Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms

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ABSTRACT

Knowledge of the incidence of clinical mastitis (CM) and the distribution of pathogens involved is essential for development of prevention and control programs as well as treatment protocols. No country-wide study on the incidence of CM and the distribution of pathogens involved has been conducted in China. Core objectives of this study were, therefore, to determine the cumulative incidence of CM and the distribution of pathogens causing CM on large Chinese (>500 cows) dairy farms. In addition, associations between the distribution of CM pathogens and bedding materials and seasonal factors were also investigated. Bacterial culture was done on a total of 3,288 CM quarter milk samples from 161 dairy herds (located in 21 provinces) between March 2014 and September 2016. Additional data, including geographical region of herds, herd size, bedding types, and number of CM cases during the last month, were also recorded. Mean cumulative incidence of CM was 3.3 cases per 100 cows per month (range = 1.7 to 8.1). The most frequently isolated pathogens were *Escherichia coli* (14.4%), *Klebsiella* spp. (13.0%), coagulase-negative staphylococci (11.3%), *Streptococcus dysgalactiae* (10.5%), and *Staphylococcus aureus* (10.2%). *Streptococcus agalactiae* was isolated from 2.8% of CM samples, whereas *Streptococcus uberis* were isolated from 2.1% of samples, and 15.8% of 3,288 samples were culture-negative. Coagulase-negative staphylococci, *E. coli*, and other *Enterobacter* spp. were more frequently isolated in the northwest than the northeast or south of China. *Streptococcus dysgalactiae*, other streptococci, and *Strep. agalactiae* were more frequently isolated in winter (October–March), whereas *E. coli* and *Klebsiella*

spp. were mostly isolated in summer (April–September). *Streptococcus dysgalactiae* was more often isolated from CM cases of herds using sand bedding, whereas *Klebsiella* spp. and other streptococci were more common in herds using organic bedding. The incidence of CM and distribution of pathogens differed among herds and better mastitis management is needed. Furthermore, geography, bedding materials, and season should be included when designing mastitis control and prevention schemes for Chinese dairies.

Key words: clinical mastitis, pathogen, distribution, season, bedding material

INTRODUCTION

Bovine mastitis is a common and costly disease for the dairy industry worldwide (Bradley, 2002), resulting in much involuntary culling. Identification of pathogens causing clinical mastitis (CM) enables appropriate choices for antibiotic treatment (Pinzón-Sánchez et al., 2011) and preventive mastitis management. The distribution of pathogens isolated from CM samples differs considerably among countries and regions (Erskine et al., 1988; Barkema et al., 1998; McDougall, 1999; Bradley et al., 2007; Olde Riekerink et al., 2008; Ericsson-Unnerstad et al., 2009; Oliveira et al., 2013; Verbeke et al., 2014). For example, in Norway and Sweden, *Staphylococcus aureus* is the most frequently isolated pathogen from CM samples, followed by *Streptococcus dysgalactiae* and *Escherichia coli*, respectively (Reksen et al., 2006; Ericsson-Unnerstad et al., 2009). In the Netherlands, *Klebsiella* mastitis occurs less frequently than CM caused by *E. coli* (Barkema et al., 1998), whereas in the United States, *Klebsiella* and *E. coli* mastitis are of equal importance (Barkema et al., 1998; Roberson et al., 2004). In New Zealand, coliforms are less prevalent as CM pathogens, but *Streptococcus uberis* is the main pathogen causing both clinical and subclinical mastitis (McDougall, 1999).

Received November 19, 2016.

Accepted March 2, 2017.

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Type of bedding and daily bedding management can have a major effect on udder health and the incidence of IMI and mastitis (Hogan et al., 1989). Organic materials are often used as bedding, but they support rapid growth of environmental mastitis pathogens, especially when mixed with manure and urine (Hogan et al., 1990; Ericsson-Unnerstad et al., 2009). Sand, an inorganic bedding material that does not support the growth of bacteria, is considered to be the gold standard bedding material (Stowell and Inglis, 2000). Fewer coliform bacteria and environmental streptococci are present in clean sand than in organic bedding (Stowell and Inglis, 2000). Exposure to lower bacteria counts is associated with reduced rates of mastitis with environmental pathogens (Hogan et al., 1989, 1999).

