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Korrespondenzadresse:
dagmar.schoder@vetmeduni.ac.at

Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animals and Veterinary Public Health of the Veterinary Medical University of Vienna, Veterinärplatz 1, 1210 Vienna, Austria

Sheep and goat raw milk consumption: a hygienic matter of concern?

Konsum von Schaf- und Ziegenrohmilch: ein Grund zur Sorge aus hygienischer Sicht?

Dagmar Schoder, Abdoulla Zangana, Martin Wagner

Summary

Within Europe, Austria has the second-highest share in direct selling of milk and milk products. All food business operators, including dairy farmers, offering on-farm sale of milk and milk products have a legal responsibility to produce safe food. The aim of this study was to determine the prevalences of major foodborne pathogens in sheep and goat bulk tank milk samples, and to evaluate whether the raw milk meets the food safety legal requirements. Sheep and goat raw milk of high-quality should have a low bacteria count (≤ 5.0 x 10^5 cfu/ml) and be free of human pathogens.

Fifty-three farms were selected from a database of registered dairies maintained at the veterinary directorate county council. Farms were selected on the basis that milk was either sold directly or processed to cheese. A total of 53 bulk tank milk (BTM) samples were collected during the hygiene inspection of each farm and examined for standard plate counts (SPC), coliforms, *E. coli* and *Staphylococcus aureus* (SA). Additionally, a total of 160 BTM samples were checked for the presence of *Salmonella* spp., *Listeria monocytogenes, Cronobacter* spp., *Campylobacter jejuni* and *Yersinia enterocolitica.*

In this study 49.1 % and 11.3 % of the BTM samples exceeded the legal limit of 5.0 x 10^5 cfu/ml for SPC and 2.0 x 10^3 cfu/ml SA, respectively. Foodborne pathogens were isolated from 6.3 % (10/160) of the BTM samples. *Campylobacter jejuni, Cronobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica* were detected in 1.3 %, 0.6 %, 1.3 % and 3.1 % of BTM samples, respectively, whereas *Listeria monocytogenes* could not be detected in any of the tested samples.

Keywords: prevalence, foodborne pathogens, goat, sheep, raw milk, hygiene

Zusammenfassung

Österreich hat innerhalb von Europa die zweithöchste Direktvermarkterquote für Milch und Milchprodukte. Alle Lebensmittelhersteller und bäuerliche Direktvermarkter sind gesetzlich verpflichtet ihrer Sorgfaltspflicht nachzukommen. Ziel dieser Studie war es, die Wiederfindungsrate der wichtigsten lebensmittelpathogenen Krankheitserreger in Schaf- und Ziegentankmilch zu bestimmen und festzustellen, ob Rohmilch den lebensmittelrechtlichen Vorschriften entspricht. Rohe Schaf- und Ziegenmilch von hoher Güteklasse darf nur wenige Bakterien (≤ 5.0 x 10^5 KBE/ml) und keine humanpathogenen Krankheitserreger enthalten.

53 bäuerliche Milchdirektvermarkter wurden aus der entsprechenden Datenbank der Landesveterinärbehörde ausgewählt und besucht. Im Rahmen einer Hygieneinspektion wurden insgesamt 53 Tankmilchproben (BTM) gezogen und die Gesamtkeimzahl (SPC), der Gehalt an coliformen Keimen, *E. coli* und *Staphylococcus aureus* (SA) bestimmt. Zusätzlich wurden insgesamt 160 BTM-Proben auf das Vorkommen von *Salmonella* spp. *Listeria monocytogenes, Cronobacter* spp., *Campylobacter jejuni* und *Yersinia enterocolitica* untersucht.

