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Research Note

Occurrence of Enterotoxic *Staphylococcus aureus* in Raw Milk from Yaks and Cattle in Mongolia

URANCHIMEG TSEGMED,[1,2] GIOVANNI NORMANNO,[3] MARIT PRINGLE,[1] AND KAREL KROVACEK[1]*

[1]*Department of Biomedical Sciences and Veterinary Public Health, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Box 7036, 750 07 Uppsala, Sweden;* [2]*Department of Veterinary Sanitation and Hygiene, Institute of Veterinary Medicine, Zaisan-210253, Ulaanbaatar, Mongolia; and* [3]*Department of Health and Animal Welfare, Faculty of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy*

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ABSTRACT

Staphylococcal food poisoning is considered one of the leading foodborne illnesses in humans worldwide and is associated with contaminated foods of animal origin, such as milk and dairy products. In this study, we investigated the occurrence of staphylococci and the enterotoxigenic properties of *Staphylococcus aureus* isolated from raw milk from yaks (*Bos mutus*) and cattle in Mongolia. Staphylococci were isolated from 72 (74%) of the 97 raw milk samples. Of the samples containing staphylococci, 69% (50 of 72) were from yaks and 30.5% (22 of 72) were from cattle. *S. aureus* was detected in 10% of yak (7 of 72) and 21% of cattle (15 of 72) milk samples. Staphylococcal enterotoxin C was detected in 23% (5 of 22) of the *S. aureus* strains investigated, based on the reverse passive latex agglutination technique. Three of the five enterotoxigenic strains were from yaks and two were from cattle. None of the *S. aureus* strains tested produced staphylococcal enterotoxins A, B, or D. To our knowledge, this is the first report of the occurrence of staphylococci and enterotoxigenic *S. aureus* in milk from yaks and cattle in Mongolia.

Staphylococci are among the most significant pathogens that cause a wide spectrum of diseases in both humans and animals. In humans, nosocomial and community-acquired staphylococcal infections are the most frequently reported *(17, 35).* Coagulase-positive and coagulase-negative staphylococci are important mastitis pathogens in animals *(1, 7, 8, 12, 22, 36, 40). Staphylococcus aureus* is one of the most significant foodborne pathogens *(18, 38).* Raw milk and unpasteurized dairy products may contain enterotoxigenic strains of *S. aureus (23),* which may be associated with staphylococcal infections of the mammary gland *(4, 10, 19). S. aureus* can produce different exotoxins such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 *(7, 8, 15, 16, 18, 28, 37, 41).* Twenty serologically distinct SEs have been identified *(20).* However, the most common SE involved in *S. aureus* food poisoning is SEA *(23).* Various approaches such as bioassays, immunoassays, tissue culture cell tests, and PCR techniques are widely used to detect SEs in isolates and food samples *(3, 6, 17, 20, 23, 24, 27, 29, 34, 35).*

The ability of *S. aureus* strains to produce one or more SEs in food products is linked to staphylococcal food poisoning (SFP). This infection is characterized by an acute onset of nausea, vomiting, abdominal cramps, and diarrhea. The symptoms occur when foods containing SEs are ingested. The amount of SE capable of causing intoxication is uncertain, but an enterotoxin dose of less than 1.0 μg in contaminated food can cause symptoms of food poisoning *(3, 11).* Although *S. aureus* is not considered especially lethal, death can ensue when large amounts of SEs are ingested; fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immunocompromised persons, elderly people, and children *(3).*

Food poisoning is an important public health concern *(21, 32).* Therefore, foods and raw ingredients, including milk, must be subjected to microbiological controls (including the detection of free bacterial toxins) to reduce the health risks for consumers. To date, there are no reports on the occurrence of enterotoxigenic staphylococci in raw milk from Mongolia. Mongolia is a pastoral country, and 80% of the milk comes from free-grazing cattle and yaks. Dairy products, including milk, cheese, cream, butter, and yoghurt, are important primary sources of nutrition in Mongolia. The aim of this study was to investigate the occurrence of staphylococci and the enterotoxic properties of *S. aureus* isolated from raw milk from yaks and cattle in Mongolia.

MATERIALS AND METHODS

Study area. The study region for the present work was an area of approximately 50 km^2 in the Sharhooloi and Bayan Dohom valleys, Gachuurt village, Mongolia. Gachuurt village is in

* Author for correspondence. Tel: + 46-18-67 31 91; Fax: + 46-18-67 33 34; E-mail: karel.krovacek@vmm.slu.se.

a major milk-producing region near Ulaanbaatar City. Dairy cattle and yaks were kept in open housing and milked twice daily.

Sampling. Between July and August 2000, 97 milk samples were collected from individual animals: 65 samples from yaks in six herds and 32 samples from cattle in three herds. For this sampling procedure, the teat ends and the hands of the milker were cleansed with alcohol and allowed to dry. After discarding the first stream, 10 ml of milk (about 2.5 ml per single mammary quarter) was collected in 15-ml disposable sterile screw-cap tubes. Samples were immediately transported under refrigeration to the Veterinary Sanitation and Hygiene Laboratory (Institute of Veterinary Medicine, Ulaanbaatar City, Mongolia) and kept at 4°C. From each sample, 1.5 ml was pipetted into sterile microcentrifuge tubes and centrifuged at 10,000 rpm for 5 min at room temperature. The supernatant was then discarded, and the pellet was stored at −20°C until processed. At the end of the collection period, the samples were transported to the Department of Biomedical Sciences and Veterinary Public Health at the Faculty of Veterinary Medicine and Animal Science (Swedish University of Agricultural Sciences) in coolers containing frozen cold packs so that they would still be frozen when they arrived about 3 days later.

