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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).

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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

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