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Korrespondenzadresse:
roger.stephan@uzh.ch

Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

Microbiological quality of raw milk sold directly from farms to consumers in Switzerland

Mikrobiologische Qualität von direkt ab Hof an den Konsumenten verkaufter Rohmilch in der Schweiz

Marc Zulauf, Claudio Zweifel, Roger Stephan

Summary

Seventy-three samples of raw cow milk marketed at farm level (12 pre-filled bottles, 61 from vending machines) were investigated for their microbiological quality and the occurrence of bacterial foodborne pathogens. Total viable counts (TVC) were mainly (67.1 %) in the range from 10^3 to 10^5 CFU/ml, while *Escherichia coli* and coagulase-positive staphylococci were each detected in 30.1 % of the samples. TVC results for raw milk from vending machines (34.4 % above 10^5 CFU/ml) were clearly higher than those from pre-filled bottles, emphasizing the importance of ensuring correct cleaning and disinfection procedures of vending machines. Moreover, regular monitoring of the microbiological quality of raw milk from vending machines should be considered. With regard to foodborne pathogens in raw milk marketed at farm level, 24.7 % of all samples were positive for *Staphylococcus aureus* harboring staphylococcal enterotoxin (SE) genes. Genes for SEA, SEC, and SED were thereby also detected. On the other hand, *Campylobacter* spp., *Listeria monocytogenes,* and Shiga toxin-producing *Escherichia coli* were not detected. But because the occurrence of foodborne pathogens can never be ruled out, raw milk should always be properly heated before consumption.

Keywords: Raw milk, farm level, indicator bacteria, foodborne pathogens, *Staphylococcus aureus*

Zusammenfassung

Dreiundsiebzig Proben von ab Hof verkaufter Rohmilch (12 vorgefüllte Flaschen, 61 aus Milchautomaten) wurden auf ihre mikrobiologische Qualität und das Vorkommen von bakteriellen Krankheitserregern untersucht. Die Gesamtkeimzahl (GKZ) von 67.1 % der Proben lag im Bereich von 10^3 bis 10^5 KbE/ml. *Escherichia coli* und Koagulase-positive Staphylokokken wurden jeweils in 30.1 % der Proben nachgewiesen. Die GKZ-Ergebnisse für Rohmilch aus Verkaufsautomaten (34.4 % über 10^5 KbE/ml) waren deutlich höher als diejenigen aus vorgefüllten Flaschen. Dies unterstreicht die zentrale Bedeutung einer korrekten Reinigung und Desinfektion von Rohmilchautomaten. Zudem ist eine regelmäßige Überwachung der mikrobiologischen Qualität von Rohmilch aus Verkaufsautomaten zu empfehlen. Im Hinblick auf Lebensmittel-assoziierte Krankheitserreger in ab Hof verkaufter Rohmilch erwiesen sich 24.7 % aller Proben als positiv für *Staphylococcus aureus* mit Staphylokokken-Enterotoxin (SE) Genen. Dabei wurden auch Gene für SEA, SEC und SED nachgewiesen. *Campylobacter* spp., *Listeria monocytogenes* und Shigatoxin-bildende *Escherichia coli* wurden in den untersuchten Rohmilchproben nicht gefunden. Da aber das Vorkommen Lebensmittel-assoziierter Krankheitserreger nie ausgeschlossen werden kann, ist Rohmilch vor dem Verzehr immer ausreichend zu erhitzten.

Schlüsselwörter: Rohmilch, Direktverkauf ab Hof, Indikatorkeime, pathogene Bakterien, *Staphylococcus aureus*

Introduction

In recent years, food consumption habits have dramatically changed under the influence of lifestyle changes and new technologies. In the context of the trend toward "consuming natural" and "purchasing locally" (Campbell et al., 2013; Van Asselt et al., 2015), the popularity of raw milk has increased in many industrialized countries. Consumers tend to prefer raw milk due to better taste and believe in better nutritional values (Claeys et al., 2013; Bigouette et al., 2018). Moreover, a variety of health benefits associated with raw milk consumption (as improved immunity, less lactose intolerance, less diabetes, and many others) are propagated, but convincing scientific evidence is hardly available (Claeys et al., 2013). On the other hand, there is a well-established association between raw milk consumption and infection with pathogenic bacteria as e.g. *Campylobacter, Salmonella,* or Shiga toxin-producing *Escherichia (E.) coli* (EFSA, 2015; Mungai et al., 2015; Costard et al., 2017). Of the raw milk-associated outbreaks in Europe, the great majority has been attributed to *Campylobacter (C.),* predominantly *C. jejuni* (EFSA, 2015).