Climatologic factors affect the incidence of CM (Morse et al., 1988). Seasonal effects are also an issue for pathogen-specific CM, with a higher incidence of streptococcal and coliform CM in the summer (Hogan et al., 1989; Makovec and Ruegg, 2003; Olde Riekerink et al., 2008). Higher humidity and temperature in summer increase bacterial counts in bedding material, resulting in an increased incidence rate of CM caused by these pathogens (Smith et al., 1985; Erskine et al., 1988).

In China, the number of large dairy farms is increasing. As management systems differ among regions, regular national-scale studies on the incidence of CM are indispensable to devise appropriate mastitis-prevention programs. The distribution of pathogens causing subclinical mastitis in Chinese dairy cows was recently reported (Bi et al., 2016), with *Strep. agalactiae* the most frequently isolated pathogen. However, in contrast to subclinical mastitis, a lack of national information exists on pathogens associated with CM in large Chinese dairy farms. The primary objective of our study was therefore to determine the cumulative incidence of CM (CICM) and distribution of pathogens that cause CM on large Chinese dairy farms. Another objective was to determine associations between distribution of pathogen-specific CM and bedding material and seasonal factors.

MATERIALS AND METHODS

Herds

Herds included in the study voluntarily participated in a large dairy farm mastitis program of the Animal Health Department of Boehringer Ingelheim (BI) in China. Herds involved had a minimum of 500 lactating Holstein-Friesian cows that were fed a TMR, milked in a milking parlor, and housed in freestalls. Herds were

added and some stopped participating during the 2.5-yr study period. Herds were located in 3 major geographic regions of China (south, northeast, and northwest; Figure 1).

Samples

Herd veterinarians were asked to collect a milk sample aseptically from quarters that had visible signs of CM before antibiotic treatment was initiated. Cases of CM were detected routinely by herd supervisors at milking time and confirmed by a veterinarian. Veterinarians collected milk samples from every quarter that had visible signs of clinical mastitis, abnormal milk, or swollen quarter(s) before treatment. Aseptic sampling procedures were discussed before the start of the project. No fixed number of CM samples to be submitted was used, and, in general, larger herds were encouraged to submit more samples. Herds often submitted several batches of samples. However, only the first submitted batch of CM samples was included in the current study. Sampling instructions were provided by the Mastitis Diagnostic Laboratory of China Agriculture University (MDL-CAU; Beijing, P.R. China). Producers received a milk sampling package consisting of sample tubes, recording forms, labels, and a protocol for aseptic collection of milk samples. Milk samples were commonly stored in a freezer on the farm (at approximately -20°C), then placed on an ice pack and sent (express mail) to MDL-CAU for bacterial culture. Herds were advised not to store the samples for a prolonged interval (in general not >3 d). Basic farm information, including sampling date, herd size, bedding material and number of cases of CM in the last month (30 d before sampling time) extracted from the farm records, were also recorded, although parity, DIM, quarter location, severity of clinical signs and treatment procedures were not requested.

Microbiological Culture

Upon arrival at MDL-CAU, all frozen samples were thawed at room temperature. Microbiologic procedures were conducted according to guidelines of NMC (1999). In short, 10 μL of milk from each sample was plated onto a blood agar and a MacConkey agar plate (Luqiao, Beijing, China), and plates were incubated at 37°C for 24 to 48 h. Samples were considered culture-positive if 1 or more colonies were observed (≥ 100 cfu/mL; Verbeke et al., 2014). Milk samples with 3 or more species were considered contaminated, unless *Staph. aureus* or *Strep. agalactiae* were isolated. *Staphylococcus aureus* was identified by α - and β -hemolysis on blood agar, Gram staining, a positive catalase test, a positive tube

coagulase test, mannitol reaction, and presence of *nuc* gene (Gao et al., 2011). Suspected *Streptococcus* spp. were identified as catalase-negative and gram-positive cocci. Streptococci were differentiated as esculin-positive (*Strep. uberis* and other esculin-positive cocci) or esculin-negative cocci (*Strep. dysgalactiae* and *Strep. agalactiae*). Christie, Atkins, and Munch-Petersen (CAMP) tests (Tianhe, Hangzhou, China) were used to distinguish *Strep. dysgalactiae* (CAMP-negative) from *Strep. agalactiae* (CAMP-positive). Streptococci not identified by the above procedures were confirmed by 16S rDNA sequencing (Frank et al., 2008). Gram-

negative bacteria were identified using MacConkey agar, Gram staining, motility, indole, ornithine reactions, oxidase, and growth on triple sugar iron slants. The API 20 E system (bioMérieux SA, Marcy-l'Étoile, France) was used to identify gram-negative bacteria that could not be identified by the above procedures. *Prototheca* and yeasts were identified by visual assessment of colony morphology and microscopic examination at 400× magnification. Species that could not be classified using previously mentioned test procedures were subjected to 16S rDNA sequencing (Frank et al., 2008).

