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 $<1 \times 10^5$ cfu/mL was 146 (91.25%) and 70 (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, including Beijing and Inner Mongolia, is the nation's major milk-producing area. However, information about the

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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°C) and winter (October and November 2014; average daily temperature <10°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 (log₁₀ 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_j is the effect of the j th area; HS_k is the effect of the k th herd size; D_l is the effect of the l th level of implementation of DHI; MF_m is the effect of the m th frequency of milking per day; $DisF_n$ is the effect of the n th level of frequency of disinfection of the milking machine unit liner per day; H_{jklmno} is the effect of the o th herd within the j th area, k th herd size, l th DHI level, m th milking frequency level, and n th 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 log₁₀ cfu/mL), and only samples with positive counts of *S. aureus* and *E. coli* were used for these analyses. The factors

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 cfu/mL)	Average count in positive samples (log ₁₀ cfu/mL)	Average count in total samples (log ₁₀ cfu/mL)
TBC	ND ¹	3.15–6.61	ND	5.10
Bacteria				
<i>Staphylococcus aureus</i>	84 (52.5)	1–4.73	2.79	1.47
<i>Escherichia coli</i>	72 (45)	1–4.83	2.91	1.31
<i>Salmonella</i>	2 (1.25)	ND	ND	ND
<i>Listeria monocytogenes</i>	2 (1.25)	ND	ND	ND
<i>Campylobacter</i>	3 (1.88)	ND	ND	ND

¹ND = not determined.

listed previously (season, area, herd size, DHI implementation, frequency of milking per day, and frequency of disinfection of milking machine unit liner per day) were included in the model, one at a time, to determine effects on slopes and intercepts. The zero value was not analyzed.

Among all 160 raw milk samples, the TBC varied from 3.15 to 6.61 log₁₀ cfu/mL, with the average of 5.10 log₁₀ cfu/mL (Table 1). The average TBC in our study is lower than that in Morocco (5.87 log₁₀ cfu/mL; Sraïri et al., 2009) and Zimbabwe (6.4 log₁₀ cfu/mL; Mhone et al., 2011) but higher than that in Belgium (3.96 log₁₀ cfu/mL; Piepers et al., 2014), the United States (4.06 log₁₀ cfu/mL; van Schaik et al., 2002), or Finland (4.11 log₁₀ cfu/mL; Ruusunen et al., 2013). Approximately 92% of raw milk samples met the TBC of the China national standard (<200 × 10⁴ cfu/mL), and approximately 44% of the raw milk samples met the TBC standards (<10 × 10⁴ cfu/mL) of the United States and Europe (Piepers et al., 2014; Table S2; <https://doi.org/10.3168/jds.2016-11631>).

Staphylococcus aureus, *E. coli*, *Salmonella*, *L. monocytogenes*, and *Campylobacter* were positive in 84 (52.50%), 72 (45.00%), 2 (1.25%), 3 (1.88%), and 2 (1.25%) raw milk samples, respectively (Table 1). The prevalence of *S. aureus* in our study is lower than that in Italy (83%; Bartolomeoli et al., 2009) but higher than that in Finland (34.4%; Ruusunen et al., 2013). The prevalence of *E. coli* in raw milk in our study was lower than that in Malaysia (64.5%) but higher than that in the United States (3.8%; Jayarao and Henning, 2001). The prevalence of *Salmonella* in our study is lower than that in Malaysia (1.4%) and the United States (2.6%; Van Kessel et al., 2004) but higher than that in New Zealand (0%; Hill et al., 2012) and Italy (0.3%; Bianchi et al., 2013). The prevalence of *L. monocytogenes* in our study is lower than that in Finland (5.5%; Ruusunen et al., 2013) but higher than that in New Zealand (0.68%; Hill et al., 2012). The prevalence of *Campylobacter* in our study is lower than that in Poland (4.6%; Wysok et al., 2011) and the United States (36.6%; Sanad et al., 2014) but higher than that in New Zealand (0.34%; Hill et al., 2012).

The TBC and prevalence of *S. aureus* and *E. coli* in raw milk samples from winter were significantly lower than in samples from summer (Table 2), which was not surprising considering the longer generation time of bacteria in a bulk tank in winter. Frequency of milking was a significant effector of TBC such that herds that milked 3 times/d had a lower TBC level than herds that milked 2 times/d. The prevalence of *S. aureus* was lower (*P* < 0.01) in large dairy herds than in small herds, lower (*P* < 0.05) with an implemented DHI system than without a DHI system, and lower (*P* < 0.01)

Table 2. Association of risk factors and total bacteria counts (TBC) or prevalence of pathogens in raw milk samples in Northern China

