

Prevalence and Major Pathogen Causes of Dairy Cows Subclinical Mastitis in Northeast China

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Abstract: The prevalence of subclinical mastitis in dairy cows was surveyed in Northeast China between June, 2011 and September, 2011. A total of 1860 apparently healthy dairy cows were detected from three provinces (Heilongjiang, Jilin and Liaoning) that locate in Northeast China. All milk samples were examined by California Mastitis Test (CMT). All positive milk samples were analyzed by microbiologically. The results showed that the overall prevalence of subclinical mastitis in dairy cows was 34.0% and the prevalence is similar in 3 different province which Heilongjiang (32.0%, 236/738), Jilin (36.9%, 173/469) and Liaoning (34.3%, 224/653), respectively. The prevalence in intensively reared dairy cows (15.3%, 155/1016) was lower than that in semi-intensively reared dairy cows (56.6%, 478/844), ($p < 0.05$). The dominant bacterial isolates in the study were *Staphylococcus* species (32.0+26.9 = 62.9%), *Streptococcus* species (6.2+7.3+2.0 = 15.5%) and *Escherichia coli* species (12.4%). The results of this survey indicated the presence of subclinical mastitis in dairy cows in Northeast China and rearing system is an important factor to affect the subclinical mastitis in dairy cows in Northeast China and *Staphylococcus* specie was major pathogen.

Key words: Subclinical mastitis, prevalence, major pathogen, Northeast China, *Escherichia coli*, China

INTRODUCTION

Bovine mastitis is a complex and economically important infectious disease for dairy cows throughout the world which can result in substantial losses due to reduced milk yield and increase culling rates and veterinary expenses (Miles *et al.*, 1992). Subclinical mastitis is a condition in which there is no detectable inflammatory change in the udder and no observable abnormalities in the milk. Often it precedes the clinical form, it reduces milk production and adversely affects milk quality. Subclinical mastitis is 3-4 times more common than clinical mastitis and causes great losses in the dairy cows (Joshi and Gokhale, 2006). Subclinical mastitis is often undetectable and has the most serious economic consequences for long term effects on milk yields which may lead to clinical mastitis (Wu *et al.*, 2007).

Bovine mastitis can be caused by physical or chemical agents but the majority of cases are infectious and usually caused by bacteria which consisting mainly of Staphylococci, Streptococci and *Escherichia coli*

forms (Capurro *et al.*, 2010). So, it is of great significance for prevention and treatment mastitis in cows to understand dairy cow subclinical mastitis pathogen. Northeast China, one of the most important milk-producing regions has the largest number of dairy cows and produces more than one-sixth of milk in the whole nation. In view of this background, the objective of the present investigation was determine the prevalence of infection, impact of risk factors and isolate the dominant bacterial causal agents of subclinical mastitis in dairy cows in Northeastern China.

MATERIALS AND METHODS

Milk sample collection: A total of 1860 cows which did not have clinical mastitis were detected from Heilongjiang, Jilin and Liaoning province in Northeast China between June, 2011 and September, 2011. Milk sample were collected as described by Quinn *et al.* (1994). About 100 mL were collected in sterile universal tube and transported in ice immediately to the Veterinary Clinical laboratories of Jilin University.

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California Mastitis Test (CMT): The procedures and interpretations were performed according to Quinn *et al.* (1994) and the results being scored as 0, Trace (T), 1, 2 or 3 depending on the intensity of reaction. Sample with a CMT score of 0 or T were considered negative while those with CMT scores of 1, 2 or 3 were considered as positive. If more than one quarters of cow were positive, it was pooled sample of the quarters subjected to culture. Any samples that were not processed immediately after the CMT test were kept at 4°C for microbiological examination within 24 h of collection.

Microbiological procedures: All CMT positive samples were analyzed microbiologically as described by Quinn *et al.* (1994). Positive samples were cultured on a blood agar plate and MacConkey agar plate. All plates were incubated aerobically at 37°C and examined for growth at 24 h. Bacteria were identified by using colony morphology, hemolytic pattern on blood agar media and further microscopic examination (Gram staining), standard biochemical methods (catalase, haemolysis, coagulase test with rabbit plasma and so on).

Statistical analysis: Statistical analysis of subclinical mastitis in dairy cows from different administrative regions and management systems were analyzed by χ^2 -test with excel (Microsoft® Excel 2003). The differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Prevalence: In the present study, the prevalence of subclinical mastitis in dairy cows was 34.0% with the following distributions: 32.0% in Heilongjiang province, 36.9% in Jilin province and 34.3% in Liaoning province (Table 1). Zhang (2010) reported that the prevalence of subclinical mastitis ranged from 20.7-61.7% in China. The result in present study was relative lower than average level in China. The result show that three different province with similar lower prevalence (32.0, 36.9 and 34.3%) indicated the raising management level were better than other province in China.