In dieser Studie überstiegen 49.1 % bzw. 11.3 % der BTM-Proben die gesetzlichen Grenzwerte von 5.0 x 10^5 cfu/ml für die Gesamtkeimzahl (SPC) und 2.0 x 10^3 cfu/ml für *Staphylococcus aureus.* Lebensmittelpathogene Krankheitserreger wurden in 6.3 % (10/160) aller BTM-Proben nachgewiesen. *Campylobacter jejuni, Cronobacter* spp., *Salmonella* spp. und *Yersinia enterocolitica* wurden in 1.3 %, 0.6 %, 1.3 % and 3.1 % der BTM-Proben isoliert, hingegen konnte *Listeria monocytogenes* in keiner der getesteten Proben nachgewiesen werden.

Schlüsselwörter: Prävalenz, lebensmittelbedingte Krankheitserreger, Ziege, Schaf, Rohmilch, Hygiene

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Introduction

In recent decades, the safety of the food supply has become a focal point for public concern. Consequently, prevention of foodborne illness is a major issue that affects all aspects of food production, including direct marketing of dairy products (Wansink, 2004).

Raw milk has been a known vehicle for pathogens for centuries (Oliver et al., 2009). Outbreaks associated with the consumption of raw milk caused by *Salmonella* spp. (Mazurek et al., 2004), *Listeria monocytogenes* (*L. monocytogenes;* CDC, 2004), and *Campylobacter jejuni* (*C. jejuni;* Jiménez et al., 2005; Heuvelink et al., 2009) have been reported in recent years. Generally, the prevalence of major foodborne pathogens in raw bovine milk is well documented (Oliver et al., 2009). On the other hand, literature on prevalence data in caprine and ovine milk is scarce (Muehlherr et al., 2003; Rey et al., 2006).

Although proper pasteurization minimizes these risks to the public, there is a small but growing group of people who choose unpasteurized milk or milk products. In Austria consumption of raw bulk tank milk (BTM) is common practice among farming families. Among the nonfarming population, a growing number of consumers are claiming that raw milk is healthier and are choosing raw milk over pasteurized milk (Schoder et al., 2008).

Additionally, fresh ovine or caprine milk is consumed by infants and others with allergies to cow milk and is also used for on-farm manufactured cheese, with or without thermal treatment. Goats and sheep rank third and fourth in terms of global milk production from different species (Anonymous, 2008), respectively, but unlike cow milk, which is associated with stringent hygiene and quality regulations, microbiological standards for the production and distribution of goat and sheep milk are less stringent (Zangerl and Kupfner, 2009).

Within Europe, Austria has the second-highest share in direct selling of milk and milk products (Anonymous, 2009). In recent years on-farm cheese-making has become a thriving business (Schoder et al., 2008). A previous Austrian study revealed that 48 % of the samples of raw milk intended for direct consumption and 34 % of the samples of fresh sheep cheese made from raw milk did not satisfy legal requirements (Pfleger 2002). Consequently, the aim of this study was to determine the prevalences of major foodborne pathogens in sheep and goat bulk tank milk samples, and to evaluate whether the raw milk meets the food safety legal requirements.

Material and Methods

Abbreviation key: BTM = bulk tank milk, **CC** = coliform count, **EC** = *Escherichia coli;* **SA** = *Staphylococcus aureus,* **SPC** = standard plate count.

Farm selection and inspection

Lower Austria is a dairy intensive area with 20.1 % of the national dairy output and 31.6 % of the gross agricultural output. Agricultural statistics show that, in 2009, Austria had 22 400 dairy sheep and 28 900 dairy goats, whereas Lower Austria contained 46.7 % and 29.5 % of the national herd (Anonymous, 2009). Fifty-three farms were selected from a database of registered dairies maintained at the veterinary directorate county council. Farms were selected on the basis that milk was either sold directly or processed to cheese. In general, farms were visited once. The farms were notified of the forthcoming inspection, and the proprietors were told to maintain routine cleaning procedures.