Factor	Category	Samples (no.)	TBC		Staphylococcus aureus			Escherichia coli				
			LSM ¹ ± SE	P-value	Positive samples (no.)	PR ²	χ ²	P-value	Positive samples (no.)	PR	χ ²	P-value
Season	Summer	80	5.69 ± 0.01	<0.01	52	32.50	9.05	<0.01	48	30.00	13.36	<0.01
Area	Winter	80	4.73 ± 0.01		32	20.00			24	15.00		
	Inner Mongolia	80	5.30 ± 0.01	0.20	40	27.50	0.23	0.63	37	23.13	0.03	0.87
	Beijing	80	5.12 ± 0.01		44	25.00			35	21.88		
Herd size (no.)	1–199	26	5.30 ± 0.04	0.19	20	12.50	20.35	<0.01	15	9.38	3.29	0.35
	200–499	63	5.40 ± 0.02		40	25.00			20	17.50		
	500–999	29	5.10 ± 0.03		12	7.50			14	8.75		
	>1,000	42	5.04 ± 0.02		12	7.50			15	9.38		
Milking frequency (times/d)	2	82	5.57 ± 0.02	0.04	54	33.75	10.95	<0.01	42	26.25	2.14	0.14
	3	78	4.85 ± 0.02		30	18.75			30	18.75		
Disinfection frequency ³ (times/d)	1	8	5.47 ± 0.08	0.23	7	4.38	14.18	<0.01	5	3.13	2.98	0.23
	2	78	4.94 ± 0.02		51	31.88			40	25.00		
	3	74	5.22 ± 0.02		26	16.25			27	16.88		
DHI system	Yes	64	5.40 ± 0.02	0.09	27	16.88	3.89	0.04	23	14.38	2.96	0.09
	No	96	5.02 ± 0.01		57	35.63			49	30.63		

¹Log₁₀ cfu/mL.
²PR = prevalence rate (%).
³Disinfection of milking machine unit liner.

Figure 1. Regression analyses of relationships for (A) *Staphylococcus aureus* counts and total bacteria counts and (B) *Escherichia coli* counts and total bacteria counts (TBC) of raw milk in Northern China. SE = season; 1 = summer; 2 = winter. Color version available online.

Figure 2. The relative importance of factors affecting total bacteria counts in raw milk expressed as semipartial eta values (η^2), which represent percentages of the Type III sums of squares attributed to the various effects in the model. Color version available online.

with increasing milking frequency and frequency of disinfection of milking machine unit liner. The prevalence of *S. aureus* in raw milk commonly indicates mastitis in dairy herds (Cicconi-Hogan et al., 2014). The lower prevalence of *S. aureus* from large dairy herds may be attributable to more stringent hygienic and managerial practices implemented in the large herds (Cicconi-Hogan et al., 2013; Toth et al., 2013). Approximately 80% of our selected large dairy herds implemented DHI, which was associated with a lower prevalence of *S. aureus*. The prevalence and high level of *E. coli* in food of animal origin implies environmental and fecal contamination (Mhone et al., 2011). In this study, only season was found to be a significant effect factor for the presence of *E. coli*. However, others have reported that some herd management practices were associated with *E. coli* contamination, such as milking machine, milking parlor type, and milking hygiene (Pantoja et al., 2009; Mhone et al., 2011; Piepers et al., 2014). A significant correlation was found between TBC and prevalence of *S. aureus* ($P < 0.01$, $R^2 = 0.23$) and *E. coli* ($P < 0.01$, $R^2 = 0.41$; Figure 1). This suggests that more attention should be paid to *S. aureus* and *E. coli* when TBC counts of milk are unusually high.

Considering the relative importance of factors affecting TBC expressed as semipartial eta values (η^2), we see that season ($\eta^2 = 30.0\%$) and herd ($\eta^2 = 39.8\%$) were most important, whereas area, herd size, and herd management factors such as use of DHI, milking frequency, and disinfection frequency were relatively unimportant (Figure 2). The proportion of variance at-

tributed to error in this analysis was found to be $\eta^2 = 15.2\%$. Because the relative importance of herd-to-herd differences is so large ($\eta^2 = 39.8\%$), important elements of herd management that are not presently known must be affecting TBC. Previous studies revealed that herd size and cleaning frequency were significantly associated with TBC in raw milk (Piepers et al., 2014). This suggests that TBC in raw milk is primarily driven by herd factors other than the ones included in this study, potentially including the length of time in the bulk tank, storage temperature, methods of premilking and postmilking teat sanitation, or incidence of mastitis.

In conclusion, we found that the majority of raw milk samples met the TBC of the China national standard. The prevalence of *S. aureus* and *E. coli* in raw milk was much higher than that of other bacterial species. Season, herd size, DHI implementation, milking frequency, and disinfection frequency were associated with microbiological quality in raw milk. However, those herd management factors could not explain all variations of TBC in raw milk. Therefore, further research should analyze how other herd factors affect TBC in raw milk.

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