Figure 2. Cumulative incidence of clinical mastitis (CICM) in 115 of 161 Chinese dairy herds that submitted milk samples.

Statistical Analyses

All statistical analyses were done with SPSS v20.0 (SPSS Inc., Chicago, IL). A P -value <0.05 was considered significant. Means and standard deviations were calculated. One-way ANOVA and 2-tailed Student's t -tests were used to compare distributions of CM pathogens with regards to region, season, bedding type, and CICM between the 2 seasons. Regions were defined as northeast, northwest, and south China (Figure 1). The CICM was calculated as the number of cows with CM per 100 cows present per month in a herd.

RESULTS

Herd Characteristics

A total of 3,288 quarter milk samples collected from 3,190 dairy cows in 161 herds were submitted to the MDL-CAU for bacterial culture between March 2014 and September 2016. Herds were located in 21 Chinese provinces (Figure 1), which contained $>90\%$ of Chinese dairy farms. There were 72 farms in 7 provinces of northeast China, 51 farms were in 5 provinces of northwest China, and 38 farms in 9 provinces of southern China (Figure 1). Inner Mongolia (25% of herds) and Hebei (22% of herds) had the largest proportion of participating herds (40 and 36, respectively). Average and median herd size were 1,912 and 1,150 lactating cows (range, 511 to 9,032 cows). A total of 72 (45%) dairy farms used organic materials for bedding, whereas the other 89 (55%) farms used sand. Organic bedding included recycled manure, saw dust, rice hull

(or a mixture of those materials), whereas inorganic bedding was exclusively sand. All participating herds used new sand. Sand bedding was cleaned 3 times per day (during milking) to remove all organic materials. In general, fresh new sand was added to the bedding once per week, according to the bedding thickness.

Incidence of Clinical Mastitis

A total of 115 (71%) of 161 farms supplied number of cases of CM in the 30 d before sampling (Figure 2). Mean herd CICM was 3.3% per month in the herds and median CICM was 3.0% per month (range = 1.7 to 8.1 per herd).

Culture Results

Among all enrolled cows, 98 (3.1%) of 3,190 cows had 2 quarters affected with CM at sampling. In total, 2,767 CM samples (84.2%) were culture positive, whereas 521 (15.8%) of CM samples yielded no growth. Of 3,288 samples, 3.8% were defined as contaminated. Among culture-positive samples, *E. coli* was most frequently isolated (14.4%), followed by *Klebsiella spp.* (13.0%), CNS (11.3%), *Strep. dysgalactiae* (10.5%), *Staph. aureus* (10.2%), and other streptococci (8.0%). *Enterobacter spp.*, *Strep. agalactiae*, and *Strep. uberis* were isolated in 5.5, 2.8, and 2.1% of samples, respectively (Table 1). Mixed cultures were isolated in 120 (3.6%) CM samples. Yeasts were the most frequently isolated nonbacterial pathogens (1.0%), whereas other pathogens were isolated in 5.1% of samples.

Table 1. Distribution of pathogens isolated from 3,288 clinical mastitis samples from 161 large (>500 cows) Chinese dairy herds

Pathogen	Samples		Herds ¹	
	No.	%	No.	%
<i>Escherichia coli</i>	473	14.4	120	74.5
<i>Klebsiella</i> spp.	426	13.0	98	60.9
CNS	372	11.3	102	63.4
<i>Streptococcus dysgalactiae</i>	346	10.5	95	59.0
<i>Staphylococcus aureus</i>	337	10.2	90	55.9
Other streptococci	264	8.0	85	52.8
<i>Enterobacter</i> spp.	182	5.5	74	46.0
<i>Streptococcus agalactiae</i>	92	2.8	25	15.5
<i>Streptococcus uberis</i>	70	2.1	23	14.3
<i>Pseudomonas</i> spp.	45	1.4	25	15.5
<i>Bacillus hemolysis</i>	41	1.2	18	11.2
Yeast	32	1.0	15	9.3
<i>Trueperella pyogenes</i>	30	0.9	16	9.9
<i>Aerococcus viridans</i>	21	0.6	16	9.9
<i>Corynebacterium bovis</i>	20	0.6	20	12.4
<i>Pasteurella</i> spp.	5	0.2	4	2.5
Others	6	0.2	5	3.1
Mixed culture	120	3.6	61	37.9
Culture negative	521	15.8	120	74.5
Contamination	125	3.8	64	39.8

¹Number and percentage of herds with at least one sample positive for a pathogen.