Collection of BTM

BTM samples (each 1000 ml) were collected in sterile flasks from each of the 53 producers by the authors. Each farmer was given a training demonstration in methods required to collect and dispatch the BTM samples to be analysed by the laboratory hygienically, and was asked to send BTM samples at regular intervals until the animals were dried off. Milk samples were collected in the morning, one to two hours after milking. The milk in the bulk tank was inclusive of that day's morning and previous evening's milk. Briefly, milk in the bulk tank was agitated for at least five minutes and collected with a sterile dipper. Samples were transported to the laboratory at 4 °C within 12 hours and processed within one to two hours after receipt. Only those samples that recorded a temperature of < 7 °C were processed.

Analysis of SPC, CC and SA

The milk samples were thoroughly mixed by gently shaking the sample flasks 20 to 25 times. One millilitre of milk was transferred to a sterile tube containing 9 ml of sterile phosphate buffered saline (Sigma, Buchs, Switzerland). The 10-fold diluted sample was then vortexed at high speed for 15 seconds. SPC and CC were determined by the pour plate method. Appropriate dilutions up to 10^{-6} were plated on standard plate count (PCA) agar (Difco, Becton Dickinson and Company, Sparks, MD, USA) and violet red bile (VRB) agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), according to international standard methods ISO 4833 and ISO 4832 (Anonymous, 2003; Anonymous 2006a). EC were determined by plating 100 µl of the sample and appropriate dilutions on chromID™ Coli (COLI-ID-F) agar (BioMerieux, Marcy l Etoile, France). The plates were incubated for 24 hours at 37 °C and colonies typical of *E. coli* (pink/violet coloured colonies) were counted.

The isolation of SA from bulk tank milk was performed according to the ISO 6888-1 standard procedure of the International Organization for Standardization (Anonymous, 1999), using Baird Parker Agar supplemented with egg yolk tellurite emulsion (Oxoid). The plates were incubated under aerobic conditions at 37 °C for 24 to 48 hours. If present, five egg yolk reaction-positive and five egg yolk reaction-negative colonies were chosen from each sample for further identification. All suspect colonies were grown aerobically in Brain Heart Infusion Broth (Oxoid) at 37 °C for 18 to 24 hours and were then spread-plated onto Columbia Blood Agar (Oxoid). The isolates were identified as SA on the basis of their colony morphology, gram-staining, catalase reaction, tellurite reduction, lecithinase activity, hemolytic properties and by their ability to coagulate rabbit plasma (tube coagulase test) and to produce clumping factor (Staphylase test; Oxoid).

Isolation of foodborne pathogens from BTM

Salmonella spp.

Isolation of *Salmonella* spp. from raw milk was performed according to ISO 6579 (Anonymous, 2002). Twenty-five ml of milk was added to 225 ml of buffered peptone water (Merck, Darmstadt, Germany) and incubated for 24 hours at 37 °C. Briefly, 0.1 and 1 milliliters of pre-enriched

samples were transferred to Rappaport-Vassiliadis medium (Merck) and Müller-Kauffmann tetrathionate-novobiocin (MKTTn) (Oxoid), followed by 24 hours of incubation at 42 and 37 °C, respectively. The enrichments were streaked on xylose lysine desoxycholate agar (XLD agar; Oxoid) and Brilliance™ Salmonella Agar (Oxoid) and incubated for 24 hours at 37 °C. Typical *Salmonella* colonies on selective agar were subcultured onto non-selective media prior to confirmatory testing. All presumptive Salmonella colonies were tested with Microbact™ Biochemical Identification Kit (Oxoid) and with polyvalent antisera for flagella (H) and somatic (O) Salmonella Latex Test (Oxoid). Isolates with a typical biochemical profile, which agglutinate with both H and O antisera, were identified as *Salmonella* spp.