Raw milk may be sold to consumers by several means, but legal specifications vary between European countries (EFSA, 2015). Traditionally, raw milk is sold directly to consumers from a farm shop or via local delivery. In some countries, raw milk vending machines are allowed and Internet sales have increased product availability. In Europe, Italy has the largest number of raw milk vending machines (EFSA, 2015). Correspondingly, most studies on the microbiological quality and safety of raw milk from vending machines originate from Italy (Giacometti et al., 2012a,b; Bianchi et al., 2013; Giacometti et al., 2013; Gasperetti et al., 2014; Tremonte et al., 2014; Bertasi et al., 2016). Other studies examining the microbiological status of raw milk sold to consumers are restricted to England (Willis et al., 2017), Estonia (Kalmus et al., 2015), Lower Saxony (Brix and Thielke, 2016), and Poland (Pyz-Łukasik al., 2015).

In Switzerland, about 500 raw milk sales points, mainly vending machines, are currently present on farms. As in many other countries, purchasers must be informed that the raw milk must be heated (at least to 70 °C) before consumption (VLtH, 2017). However, studies from Italy showed that about 40 % of consumers did not (properly) follow the heating recommendations (Giacometti et al., 2012b; Giacometti et al., 2013) and the behavior of Swiss consumers is probably comparable. Moreover, the microbiological status of raw milk sold from Swiss farms to consumers has so far been unknown and a specific monitoring program is currently lacking. Thus, the aim of the present study was to generate initial baseline data on hygiene parameters, selected bacterial foodborne pathogens, and antibiotic-resistant bacteria in raw milk marketed at farm level.

Materials and methods

Analyzed raw milk and sampling

In this study, 73 raw milk samples sold directly from farms to consumers were analyzed. They originated from 73 farms located in the northern and central parts of Switzerland. Samples consisted of cow milk and were grouped into two categories: raw milk sold in pre-filled bottles (n = 12) and raw milk sold from self-service automatic vending machines (n = 61; Brunimat Milchautomaten, Muolen, CH; www.brunimat.ch). Sampling was performed during two months (September to October 2017) and samples were transported chilled to the laboratory.

Total viable counts (TVC), *E. coli,* and coagulase-positive staphylococci (CPS)

Samples were quantitatively analyzed by the spreading technique. The following agars and conditions were used: plate count agar (Oxoid, Pratteln, CH; 72 h, 30 °C) for TVC, RAPID *E. coli* 2 agar (Bio-Rad, Reinach, CH; 24 h, 37 °C) for *E. coli,* and Baird Parker + RPF (rabbit plasma fibrinogen) agar (Bio-Rad; 48 h, 37 °C) for CPS. The detection limit was 1.0 x 10^2 CFU/ml for TVC and 1.0 x 10^1 CFU/ml for *E. coli* and CPS.

Staphylococcus (S.) aureus

For confirmation of CPS as *S. aureus* and for further strain characterization, the StaphType DNA microarray assay was used (Alere Technologies, Jena, D). This assay covers a variety of target sequences, including species markers, enterotoxin genes, and resistance-associated genes. Resulting DNA microarray profiles were used to assign the *S. aureus* isolates to clonal complexes (Ebner et al., 2013).

Campylobacter spp.

10 ml of each sample were enriched at a 1:10 ratio in CampyFood broth (bioMérieux, Geneva, CH; 24 h, 37 °C, microaerophilic conditions). The enrichment broths were subcultured on CampyFood agar (bioMérieux; 24 h, 37 °C, microaerophilic conditions).

Listeria (L.) monocytogenes

10 ml of each sample were first enriched at a 1:10 ratio in Half-Fraser broth (Oxoid; 24 h, 30 °C). From the first enrichment, 0.1 ml were incubated in 10 ml of Fraser broth (Oxoid; 24 h, 37 °C). The enrichment broths were subcultured on Rapid'L.mono agar (Bio-Rad; 48 h, 37 °C).