Region

Across China, 1,063 CM samples were collected from southern China, whereas 1,271 and 954 samples were obtained from the northeast and northwest, respectively. Mean monthly CICM was 3.1, 2.7, and 4.3% per month for herds in the northeast, northwest, and south, respectively. Distribution of major CM pathogens differed by region (Table 2). Percentage of CNS (6.7%) was lower, but *E. coli* (20.5%) and other *Enterobacter* spp. (8.3%) were higher in northwest herds than in northeast and southern herds. Proportion of culture-

negative samples (20.6%) was higher in herds from southern China. Isolation rates of *Strep. dysgalactiae* differed among the 3 regions.

Sampling Season

A total of 1,287 CM samples were collected in winter (October–March), whereas 2,001 samples were obtained in summer (April–September; Table 3). In winter (October–March) mean monthly CICM was 3.0% per month, whereas in summer CICM was 3.4% per month (Figure 3; $P < 0.05$). Distribution of major pathogens differed by sampling season (Table 3). *Streptococcus dysgalactiae*, other streptococci, and *Strep. agalactiae* were more frequently isolated in winter, whereas *E. coli* and *Klebsiella* spp. were more prevalent in summer.

Bedding Material

Of 3,288 CM samples, 1,750 were collected from herds using sand bedding, whereas 1,538 samples were from herds using organic bedding materials (Table 4). None of the 161 herds used straw as a bedding material. Mean monthly CICM was 2.9 and 3.7% for herds using sand and organic bedding material, respectively. *Streptococcus dysgalactiae* was more frequently isolated in herds using sand bedding than those using organic bedding, whereas *Klebsiella* spp. and other streptococci were more prevalent in herds using organic bedding.

DISCUSSION

In the current study on a considerable number of large Chinese dairy herds, average CICM was 3.3% per month, and *E. coli*, *Klebsiella* spp., CNS, *Strep. dysgalactiae*, and *Staph. aureus* were the most com-

Table 2. Distribution of selected pathogens isolated from clinical mastitis samples in 3 regions of China from 3,288 milk samples collected from 161 large (>500 cows) dairy herds

Pathogen	Region					
	Northeast (n = 1,271)		Northwest (n = 954)		South (n = 1,063)	
	No.	%	No.	%	No.	%
<i>Staphylococcus aureus</i>	179	13.1	85	8.8	73	7.3
CNS	146	12.4 ^a	58	6.7 ^{ab}	168	14.5 ^b
<i>Streptococcus dysgalactiae</i>	143	10.0 ^a	138	14.8 ^a	65	6.0 ^a
Other streptococci	109	8.5	57	6.6	98	10.6
<i>Streptococcus agalactiae</i>	46	4.8	23	2.2	23	2.8
<i>Escherichia coli</i>	171	14.5 ^a	175	20.5 ^{ab}	127	12.3 ^b
<i>Klebsiella</i> spp.	183	14.4	120	10.5	123	12.1
<i>Enterobacter</i> spp.	51	4.7 ^a	73	8.3 ^{ab}	58	4.5 ^b
Culture negative	146	10.0 ^a	148	14.1 ^b	227	20.6 ^{ab}

^{a,b}Within a row, percentage of pathogens without a common superscript differed ($P < 0.05$).

Table 3. Distribution of major mastitis pathogens in winter and summer from 3,288 clinical mastitis milk samples in 161 large (>500 cows) Chinese dairy herds

Pathogen	Sampling season ¹			
	Winter (n = 1,287)		Summer (n = 2,001)	
	No.	%	No.	%
<i>Staphylococcus aureus</i>	144	11.3	193	9.8
CNS	120	9.2	252	12.1
<i>Streptococcus dysgalactiae</i>	203	16.3 ^a	143	7.5 ^a
Other streptococci	122	11.1 ^a	142	6.9 ^a
<i>Streptococcus agalactiae</i>	63	6.3 ^a	29	2.0 ^a
<i>Escherichia coli</i>	138	10.6 ^a	335	18.8 ^a
<i>Klebsiella</i> spp.	112	8.3 ^a	314	15.0 ^a
<i>Enterobacter</i> spp.	75	5.5	107	5.9
Culture negative	205	13.6	316	13.9

^aWithin a row, percentage of pathogens without a common superscript differed ($P < 0.05$).