L. monocytogenes

Detection and enumeration of *L. monocytogenes* were carried out according to ISO 11290-1 and 11290-2 methods, respectively (Anonymous, 1996; Anonymous, 1998). All culture media and selective supplements were from Oxoid Ltd. (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). To improve the detection limit, the sample volumes of BTM were increased from 25 to 100 ml, respectively. Briefly, 25 and 100 ml of milk were added to 225 and 900 ml Half-Fraser (HF) broth and incubated for 24 hours at 30 °C. Then, 0.1 ml of the HF broth was transferred to tubes containing 10 ml of Fraser Broth and incubated at 37 °C for 48 hours. Both enrichment broths (HF and Fraser) were streaked onto duplicate plates of Palcam and OCLA agar. The plates were incubated for 48 hours at 37 °C and observed for the presence of typical *Listeria* spp. colonies. Presumptive *Listeria* colonies were identified by species-specific PCR, according to Bubert et al. (1999). In addition, 0.1 ml BTM samples were directly streaked onto Palcam and OCLA agar in accordance with ISO-11290-2.

Campylobacter spp.

Isolation procedures were based on ISO 10272-1 (Anonymous, 2006b). Bolton broth, containing the Bolton antibiotic supplement, and 5 % lysed horse blood and Preston broth, i.e. nutrient broth containing the Preston *Campylobacter* selective supplement and 5 % lysed horse blood, were prepared according to manufacturer recommendations (Oxoid) and kept for a maximum of four weeks at 4 °C before use. Briefly, 10 ml of milk was added to 90 ml Preston and Bolton broths, respectively, and incubated for 18 hours at 42 °C. The enrichments were streaked onto modified charcoal cefoperazone deoxycholate agar (mCCDA) and Karmali agar (Oxoid) and incubated at 42 °C. Plates were examined after 24 and 48 hours. Enriched broth and plates were incubated in a microaerophilic atmosphere that was created by using the gas-generating kit for *Campylobacter,* CampyGen gas-generating system (Oxoid) in an anaerobic jar. All presumptive *Campylobacter* isolates were confirmed to species by use of the API-CAMPY identification kit (BioMerieux).

Yersinia enterocolitica (Y. enterocolitica)

Samples were analysed according to ISO 10273 (Anonymous, 2001). Briefly, 10 ml of raw milk was added to 90 ml of peptone sorbitol bile broth (Sigma) and incubated at 25 °C for five days. On day five, the enriched broth was treated with 0.5 % KOH (Merck) and plated onto Cefsulodin-Irgasan-Novobiocin (CIN) agar (Oxoid). Additionally, 1 ml of raw milk was transferred to 99 ml Irgasan-Ticarcillin-potassium chlorate (ITC) enrichment broth (Merck). The ITC enrichment broth was incubated for two days at 25 °C when a 10 µl volume was streaked for selective isolation onto *Salmonella-Shigella*-desoxycholate-calcium-chloride (SSDC, Yersiniaagar; Merck) and Cefsulodin-Irgasan-Novobiocin (CIN) agar (Oxoid) plates. SSDC were incubated for 24 hours and CIN plates for 18–

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22 hours at 30 °C. All presumptive *Y. enterocolitica* isolates were biochemically confirmed, first selecting only lactose-negative, urease-positive colonies, and later with API-50E identification kit (BioMerieux).

Cronobacter spp.

Ten milliliters of raw milk was added to 90 ml of EE broth (Merck) and incubated at 36 °C for 24 hours. The enrichments were streaked onto violet red bile glucose (VRBG) agar plates (Merck). Five presumptive *Cronobacter* spp. colonies, that appear as purple colonies surrounded by a purple halo of precipitated bile acids on VRBG plates, were streaked onto TSA agar (Tryptic Soy Agar; Merck) supplemented with 0.5 % yeast extract (Merck), and incubated at 25 ± 1 °C for 48–72 hours. Yellow pigmented colonies from the TSA plates were selected and confirmed using the API 20E biochemical identification system, according to the manufacturer's instructions (BioMerieux).

Results and discussion

The concept of "produce, sell, and buy local" and the demand for natural and unprocessed foods are growing consumer trends that have resulted in an increased interest in raw milk. All 53 dairy farms, which were included in this study, sold milk and milk products directly to the consumer. One farm sold cheese made from pasteurized milk, all other 52 farms sold raw milk or produced fresh cheese from raw milk.

