Prevalence and Risk Factors of Subclinical Mastitis in Lactating Cows in Northwest China

Wang, L., Yang, F.,* Wei, X.J., Luo Y.J., Guo, W.Z., Zhou, X.Z. and Guo, Z.T.

Key Laboratory of Veterinary Pharmaceutics Discovery, Ministry of Agriculture, Lanzhou Institute of Animal Science and Veterinary Pharmaceutics, Chinese Academy of Agricultural Sciences (CAAS), Lanzhou, Gansu, 730050, PR China.

* **Corresponding author:** Dr. Feng Yang, Lanzhou Institute of Animal Science and Veterinary Pharmaceutics, Chinese Academy of Agricultural Sciences (CAAS), Lanzhou, Gansu, 730050, PR China. E-mail: yangfeng@caas.cn

ABSTRACT

The aim of this study was to investigate the prevalence and risk factors of subclinical mastitis (SCM) in lactating cows in northwest China. A total of 1,590 lactating cows from 38 different-scale dairy farms located in Gansu province and Ningxia Hui autonomous region were selected for this study. SCM was confirmed by California Mastitis Test. Cow-level risk factors including age, parity, lactation stage, milk yield, clinical mastitis history, teat damage, hygienic-sanitary quality, milking method and season were identified through univariate analysis and multivariate analysis using SPSS 19.0. In the two regions, the prevalence of SCM was 25.92% at cow level and 16.40% at quarter level. On large-, medium- and small-scale dairy farms, the SCM incidence was 21.46%, 26.74% and 33.16%, respectively. Among the potential risk factors, parity, lactation stage, clinical mastitis history and teat damage significantly influenced the occurrence of SCM ($P<0.01$) at cow level. This study indicated high-level prevalence of SCM and identified four risk factors for SCM which can be addressed by practical management of dairy cows for the local farms.

Keywords: Subclinical Mastitis; Risk Factors; California Mastitis Test; Lactating Cows.

INTRODUCTION

Bovine mastitis is one of the most frequent diseases of dairy cattle, causing vast economic losses due to reduction in milk production and treatment costs (1,2). In China, the annual average losses of mastitis were calculated to be 15-45 billion Chinese Yuan (CNY) (3). Beside the economic pressures of mastitis, the effects of mastitis on public health should not be overlooked. Antibiotics abuse in prevention and treatment of mastitis has possible implications for human health through an increased risk of antibiotic resistant bacteria emerging that may then enter the food chain, which could make antimicrobial resistance transferred from animals to people. Although the transmissions are rare, based on the modern techniques of pasteurization, failure of pasteurization still poses a serious threat to human health (1,4).

Subclinical mastitis (SCM) is a major problem affecting the dairy industry worldwide. It is difficult to detect because there are no visible signs in the milk or udder (5), resulting in major cost implications (6). Knowledge of risk factors associated with SCM will be of importance for design and implementation of effective prevention and control measures. Many of the risk factors have been identified that influence the occurrence of SCM, including breed, floor type, age, lactation stage, milk yield and history of clinical mastitis. (7).

However, very few articles have been published on the subjects of risk factors of SCM in China to date. Therefore, the aim of the present study was to investigate the prevalence of SCM at cow and quarter level, and to identify associated risk factors for SCM in lactating cows form Gansu province and Ningxia Hui autonomous region, northwest China.