Isolation and identification. The sediment was resuspended in 1 ml of sterile phosphate-buffered saline, and 10 μl of this milk suspension was pipetted, streaked onto blood agar plates (5% [vol/vol] bovine erythrocytes in heart infusion agar; Difco, Becton Dickinson, Sparks, Md.), and incubated at 37°C for 24 h under aerobic conditions. Five presumptive staphylococcal colonies (creamy grayish white or yellow colonies 2 to 5 mm in diameter) were then randomly selected and examined by Gram staining. Gram-positive and catalase-positive cocci were further subjected to coagulase, maltose, mannitol, and DNase tests and further identified using the API Staph system (bioMérieux, Marcy l'Etoile, France). *S. aureus* isolates were confirmed with the Phadebact Staph Aureus Test (Boule Diagnostics AB, Huddinge, Sweden) and by PCR amplification of the *nuc* gene as described elsewhere *(5).* The DNA extraction was carried out using the Genomic Prep Tissue DNA isolation kit (Amersham Pharmacia Biotech, Piscataway, N.J.) following the manufacturer's instructions. The hemolytic properties of isolates were tested on blood agar plates (5% [vol/vol] bovine erythrocytes in heart infusion agar).

Detection of SEs. Presence of SEs was determined using the reverse passive latex agglutination test. The culture fluid from each *S. aureus* isolate (one per positive sample) was tested for SEs A, B, C, and D with the SET-RPLA kit (Oxoid, Basingstoke, UK). The isolates to be tested were grown in tubes containing 10 ml of brain heart infusion broth (Oxoid) for 24 h at 37°C under aerobic conditions, then the broth was filtered through a membrane with a pore diameter of 45 μm (194-2545, Nalgene Labware, Rochester, N.Y.). The filtrates were then pipetted into 96-well microtiter plates, and the test was performed following the manufacturer's instructions. *S. aureus* strains ATCC 13565, ATCC 14458, ATCC 19095, and ATCC 23235 were used as positive controls for SEA, SEB, SEC, and SED, respectively.

RESULTS AND DISCUSSION

Of the 97 raw milk samples examined, 72 (74%) yielded staphylococci, of which 69% (50 of 72) were from yaks and 30.5% (22 of 72) were from cattle. *S. aureus* was detected in 10% (7 of 72) of the yak milk samples and in 21% (15 of 72) of the cattle milk samples. All presumptive *S. aureus* isolates were confirmed using the Phadebact *S. aureus* test and PCR assay. Three isolates were mannitol negative, and four were nonhemolytic. Five isolates (three from yaks and two from cattle) were enterotoxigenic, and all of these isolates produced SEC. None of the *S. aureus* strains produced SEA, SEB, or SED.

In a recent report, *S. aureus* was detected in 75% of 220 bovine bulk milk samples *(14).* Several investigators have described prevalence rates of 20 to 38% for *S. aureus* in raw milk products in Norway *(14).* In one Swedish report, coagulase-positive staphylococci were detected in 38% of raw goat cheeses *(33).*

Olson et al. *(26)* found that 15% of 157 *S. aureus* isolates from mastitic cattle were enterotoxigenic, whereas Kenny et al. *(16)* reported that 28.6% of bovine *S. aureus* strains were enterotoxin producers. Stephan et al. *(30)* reported that 54% of *S. aureus* isolates from bovine mastitic milk were enterotoxigenic, and Normanno et al. *(23)* reported that 55.9% of *S. aureus* isolates from raw milk in Italy produced enterotoxins. In contrast, Danish investigators reported that none of 160 *S. aureus* strains isolated from mastitic samples of bovine milk produced enterotoxins *(1).* Similarly, Jörgensen et al. *(14)* in Norway failed to detect SE (SEs A through D) in 75 *S. aureus* isolates from a farm producing small quantities of raw milk cheese. The different rates of enterotoxin production found in these reports might be explained by the different techniques used in these studies, differences in the origin of the isolates, or geographical differences. Various investigators have reported that *S. aureus* isolated from dairy products of bovine and ovine origin are able to produce SEC and SED *(25).*

In the present investigation, *S. aureus* was found in raw milk samples from yaks and cattle in Mongolia, and 23% (5 of 22) of the isolates produced SEC. This finding is not surprising because SEC is the SE most frequently produced by *S. aureus* from milk-producing animals *(13, 39).* This finding may be important in terms of food safety because although the SEs most commonly involved in cases of SFP are SEA and SED *(2),* SEC also has been recognized as an important cause of SFP associated with the consumption of dairy products *(31).* Analysis of the data obtained in this study resulted in a probable underestimation of the occurrence of staphylococci and *S. aureus* in the samples because of the need to centrifuge and freeze the samples before analysis. The enterotoxigenic properties of the analyzed isolates also may have been underestimated because the possible presence of newly described SEs was not considered.

There are no data available on the occurrence of SFP in Mongolia. However, the presence of enterotoxigenic *S. aureus* in milk, one of the most important foods associated with SFP, may well constitute a potential hazard for consumers, especially when good hygiene practices such as the rapid refrigeration of milk after milking are not utilized. Because humans are the primary vehicles for contaminating foods with enterotoxigenic *S. aureus (9),* the potential roles of milkers, food handlers, and consumers in spreading enterotoxigenic *S. aureus* must be better defined to avoid SFP cases.

To the best of our knowledge, this is the first report on the occurrence of staphylococci and enterotoxigenic *S. aureus* in raw milk from Mongolia. These results warrant further investigation to elucidate the public health significance of enterotoxigenic *S. aureus* and other milkborne pathogens that may be present in Mongolian raw milk.

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