All food business operators, including dairy farmers, offering on-farm sale of milk and milk products, have a legal responsibility to produce safe food. The regulation on microbiological criteria for foodstuffs (Commission Regulation (EC) No 2073/2005) contains microbiological criteria for specific food/microorganism combinations and the implementing rules to be complied with by food business operators at all stages of the food chain. This means that high-quality milk contains a low number of somatic cells, a low bacteria count, and is free of human pathogens and antibiotic residues.

Hygienic quality of BTM

A total of 53 BTM samples were collected during the hygiene inspection of each dairy farm. 60.4 % of the BTM samples were sheep-, 32.1 % goat- and 7.5 % were mixed sheep-goat-milk samples, respectively. The BTM samples were examined for standard plate counts (SPC), coliforms (CC), *E. coli* (EC) and *Staphylococcus aureus* (SA).

The most important requirement in the dairy sector is meeting the standards for raw milk quality, according to Annex III, Section IX, Chapter I (III, 3) of the Regulation 853/2004/EC and particularly as regards to: (i) Plate count at 30 °C up to 1.5 x 10^6 cfu/ml for raw milk from other species, (ii) Plate count at 30 °C up to 5.0 x 10^5 cfu/ml for raw milk from species other than cows intended for the manufacturer of products made with raw milk by a process that does not involve any heat treatment.

In this study 49.1 % of BTM samples (26/53) exceeded this limit of 5.0 x 10^5 cfu/ml. The SPC of this subset of samples ranged from 1.0 x 10^3 cfu/ml to 6.0 x 10^7 cfu/ml showing a median and mean value of 4.2 x 10^5 cfu/ml and 1.2 x 10^7 cfu/ml, respectively. Figure 1 shows the distribution of SPC among the raw milk samples. SPC were categorized into four groups (low ≤ 50 000, medium > 50 000 to 500 000, high > 500 000 to 1 500 000, very high > 1 500 000). Only 20.8 % of the samples showed a SPC ≤ 5.0 x 10^4 cfu/ml, 30.2 % of the samples fell within 50 000 to 500 000 cfu/ml and almost half of the samples, namely 47.2 % even exceeded > 1.5 x 10^6 cfu/ml.

The SPC is an estimate of the total number of viable aerobic bacteria present in raw milk. The most frequent cause of high SPC is poor cleaning of milking systems (Hayes et al., 2001; Jayarao et al., 2004). With regard to cow milk, most Austrian farms can produce milk with counts of < 50 000 cfu/ml (Grade A milk). High bacterial counts (> 100 000 cfu/ml for cow milk and > 500 000 cfu/ml for sheep and goat milk, respectively) suggest that bacteria are