¹Winter = October–March; Summer = April–September.

monly isolated bacteria. To our knowledge, this was the first China-wide study on incidence rate of CM and associated pathogens. In recent years, the Chinese dairy industry has experienced substantial structural changes, with a rapid increase in number of large herds. It was noteworthy that many large-scale farms were included in our study. The new knowledge generated in our study should be indispensable for preventing and treating CM cases, including development of regional and national mastitis prevention and control programs.

The average CICM in our study of 3.3% per month equates to approximately 37 cases per year; this incidence falls in the range of previous reports (Daniel et al., 1982; Wilesmith et al., 1986; Erskine et al., 1988; Schukken et al., 1989a; Barkema et al., 1998; Bradley et al., 2007; Olde Riekerink et al., 2008). Because this was apparently the first nationwide study in China, no other Chinese studies are available for comparison.

In our study, most (45%) cases of CM were caused by gram-positive cocci, followed by gram-negative

Figure 3. Distribution of cumulative incidence of clinical mastitis (CICM) in 115 large Chinese dairy herds over the year. No samples were submitted in February due to the Spring Festival.

Table 4. Distribution of major clinical mastitis pathogens from 3,288 milk samples in 161 large Chinese dairy herds using 2 kinds of bedding material

Pathogen	Bedding material			
	Sand (n = 1,750)		Organic (n = 1,538)	
	No.	%	No.	%
<i>Staphylococcus aureus</i>	196	11.3	141	9.2
CNS	189	10.8	183	11.5
<i>Streptococcus dysgalactiae</i>	225	13.4 ^a	121	7.0 ^a
Other streptococci	112	6.6 ^a	152	10.6 ^a
<i>Streptococcus agalactiae</i>	73	4.6	19	2.2
<i>Escherichia coli</i>	264	16.9	209	14.7
<i>Klebsiella</i> spp.	177	10.2 ^a	249	15.7 ^a
<i>Enterobacter</i> spp.	101	6.3	81	5.1
Culture negative	273	12.8	248	14.9

^aPercentage of pathogens on the same row having a common superscript differed ($P < 0.05$).

pathogens (33%), which was the opposite of a study that included milk samples from CM cases in large US dairy farms (Oliveira et al., 2013). Environmental pathogens appeared to be the most frequent cause of CM in Chinese farms, similar to other regions in the world (Bradley et al., 2007; Olde Riekerink et al., 2008; Oliveira et al., 2013; Verbeke et al., 2014). However, *Staph. aureus* was isolated from 10% of samples collected from 56% of studied herds, similar to results of CM studies conducted in Canada (Olde Riekerink et al., 2008; Levison et al., 2016), lower than reported in a Swedish study (Ericsson-Unnerstad et al., 2009), but higher than other CM studies (McDougall, 1999; Bradley et al., 2007; Oliveira et al., 2013). A high prevalence of *Staph. aureus* and *Strep. agalactiae* was recently reported in bulk milk samples (Bi et al., 2016), as well as *Strep. agalactiae*, *Staph. aureus*, and *Strep. dysgalactiae* in IMI from Chinese herds (Zhang et al., 2016); therefore, these 3 bacterial species were considered the predominant pathogens associated with subclinical mastitis. Based on high isolation rates of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. agalactiae* from both subclinical and clinical mastitis samples (an isolation rate in CM samples of 2.8% is relatively high for *Strep. agalactiae*, a predominantly subclinical pathogen), we inferred that better contagious mastitis-control programs should be implemented in Chinese herds. Several studies reported that *Strep. uberis* was the most prevalent streptococcal species isolated from CM (McDougall, 1999; Roberson et al., 2004; Bradley et al., 2007; Olde Riekerink et al., 2008; Verbeke et al., 2014). The low proportion of *Strep. uberis* in these large Chinese dairy herds was attributed to the cows being confined and not managed on pasture. Additionally, straw, which is associated with *Strep. uberis* CM (e.g., Ericsson Unnerstad et al., 2009), is in contrast to

sawdust rarely used as a bedding material on Chinese dairy farms.