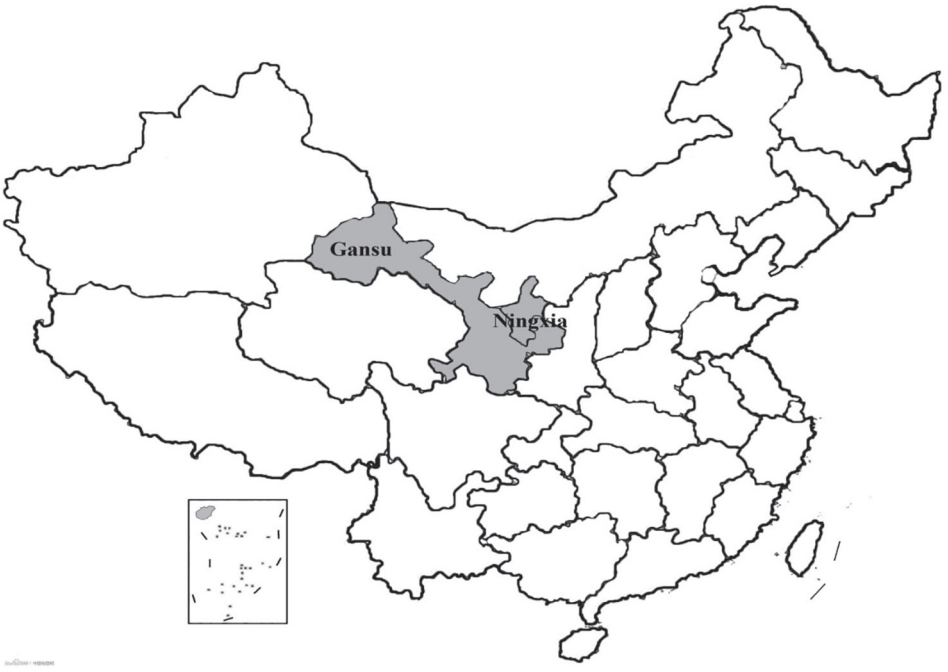

Figure 1: The present research was conducted to study the prevalence and associated risk factors with SCM in Gansu and Ningxia provinces as indicated in the map of China.

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 work, owners of all dairy farms involved in the study were contacted and their permission obtained for collection of milk samples, and the protocol was acceptable to the owners of the dairy farms under investigation.

Milk samples were obtained 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 cows

A total of 1590 Chinese Holstein cows from 38 dairy farms located in Gansu province and Ningxia Hui autonomous region were randomly selected as the experimental population over the period of 2015 to 2016. The 38 dairy farms were classified into large-scale farms (more than 500 cows), medium-scale farms (more than 50 cows and less than 500 cows), and small-scale farms (less than 50 cows) based on a previously description by Becker *et al*. in 2007 (8). The number of large-, medium- and small-scale dairy farms (cows) in Gansu province was 2 (240), 9 (560), and 13 (130), respectively. In Ningxia Hui autonomous region, the number was 2 (240), 6 (360), and 6 (60), respectively (Figure 1).

Subclinical mastitis test and risk factors survey

Quarter milk samples (n=6,032) without clinical mastitis (CM) signs were collected for detection of subclinical mastitis using California Mastitis Test (CMT) in cows without any signs of mastitis. Before sampling, the first two milk streams were discarded, and teat ends were disinfected using cotton swabs soaked in 70% alcohol and allowed to dry. A few streams of milk were collected in corresponding paddle wells with equal CMT reagent. Then circular motion was applied to mix milk with reagent for 15 seconds. The result was scored and interpreted as either 0, Trace (T), 1, 2 or 3 inflammatory response based on the viscosity of the gel formed by the mixture. Quarters with CMT score T, 1, 2, or 3 were diagnosed as infection positive while those with CMT score 0 were negative. A cow was identified as SCM if it had at least one clinically infected quarter (9).

Questionnaires was used to collect information on risk factors for SCM, including age, parity, lactation stage, milk yield, clinical mastitis history, teat damage, hygienic-sanitary quality, milking method and season. Animal age was categorized as 3-4, 5-7, and ≥8 years. Parity was classified as 1-2, 3-4, 5-6, and 7-8 calves. Stage of location was categorized as 1-3, 4-6, and 7-9 months. Previous clinical history mastitis and teat lesion were regarded as yes and no. Hygienic-sanitary quality was classified as high-, medium-, and low level. Milk yield was categorized as >25 kg/day and <25 kg/day. Season was described as winter, spring, summer, and autumn.

Table 1: Prevalence of subclinical mastitis in Gansu province and Ningxia Hui autonomous region, northwest China

Study areas	No. of farms			No. of SCM cases (%)			
	Large (cows)	Medium (cows)	Small (cows)	Cow level		Quarter level	
Gansu	2 (240)	9 (560)	13 (130)	930	243 (26.12)	3496	526 (15.05)
Ningxia	2 (240)	6 (360)	6 (60)	660	169 (25.60)	2536	463 (18.26)
Total	4 (480)	15 (920)	19 (190)	1590	412 (25.91)	6032	989 (16.40)

No.= number, CM: Clinical mastitis SCM: Subclinical mastitis CMT: California mastitis test

Statistical analysis

Risk factors associated with SCM at cow level were analyzed by SPSS 19.0 (SPSS Inc, Chicago, Illinois, USA). First the univariable analysis was used to identify and select the risk factors. Only those factors with significant associations (P<0.2) with the outcome variable were submitted to multivariable analysis in the final model. The P-values for data inclusion and exclusion were set at less than 0.05. Statistical significance in this step was assessed at P<0.05.