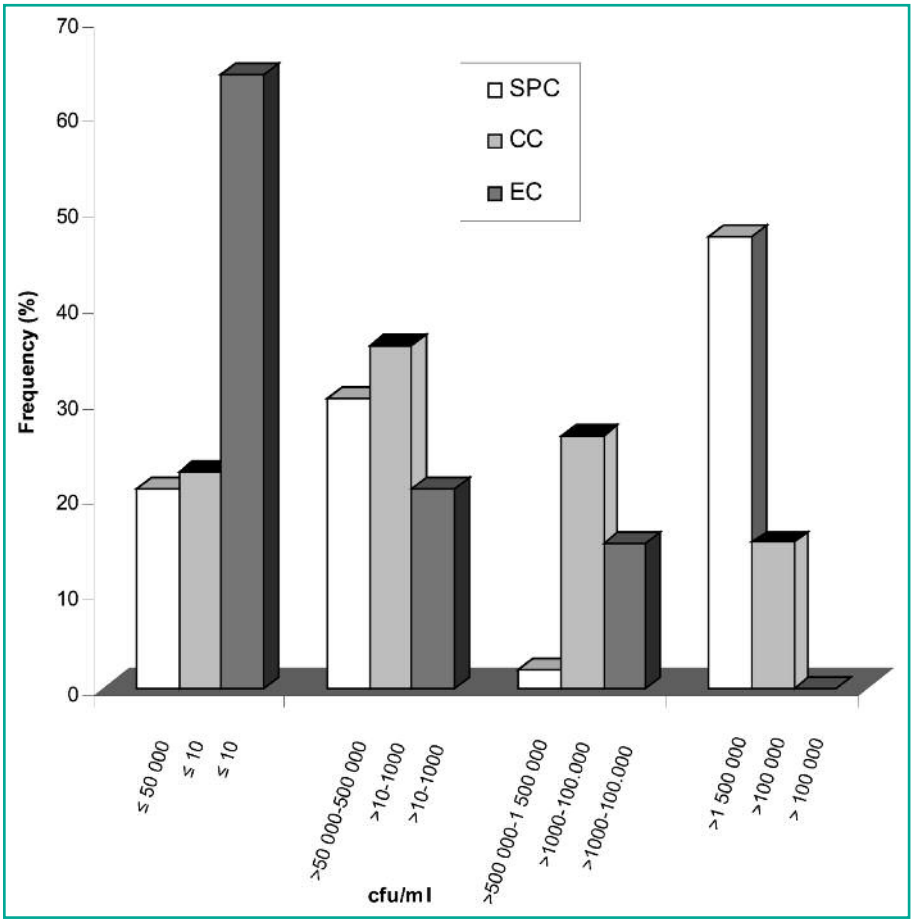

FIGURE 1: *BTM samples were collected during the hygiene inspection of 53 dairy farms. The BTM samples (n = 53) were transported to the laboratory at 4 °C within 12 h. Frequency (%) distribution of SPC (empty bar), of CC (hatched bar) and EC (filled bar). SPC were categorized into 4 groups (low ≤ 50 000, medium > 50 000 to 500 000, high > 500 000 to 1 500 000, very high > 1 500 000). CC and EC were categorized each into 4 groups (low ≤ 10, medium > 10 to 1000, high > 1000 to 100 000, very high > 100 000).*

entering milk from a variety of possible sources. Milk residues on equipment surfaces provide nutrients for growth and multiplication of bacteria that contaminate milk of subsequent milkings. Unclean milking practices and failure to cool milk rapidly to 4 °C can also contribute to high SPCs in raw milk (Hayes et al., 2001; Jayarao et al., 2004; Zadoks et al., 2004). Although it is impossible to eliminate all sources of bacterial contamination of milk, milk from clean, healthy sheep and goats that has been properly collected is able to achieve an SPC of < 5.0 x 10^4 cfu/ml. According to our study, only 20.8 % (11/53) of dairy farms could fulfil this requirement.

The presence of coliform bacteria in BTM milk is suggestive of fecal contamination. Coliforms are frequently isolated from BTM and include *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. Unlike pasteurized milk and milk products, where coliform count standards have been established, there are no regulatory standards for CC in raw milk. However, it is generally accepted that counts for coliforms > 1 000 cfu/ml and for *E. coli* > 100 cfu/ml of raw milk indicate milk produced under unhygienic conditions (Bray and Shearer, 1996). Coliforms were detected in 77.4 % (41/53) of all collected samples. The CC observed in this study ranged from < 1.0 x 10^1 cfu/ml to 7.9 x 10^5 cfu/ml, resulting in a mean CC of 7.0 x 10^4 cfu/ml. Comparable results were revealed with the EC, ranging from < 1.0 x 10^1 cfu/ml to 9.0 x 10^4 cfu/ml and showing a mean and median value of 3.0 x 10^3 cfu/ml and 1.0 x 10^1 cfu/ml, respectively. The mean coliform counts observed in this study are higher than those reported by Hogan et al. (1989) (1.0 x 10^2 cfu/ml) and about 41.6 % of the samples in this study had counts greater than 1.0 x 10^3 cfu/ml. These data clearly indicate deficiencies in the hygiene management of the inspected dairy farms, most probably associated with improper cleaning of the milking system, udder and teats before milking.

