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Short communication: Microbiological quality of raw cow milk and its association with herd management practices in Northern China

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ABSTRACT

Contamination of raw milk with bacterial pathogens is potentially hazardous to human health. The aim of this study was to evaluate the total bacteria count (TBC) and presence of pathogens in raw milk in Northern China along with the associated herd management practices. A total of 160 raw milk samples were collected from 80 dairy herds in Northern China. All raw milk samples were analyzed for TBC and pathogens by culturing. The results showed that the number of raw milk samples with TBC $<2 \times 10^6$ cfu/mL and $<1 \times 10^5 \text{ cfu/mL was } 146 \text{ (91.25\%)} \text{ and } 70 \text{ (43.75\%)},$ respectively. A total of 84 (52.50%) raw milk samples were Staphylococcus aureus positive, 72 (45.00%) were Escherichia coli positive, 2 (1.25%) were Salmonella positive, 2 (1.25%) were Listeria monocytogenes positive, and 3 (1.88%) were Campylobacter positive. The prevalence of S. aureus was influenced by season, herd size, milking frequency, disinfection frequency, and use of a Dairy Herd Improvement program. The TBC was influenced by season and milk frequency. The correlation between TBC and prevalence of S. aureus or E. coli is significant. The effect size statistical analysis showed that season and herd (but not Dairy Herd Improvement, herd size, milking frequency, disinfection frequency, and area) were the most important factors affecting TBC in raw milk. In conclusion, the presence of bacteria in raw milk was associated with season and herd management practices, and further comprehensive study will be powerful for effectively characterizing various factors affecting milk microbial quality in bulk tanks in China.

Key words: raw milk, total bacterial count, pathogen, management practice, Northern China

Short Communication

Milk is a nutritious food for humans as well as an ideal growth medium for bacterial pathogens (Ruusunen et al., 2013). Commonly, serious outbreaks of foodborne disease have been traced back to consumption of milk or dairy products contaminated by pathogens such as Escherichia coli, Salmonella, Listeria monocytogenes, or Campylobacter spp. (Quigley et al., 2013; Cerva et al., 2014). A total of 13 reported outbreaks were associated with Shiga toxin-producing E. coli in unpasteurized milk in the United States from 2007 to 2012 (Mungai et al., 2015). Salmonella outbreaks were associated with raw milk and dairy products. Dairy products have been implicated in approximately half of all listeriosis outbreaks in Europe (Parisi et al., 2013). The number of reported foodborne disease outbreaks in the United States caused by unpasteurized milk increased from 30 cases between 2007 and 2009 to 51 cases between 2010 and 2012, and the number of Campylobacter spp. outbreaks approximately doubled (Mungai et al., 2015). Although these hazards are well known worldwide, little information is available about pathogen contamination rates of raw milk in China.

Total bacterial count (**TBC**), an estimate of quality of raw milk, has become one of the accepted criteria for grading milk (Mhone et al., 2011). High-quality raw milk has a low TBC (Oliver et al., 2009). In the United States, the Pasteurized Milk Ordinance requires grade A raw milk to have a TBC of $<1 \times 10^5$ cfu/mL (FDA, 2013)—the same level as mandated in Europe (Piepers et al., 2014). In China, the national standard requires raw milk to have a TBC of $<2 \times 10^6$ cfu/mL (National Standards of the Republic of China, 2010a). Most dairy herds in China fall into 1 of 2 categories: those with modern feeding systems (most of these are in Beijing) and those with traditional feeding systems (mostly in Inner Mongolia). Northern China, includ-

Received June 17, 2016.

Accepted March 2, 2017.

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ing Beijing and Inner Mongolia, is the nation's major milk-producing area. However, information about the microbiological quality of raw milk and associated factors in Northern China is limited. The aim of this study was to investigate levels of TBC and the prevalence of Staphylococcus aureus, E. coli, Salmonella, L. monocytogenes, and Campylobacter in raw milk samples collected from dairy herds in Northern China and evaluate the factors influencing raw milk microbial quality.