RESULTS

Prevalence

In this study, 1,508 lactating cows without clinical mastitis signs, corresponding to 6,032 quarters were tested for SCM in Gansu province and Ningxia Hui autonomous region. A total of 25.92% (412) of cows and 16.40% (989) of quarters were SCM positive based on CMT (Table 1). The SCM incidences in the two regions were 26.12% and 25.60% at the cow level, respectively. However, at quarter level, the prevalence of SCM in Ningxia Hui autonomous region (18.26%) was higher than that of Gansu province (15.05%). In addition, the prevalence of SCM on large-, medium- and small-scale dairy farms in the two regions was 21.46%, 26.74% and 33.16%, respectively (data not shown).

Risk factors

The associations between SCM prevalence and the investigated variables at cow level were shown in Table 2. Based on the results, four variables were confirmed as potential risk factors for SCM, in which dairy cows with previous clinical mastitis cases had the highest odds ratio (OR=7.04; P<0.001) and had a greater risk for mastitis compared to those without. The incidence rates were higher in cows with parity number 3-4 (OR=2.08; P<0.001), parity number 5-6 (OR=2.73; P<0.001), and parity number 7-8 calves (OR=2.55; P<0.001), cows that were at the end of lactation stage 4-6 (OR=1.68; P<0.001) and 7-9 months (OR=2.56; P<0.001), and cows with teat damage (OR=1.41; P<0.01) which were high significantly associated with SCM. Among these factors, previous clinical mastitis history showed the most significant association with SCM (odds ratio, 7.04; 95% CI, 5.69 to 8.71; P<0.001), it revealed that cows with a history of previous mastitis were 7 times more susceptible to SCM than those without history of CM at cow level.

Other factors positively associated with SCM were age (5-7 years, P=0.493), milk yield (<25 Kg, P=0.388), hygiene of cows & sanitary practice (poor, P=0.509), milking method (hand milking, P=0.348) and season (summer, P=0.144; autumn, P=0.249). Moreover, in this study the incidence of SCM were more likely to be observed in summer and autumn compared to winter without any statistical significance in the incidence of SCM in different seasons (P>0.05). Furthermore, it revealed that poor management and hygiene of cows as well as milking procedures were potential risk factors for the high incidence of SCM in this survey. The incidence of SCM in the two milking method (hand or machine milking) had not significant difference (P>0.05), however, hand milking was observe to cause a higher incidence of mastitis at the cow level.

DISCUSSION

Mastitis is still a major challenge to dairy industry despite the widespread implementation of mastitis control strategies (1). SCM is considered as the most important type of mastitis because of the higher prevalence and lack of clinical symptoms and a quality control system (9). In China, the prevalence of SCM has been found to range from 40% to 80% (10), which was 2 to 3 times more prevalent than that of clinical mastitis (11). The annual economic losses caused by SCM have been estimated to be 135 million Chinese Yuan (CNY) (12). In this study, the prevalence of SCM was 25.91% at cow level and 16.40% at quarter level as measured by CMT. This result was lower than studies reported in China (13, 14) as