It was notable that *Klebsiella* spp. were isolated from 61% of studied herds and was the second most frequently isolated pathogen in the present CM study. *Klebsiella* infections are typically very difficult to treat, and infected animals or individual quarters usually have to be culled (Podder et al., 2014). In addition, economic losses due to *Klebsiella* spp. are much greater compared with losses caused by *E. coli* due to reduced cow survival and decreased milk production (Gröhn et al., 2004, 2005). Given the lower proportion of *Klebsiella*-positive CM samples in herds using sand bedding and the lower CICM in these herds, herds with a high *Klebsiella* CICM that use organic bedding should be advised to change bedding to sand (if possible). Growth of bacteria is low in sand bedding because of the low organic content (e.g., Kristula et al., 2005). It is therefore important when using sand bedding to keep it clean so that the organic content is maintained as low as possible.

As the contamination rate of samples herein seemed lower than other reports (Olde Riekerink et al., 2008; Verbeke et al., 2014), we inferred that milk samples were collected in accordance with the supplied sampling procedure. The percentage of negative samples in our study seemed slightly lower than results from other studies (Olde Riekerink et al., 2008; Oliveira et al., 2013; Verbeke et al., 2014). Perhaps this was due to storage of some samples at 4°C instead of freezing, as freezing and thawing of samples decreased culture sensitivity of *E. coli* (Schukken et al., 1989b). Culture methods used in our study were not designed to detect *Mycoplasma* spp., as this pathogen requires special media and culture conditions. Therefore, no conclusions can be drawn on the CICM of *Mycoplasma* spp.,

a pathogen that has been detected on large dairy farms in China (Qi et al., 2012).

Although the climate in northwest of China is mostly cool and dry, a high proportion of environmental pathogens, including *E. coli* and other *Enterobacter* spp., were detected. In the absence of management details of studied herds (e.g., dry cow and calving management, lactation number and lactating days of infected cows, maintenance of bedding and cleanness of cows), we were unable to interpret these findings. The higher percentage of culture-negative samples in southern herds may have been caused by death of pathogens during prolonged transportation from farms to MDL-CAU (located in the north of China).

Seasonal effects on pathogen-specific CM have been reported. In Norway, *Staph. aureus*, *E. coli*, and *Strep. dysgalactiae* were most prevalent in winter, whereas incidence of CM caused by *Strep. uberis* was higher in August (Osterås et al., 2006). China is located in the northern hemisphere; thus, it is cold and dry in winter, but hot and wet in the summer. In our study, CICM of streptococci, including *Strep. dysgalactiae*, environmental streptococci, and *Strep. agalactiae*, was higher in the winter than the summer. High prevalence of *Strep. dysgalactiae* in winter was similar to Norwegian and Canadian studies (Osterås et al., 2006; Olde Riekerink et al., 2007), but not in agreement with another study (Makovec and Ruegg, 2003). In the summer, coliforms, including *E. coli* and *Klebsiella* spp., were more frequently isolated from CM, which was not consistent with a previous study in Canada (Olde Riekerink et al., 2007) but was similar to a series of US studies (Hogan et al., 1989; Todhunter et al., 1991; Makovec and Ruegg, 2003). In the present study, all herds were confined, which was similar to herds in those US studies. It has been demonstrated that, compared with pastured herds, confined herds had a higher incidence of coliform CM in the summer (Olde Riekerink et al., 2007), whereas incidence of *E. coli* CM was lower in summer in herds that kept cows on pasture day and night (Barkema et al., 1999). In summer, heat and humidity enhance the growth of *E. coli* in the environment, resulting in high coliform counts in bedding (Smith et al., 1985; Hogan et al., 1989) and therefore greater exposure to the pathogen.

