. Concha, R. Rivero, I. Delucci and J.M. Lopez, 2002. Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. Acta Vet. Scand., 43: 221-230.

Gruet, P., P. Maincent, X. Berthelot and V. Kaltsatos, 2001. Bovine mastitis and intramammary drug delivery: Review and perspective. Adv. Drug Deliv. Rev., 50: 245-259.

Kivaria, F.M., J.P.T.M. Noordhuizen and A.M. Kapaga, 2004. Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. Trop. Anim. Health Prod., 36: 581-592.

Kivaria, F.M., J.P.T.M. Noordhuizen and M. Nielen, 2007. Interpretation of California mastitis test scores using *Staphylococcus aureus* culture results for screening of subclinical mastitis in low yielding smallholder dairy cows in the Dares Salaam region of Tanzania. Preventive Vet. Med., 78: 274-285.

Leitner, G., E. Lubashevsky and Z. Trainin, 2003. *Staphylococcus aureus* vaccine against mastitis in dairy cows, composition and evaluation of its immunogenicity in a mouse model. Vet. Immunol. Immunopathol., 93: 159-167.

Mungube, E.O., B.A. Tenhagen, T. Kassa, F. Regassa, M.N. Kyule, M. Greiner and M.P.O. Baumann, 2004. risk factors for dairy cow mastitis in the central highlands of Ethiopia. Trop. Anim. Health Product., 36: 463-472.

Pyorala, S., 2003. Indicators of inflammation in the diagnosis of mastitis. Vet. Res., 34: 565-578.

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.

Song, H.R., Z.G. Li, R.Q. Luo, H. Guang, X.L. Gu, F. Zhang and X. Zhou, 2009. Isolation and idlemirlaltion on maslilis pathogen of cow in shihezi area and bacteriostatic test with chinese herbal medicine. J. Anhui Agric. Sci., 37: 4044-4047.

Spears, J.W. and W.P. Weiss, 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J., 176: 70-76.

Sung, Y.Y., T.I. Wu and D.H. Wang, 1999. Evaluation of milk quality of Alpine, Nubian, Saanen and Toggerburg breeds in Taiwan. Small Rumin. Res., 33: 17-23.

Workineh, S., M. Bayleyegn, H. Mekonnen and L.N.D. Potgieter, 2002. Prevalence and aetiology of mastitis in cows from two major Ethiopian dairies. Trop. Anim. Health Prod., 34: 19-25.