Rearing systems: The prevalence in intensively reared dairy cows was lower than that in semi-intensively reared animals, ($p < 0.05$) with the following distributions: 15.3% in intensively raised dairy cows, 56.6% in semi-intensively dairy cows, respectively (Table 1). These finding are in close relation with earlier reports of Biffa *et al.* (2005) and

Table 1: The prevalence of subclinical mastitis in dairy cows was surveyed by CMT according regional and management systems in Northeast China

Factors	No. examined	No. positive	Prevalence (%)
Regional			
Heilongjiang province	738	236	32.0
Jilin province	469	173	36.9
Liaoning province	653	224	34.3
Rearing system			
IR ^a dairy cows	1016	155	15.3
SIR ^b dairy cows	844	478	56.6
Total	1860	633	34.0

^aIR: Intensively Raised; ^bSIR: Semi-Intensively Raised

Table 2: Dominant bacterial isolates identified from sub-mastitic cows

Types of bacterial isolates	No.	%
<i>Staphylococcus aureus</i>	221	36.0
CNS	165	26.9
<i>Streptococcus agalactiae</i>	38	6.2
<i>Streptococcus dysgalactiae</i>	45	7.3
<i>Streptococcus uberis</i>	12	2.0
<i>Escherichia coli</i>	76	12.4
Proteus	12	2.0
<i>Pseudomonas aeruginosa</i>	16	2.5
<i>Micrococcus</i> species	29	4.7
Total	614	100.0

CNS: Coagulase-Negative Staphylococci

Karimuribo *et al.* (2006). Dairy cows are raised intensively in large farms (>500) or semi-intensively by individual families (<50) in Northeast China. In intensive farms, cows were maintained under relatively good management. The cultivation environmental sanitation, the raising management level is in the advanced standard in the intensive farms has the reasonable athletic field and has the strict disinfection medicinal bath measure has stably skilled, the technical quality high milker troop and to secretes the cow to carry on the dairy herd improvement. In semi-intensive farms, cows were maintained in dirty and muddy common barns with bedding materials that favor the proliferation and transmission of mastitis pathogens.

The environmental sanitation, the raising management level to be irregular does not have the athletic field some peasant households match the concentrated feed by the dried egg yolk corn straw does not feed the thick green fodder or unusual thick green fodder supplies and transforms frequently, the non-medicinal bath measure, milks in the personnel besides the family member stable, other also when has exchanges, milks the horizontal ataxia.

Bacterial isolation: Of the 633 milk samples subjected to bacteriological examination, 3.0% (19) showed no bacterial growth. The most frequently isolated pathogen was Staphylococci (62.9%), followed by *Streptococcus* sp. (15.5%), gram-negative bacteria (12.4%) other than *Escherichia coli* were the dominant isolates identified (Table 2). In the study, Staphylococci were the major subclinical mastitis inducing dominant pathogens

detected. *Staphylococcus aureus* was the predominant pathogens involved constituting (36.0%) of all isolates. Similarly, the same findings were reported in dairy cows (Unnerstad *et al.*, 2009). Mekibib *et al.* (2010) also found *Staphylococcus aureus* was predominant cause of subclinical mastitis in dairy cows. The prevalence of Coagulase Negative Staphylococci (CNS) was 26.9% that it was the second predominant isolate next to *S. aureus*. The finding is in close relation with earlier reports of Moges *et al.* (2011). In many modern dairy herds, opportunistic bacteria such as CNS are frequently associated with bovine mastitis and CNS could be described as emerging pathogens (Pyorala and Taponen, 2008). Staphylococci were contagious mastitis pathogens that tend to be subclinical in nature (Sharif and Muhammad, 2009). Contagious mastitis can be transmitted from one cow to another during milking process and new infections are most often acquired during the lactation period. The primary reservoir of contagious pathogens is the mammary gland itself.

Frequency of contagious pathogens among mastitis cases is greater (Sori *et al.*, 2005). Therefore, the survey of subclinical mastitis in dairy cows and isolation of infected dairy cows will be an effective measure. In the study, environmental pathogens for *Streptococcus* sp. and *E. coli* were isolated. Animal management systems may be the reason that environmental pathogens were isolated in this study. The intensive and in semi-intensive dairy farms are incidence of environmental pathogens was higher than that in pastured dairy cows.

CONCLUSION

The subclinical mastitis in dairy cows is relatively low in Northeast China could lead to great economic loss and affect human health. Therefore, to reduce the prevalence of subclinical mastitis cows has become a priority. In the survey, researchers found that the prevalence significantly difference in different rearing systems. Therefore, rearing system is an important factor to affect the subclinical mastitis in dairy cows in Northeast China. In this study predominant subclinical mastitis pathogen was *Staphylococcus* sp. which is the most prevalent contagious mastitis pathogens. Therefore, the survey of subclinical mastitis in dairy cows and isolation of infected dairy cows will be an effective measure.

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