Bedding types were roughly divided into sand and organic materials, as most farms using organic materials had some mixture of materials (but detailed composition was not supplied by farm producers). In a previous study, lactating cows on farms with organic materials used for bedding had higher coliforms, *Klebsiella* species, and streptococcal counts than those bedded on inorganic materials (Hogan et al., 1989). In a Swed-

ish study, *Klebsiella* CM was associated with sawdust bedding, whereas *Strep. uberis* CM was associated with straw or peat as a bedding material (Ericsson Unnerstad et al., 2009). In the present study, *Klebsiella* spp. and environmental streptococci were more frequently isolated from herds using organic materials, whereas *E. coli* was not. Environmental pathogens usually do not colonize teat skin for prolonged intervals. Bacterial counts on teat ends are correlated with bacterial counts in organic bedding (Hogan et al., 1989, 1999; Zdanowicz et al., 2004). Counts of both *E. coli* and *Klebsiella* spp. were higher on teat ends of cows housed on sawdust compared with sand (Zdanowicz et al., 2004). It was recently reported that fewer cases of CM were caused by gram-negative bacteria for primiparous cows housed in pens containing new sand than in other bedding types (Rowbotham and Ruegg, 2016). Thus, we speculate many organic products contained high levels of a resident population of potential pathogens before they were used as bedding. A source of other streptococci was dried manure (in farms using recycled dry manure), whereas *Klebsiella* spp. mainly came from fresh sawdust (Hogan et al., 1999). Physical properties of sand (e.g., poor adhesion to skin) may lead to bacterial exposure in a more sporadic manner than is the case for sawdust (Zdanowicz et al., 2004).

Studies such as ours that rely on herd personnel and herd veterinarians to collect and submit samples of CM cases have drawbacks. Not all cases of CM were sampled and it is not known how cases of CM that were submitted for culture were selected. It is possible that particularly severe cases of CM were selected and that repeat cases were only sampled if no sample was submitted the first time the cow had CM. Also, it is likely that cows on not-to-breed or cull lists were not sampled. It was not possible to estimate the level of bias that may have been introduced, and this may have affected pathogen distribution. For example, if particularly more severe cases of CM were submitted and less so repeated cases, gram-negative pathogens that, on average, cause more severe CM and less repeated CM cases such as *E. coli* would have been overrepresented (Barkema et al., 1998; Verbeke et al., 2014).

The herds voluntarily participated in the current project, and, except for the size of the lactating herd (>500 cows), they were not purposely selected. Therefore, we expect that the participating herds were typical of large Chinese dairy herds. However, no reliable data exist on the size and management of Chinese dairy herds for comparison.

Estimation of CICM was based on recording of CM by the farms. In our opinion, the estimate of CICM was more accurate when based on the relatively short pe-

riod of 1 mo than a longer interval (e.g., the last year). Additionally, different from privately owned farms in most other countries, all of these large participating Chinese herds had dedicated personnel for data recording. Clinical mastitis cases were recorded on all farms. In an intensive Dutch study, 6% of farm CM data recording was not sufficient to be included in estimation of incidence rate of CM, and in approximately 6% of CM no sample was collected (Barkema et al., 1999). Given the less intensive oversight in our study, CICM was likely underestimated.

In the near future, the antimicrobial susceptibility of these pathogens, phenotypically and genotypically, will be determined. Additionally, in future studies, more cow information (quarter location, and parity) and more herd-level information should be collected; however, in China very few herds are on DHI, so the quality of these data will mostly depend on individual herd recording systems.

CONCLUSIONS

In large dairy herds in China, the CICM was 3.3% per month. *Escherichia coli*, *Klebsiella* spp., CNS, *Strep. dysgalactiae*, *Staph. aureus*, and other streptococci were the most frequently isolated pathogens from bovine CM. Based on a high proportion of *Staph. aureus*, we inferred that routine monitoring for this contagious pathogen was essential and a prevention program should be developed and implemented for affected herds. The CICM was higher in summer, and distribution of CM pathogens was associated with season. In winter, streptococci were more prevalent, whereas coliforms were more frequently isolated in summer. The CICM was lower in herds using sand bedding. *Streptococcus dysgalactiae* was more frequently isolated in herds using sand bedding, whereas proportion of *E. coli* and *Klebsiella* spp. was higher in herds using organic bedding.

ACKNOWLEDGMENTS

This research was financially supported by the Chinese thirteen “Five-year” National Science and Technology Support Project (Beijing; No. 2016YFD0501203), Ministry of Education in China major project (Beijing; No. 313054), High-end Foreign Experts Recruitment Program (Beijing; No. GDT20171100013), and the National Natural Science Foundation of China (Beijing; No. 31572587 and No. 3151101034). The authors also thank staff of Animal Health Cattle, Boehringer Ingelheim International Trading (Shanghai) Co., as well as dairy managers and farm herdsman and veterinarians for their participation.

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