Table 2: Potential risk factors for subclinical mastitis in lactating cows

Risk factors	Subclinical mastitis		Multivariate analysis		
	Present (n=412) No. (%)	Absent (n=1096) No. (%)	OR[a]	95% CI[b]	*P* value
Age (years)					
3-4	126 (30.58)	426 (38.89)	Reference		
5-7	219 (53.16)	468 (42.70)	1.73	0.36-5.35	0.493
≥ 8	67 (16.26)	202 (18.43)	1.44	0.19-5.29	0.723
Parity					
1-2	72 (17.47)	370 (33.76)	Reference		
3-4	133 (32.28)	328 (29.93)	2.08	1.51-2.88	0.001
5-6	148 (35.92)	279 (25.46)	2.73	1.98-3.76	0.001
7-8	59 (14.32)	119 (10.86)	2.55	1.71-3.81	0.001
Lactation stage (months)					
1-3	83 (20.15)	378 (34.49)	Reference		
4-6	142 (34.47)	385 (35.13)	1.68	1.24-2.28	0.001
7-9	187 (45.39)	333 (30.38)	2.56	1.90-3.44	0.001
Milk yield (kg/d)					
>25	163 (39.56)	607 (55.38)	Reference		
<25	249 (60.44)	489 (44.62)	1.88	0.45-6.80	0.388
Clinical mastitis history					
No	136 (33.01)	943 (86.04)	Reference		
Yes	276 (66.99)	153 (13.96)	7.04	5.69-8.71	0.001
Teat damage					
No	373 (90.53)	1035 (94.43)	Reference		
Yes	39 (9.47)	61 (5.57)	1.41	0.12-5.12	0.007
Hygienic-sanitary quality					
High	150 (36.41)	477 (43.52)	Reference		
Medium	210 (50.97)	530 (48.36)	0.71	0.19-2.64	0.607
Low	52 (12.62)	89 (8.12)	0.50	0.06-3.91	0.509
Milking method					
Machine	354 (85.92)	1026 (95.44)	Reference		
Hand	58 (14.08)	70 (6.39)	1.25	0.13-4.19	0.348
Season					
Winter	52 (12.62)	306 (27.92)	Reference		
Spring	65 (15.78)	285 (26.00)	1.88	0.27-7.73	0.526
Summer	156 (37.86)	244 (22.26)	3.75	0.64-8.57	0.144
Autumn	139 (33.74)	261 (23.81)	2.89	0.48-6.10	0.249

No.= number, [a] OR: Odds ratio, [b] CI: 95% confidence interval

well as other countries (9, 15) where the prevalence of SCM was 46.6-61.9% and 20.2-51.3% at cow and quarter level, respectively. This discrepancy could be due to the fact that most of cows selected in the previous studies to investigate the prevalence of SCM, belonged to small- or medium-scale farms, whereas, in our study, more than half of the cows lived on large- and medium- scale farms. In our study, the SCM incidence in different scale dairy farms suggested that cows in the small scale farms (33.16%) are more likely to be infected than medium- (26.74%) and large-scale farms (21.46%). This can be attributed to the different management routines in these farms (16). Most management routines used having consistent associations with high-level mastitis incidence include feeding, housing, milking procedures, free stall system, sand bedding, cleaning the calving pen after each calving, frequent use of the California Mastitis Test, etc. (17,18). In the small scale dairy farms, poorly planned dairy infrastructures and lack of knowledge and skills among the producers are the major reasons for sub-optimal housing hygiene, which is associated with high incidences of mastitis (19).

Mastitis is a complex disease that is influenced by various risk factors (20). In the present study, cows with clinical mastitis history exhibited the highest odds ratio (7.04) of SCM occurrence among the four risk factors ($P<0.01$) at cow level. Similar results have been recorded by Sarker *et al*. in 2013 (7). Cows that have had clinical mastitis once have a greater risk for mastitis than those without was also reported by Steeneveld *et al*. in 2008 (21).

Cows with high parity or late lactation appeared to increase the incidence rate of mastitis (15,22). The similar trends were seen in our data. Although it was not in line with a previous study in central China (23), most of records from other countries showed similar results for bovine mastitis cases (21, 24, 25).

The incidence of SCM was significantly associated with teat damage in our study. This phenomenon is in agreement with those in other studies (9). This may be explained indirectly in that teat damage was found to be significantly

associated with tick infestation (26), and the incidence of tick infestation was significantly influenced the occurrence of subclinical mastitis (27).

In our study, no significant associations were detected between incidence of subclinical mastitis and age, milk yield, hygiene conditions, milking method, and season at cow level. Sarker *et al*. (2013) (7) and Tolosa *et al*. (2013) (9) found similar results that age, and milk yield had no significant associations with subclinical mastitis occurrence. However, several previous studies suggested that hygienic-sanitary quality (28), milking method (17, 19, 29), and season (30), significantly influenced the prevalence subclinical mastitis. These discrepancies may be attributed to the fact that most of these studies were conducted on different scale farms under varying conditions (31).

In conclusion, the present study indicates that a high proportion of cows and quarters were identified as subclinical mastitis cases as detected using CMT in Gansu province and Ningxia Hui autonomous region, northwest China. The results also confirm that factors such as parity, lactation, clinical mastitis history and teat damage significantly influenced the risk having SCM at cow level, which needs to be the focus of more formulated prevention and control programs on the local farms.

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CONFLICT OF INTERESTS

The authors have declared that no competing interests exist, and the authors contributed equally to this study.

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