A total of 160 raw milk samples were collected from 80 dairy herds from 2 regions of Northern China: Inner Mongolia and Beijing. The raw milk samples were collected in summer (August and September 2014; average daily temperature $>20^{\circ}$ C) and winter (October and November 2014; average daily temperature $<10^{\circ}$ C). All raw milk samples were sampled from a bulk tank (3–5°C), transferred to sterile bottles, stored at approximately 4°C, and transported to the local laboratory for microbiological analyses within 24 h of sampling.

Information on management practices of dairy herds was obtained by interview with farm managers during sampling. Information included total number of cattle, frequency of milking per day, frequency of disinfection of milking machine unit liner per day, DHI program, and common hygienic measures.

The raw milk samples were analyzed for TBC, S. aureus, and E. coli by plate counts. Salmonella, L. monocytogenes, and Campylobacter spp. in raw milk were analyzed by the International Organization for Standardization standard methods (National Standards of the Republic of China, 2010b; ISO, 1999, 2001, 2002, 2004, 2006; Supplemental Table S1; https://doi.org/10.3168/jds.2016-11631).

All statistical analysis was performed using SAS/Stat software (version 9.2; SAS Institute Inc., 2008). The TBC (\log_{10} cfu/mL) were analyzed using the following model:

$$Y_{ijklmno} = \mu + S_i + A_j + HS_k + D_l + MF_m + DisF_n + H_{jklmno} + E_{ijklmno},$$

where $Y_{ijklmno}$ is the dependent variable, μ is the mean, S_i is the effect of the *i*th season (summer or winter); A_i is the effect of the jth area; HS_k is the effect of the kth herd size; D_l is the effect of the lth level of implementation of DHI; MF_m is the effect of the mth frequency of milking per day; $DisF_n$ is the effect of the nth level of frequency of disinfection of the milking machine unit liner per day; H_{iklmno} is the effect of the oth herd within the jth area, kth herd size, lth DHI level, mth milking frequency level, and nth disinfection frequency level; and $E_{ijklmno}$ is the residual error. All factors except herd were considered to be fixed effects; herd was considered to be a random effect. All herds were sampled in both seasons, but for the other factors, each herd was characterized by 1 level of each factor. It was not possible to include interaction terms in the model due to the relatively small size of the data set. The relative importance of each factor was determined using the effect size option in the GLM procedure. These effect sizes were determined as the proportion of the sums of squares (Type III) for each factor in the model relative to the total sums of squares [referred to as semipartial eta squared (η^2) in SAS according to Hoefkens et al. (2011)]. The model described here was also analyzed using the MIXED procedure to test hypotheses about the significance of the fixed effects. The Kenward-Roger option was used to determine the appropriate degrees of freedom for each test.

A chi-squared test (χ^2) using the FREQ procedure of SAS was used to assess associations between the prevalence of S. aureus and E. coli pathogens and the following factors: season, region, herd size, DHI implementation, frequency of milking per day, and frequency of disinfection of milking machine unit liner per day. A regression analysis (using the REG procedure of SAS) was carried out to determine the relationship between S. aureus or E. coli and TBC (all values as \log_{10} cfu/mL), and only samples with positive counts of S. aureus and E. coli were used for these analyses. The factors listed previously (season, area, herd size, DHI implementation, frequency of milking per day, and frequency

Table 1. Total bacteria count (TBC) and pathogen prevalence in raw milk samples (n = 160) in Northern China

Item	Positive samples [no. (%)]	Count range in positive samples $(\log_{10} \text{ cfu/mL})$	Average count in positive samples $(\log_{10} \text{ cfu/mL})$	Average count in total samples $(\log_{10} \text{ cfu/mL})$
TBC	ND^1	3.15-6.61	ND	5.10
Bacteria				
Staphylococcus aureus	84 (52.5)	1-4.73	2.79	1.47
Escherichia coli	72 (45)	1-4.83	2.91	1.31
Salmonella	2(1.25)	ND	ND	ND
Listeria monocytogenes	2 (1.25)	ND	ND	ND
Campylobacter	3 (1.88)	ND	ND	ND

¹ND = not determined.

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