The bulk tank milks of 17 out of 53 farms (32.1 %) were contaminated with SA at levels of up to 5.0 x 10^5 cfu/ml, which was significantly higher than a previous report by Muehlherr et al. (2003), who determined that SA counts ranged from 1.0 x 10^1 cfu/ml to 10^4 cfu/ml in BTM samples in Switzerland. In our study, about 11.3 % and 5.7 % of the samples had counts greater than 2.0 x 10^3 cfu/ml or 1.0 x 10^5 cfu/ml, respectively. At the moment there are no regulatory standards for SA in raw milk. However, in view of the microbiological criteria laid down in the former EU Milk Hygiene Directive 92/46 (Council of the European Communities, 1992), 69.8 % of the BTM samples in this study would have been below the m value (< 5.0 x 10^2 cfu/ml). Zangerl and Kupfner (2009) in turn demand even more stringent criteria of < 1.0 x 10^2 cfu/ml SA in raw milk that is intended for the manufacture of cheese.

Prevalence of foodborne pathogens

High-quality milk should also be free of foodborne pathogens. In this study, a total of 160 bulk tank milk samples were checked for the presence of *Salmonella* spp., *L. monocytogenes, Cronobacter* spp., *C. jejuni* and *Y. enterocolitica.* 33.1 % (53/160) BTM samples were collected during the hygiene inspection by the authors and 66.9 % (107/160) BTM samples were collected by the farmers themselves and were sent to the laboratory within 12 hours. Foodborne pathogens were isolated from 6.3 % (10/160) of the BTM samples. *C. jejuni, Cronobacter* spp., *Salmonella* spp. and *Y. enterocolitica* were detected in 1.3 %, 0.6 %, 1.3 % and 3.1 % of BTM samples, respectively. *L. monocytogenes* on the other hand could not be detected in any of the tested samples.

Unfortunately, literature regarding the prevalence of milkborne pathogens in raw sheep and goat milk is scarce (Rey et al., 2006; Solomakos et al., 2009). With regard to cow milk, several surveys have detected foodborne pathogens such as *C. jejuni, L. monocytogenes, Salmonella* spp., enterotoxigenic *Staphylococcus aureus, Y. enterocolitica,* and others in bulk tank milk (Oliver et al., 2005), whereas *L. monocytogenes* and *Salmonella* spp. were the most commonly reported foodborne pathogens isolated from bulk tank milk. Isolation rates for *L. monocytogenes* and *Salmonella* spp. ranged from 2.8 to 7.0 % and 0 to 11 % respectively (Jayarao and Henning, 2001; Van Kessel et al., 2004; Jayarao et al., 2006; D'Amico et al., 2008). Jayarao and co-workers also reported on the occurrence of *C. jejuni* and *Y. enterocolitica* in bulk tank milk. Isolation rates for *C. jejuni* were 2.0 % (Jayarao et al., 2006) and 9.2 % (Jayarao and Henning, 2001). Corresponding prevalence rates for *Y. enterocolitica* were 1.2 % (Jayarao et al., 2006) and 6.1 % (Jayarao and Henning, 2001). Interestingly, with the exception of *L. monocytogenes,* our study revealed prevalence data of similar order of magnitude.

Conclusion

Based on the findings of our study, we conclude that almost half of the inspected dairy farms were not able to meet basic hygiene requirements. Our data clearly indicate that consumers are at risk of being exposed to foodborne pathogens when they consume raw milk.

Dairy producers supplying raw milk must be well informed of the risks and liabilities associated with the milk they sell. Of primary importance is the need for providing educational programs and materials that bring awareness of microbial safety hazards to dairy farmers, milk processors and consumers.

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Address of corresponding author:
Dagmar Schoder
Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animals and Veterinary Public Health of the Veterinary Medical University of Vienna,
Veterinärplatz 1, 1210 Vienna, Austria;
dagmar.schoder@vetmeduni.ac.at