Shiga toxin genes

10 ml of each sample were enriched at a 1:10 ratio in brilliant green bile broth (Oxoid; 24 h, 37 °C). The enrichment broths were subcultured on plate count agar (Oxoid; 24 h, 37 °C). After washing off the colonies with 0.85 % NaCl, samples were screened by the Assurance GDS assay for Shiga toxin genes (Bio Control Systems, Bellevue, WA, USA).

Antibiotic resistance profiles of *Escherichia coli*

From each *E. coli* positive chromogenic agar, one typical colony was subjected to susceptibility testing against 16 antimicrobial agents by the disk diffusion method according to the Clinical and Laboratory Standards Institute protocols and criteria (CLSI, 2013). The panel included amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), azithromycin (15 µg), cefazolin (30 µg), cefepime (30 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), fosfomycin (200 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), streptomycin (10 µg), sulfamethoxazole/trimethoprim (23.75/1.25 µg), and tetracycline (30 µg) (Becton Dickinson, Allschwil, CH).

Extended-spectrum ß-lactamases (ESBL)-producing Enterobacteriaceae

Of the enrichments prepared for the detection of Shiga toxin genes, one loopful was subcultured on chromogenic Brilliance ESBL agar (Oxoid; 24 h, 37 °C).

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Results and Discussion

Hygiene parameters

Total viable counts (TVC) of the 73 raw milk samples sold directly from farms to consumers ranged from 9.0 x 10^2 to 3.9 x 10^6 CFU/ml (Table 1). For raw milk in pre-filled bottles, TVC ranged from 9.0 x 10^2 to 1.6 x 10^5 CFU/ml (median: 4.3 x 10^3 CFU/ml). The majority (66.7 %) of these results were in the range from 10^3 to 10^4 CFU/ml. For raw milk from vending machines, TVC ranged from 9.0 x 10^2 to 3.9 x 10^6 CFU/ml (median: 3.1 x 10^4 CFU/ml). About a third (34.4 %) of these results were >10^5 CFU/ml. With regard to *E. coli,* 30.1 % of the 73 samples showed results above the detection limit (1.0 x 10^1 CFU/ml), namely four bottle samples (33.3 %) and 18 vending machine samples (29.5 %). Counts of *E. coli* positive (≥1.0 x 10^1 CFU/ml) samples were mainly in the range from 10^1 to 10^2 CFU/ml (Table 1).

Due to varying framing conditions and evaluation criteria, comparisons with literature data on raw cow milk sold to consumers are hampered. For Switzerland, respective data have so far been lacking, but TVC median values of 2.1 x 10^3 and 6.0 x 10^3 CFU/ml were reported for bulk-tank milk (Stephan and Buehler, 2001; Zweifel et al., 2006). In a Polish study investigating raw milk sold to consumers, Pyz-Łukasik al. (2015) reported TVC between 9.2 x 10^4 and 3.6 x 10^7 CFU/ml and *E. coli* were detected in 12 (24.0 %) samples with counts ranging from 5.0 x 10^0 to 1.1 x 10^2 CFU/ml. Similarly, Kalmus et al. (2015) found TVC between 5.0 x 10^3 and 2.0 x 10^7 CFU/ml (median 5.4 x 10^5 CFU/ml) for raw milk from sales points in Estonia. Furthermore, some studies determined the proportion of samples exceeding certain TVC limits. In England, 19.3 % of 902 raw drinking milk samples exceeded 2.0 x 10^4 CFU/ml (Willis et al., 2017). For raw milk from vending machines, the proportion of non-compliance was 44.8 % (limit: 5.0 x 10^4 CFU/ml) in northern Italy (Giacometti et al., 2012a), 33.3 % (limit: 1.0 x 10^5 CFU/ml) in southern Italy (Tremonte et al., 2014), and 20.0 % (limit: 1.0 x 10^5 CFU/ml) in Lower Saxony (Brix and Thielke, 2016). In Switzerland, legislation defines a TVC limit of 8.0 x 10^4 CFU/ml for bulk-tank milk (VHyMP, 2005). Of the 73 raw milk samples examined in the present study, 31.5 % exceeded this limit, namely one bottle sample (8.3 %) and 22 vending machine samples (36.1 %).

Striking were in the present study the TVC differences between bottle and vending machine samples: TVC results for raw milk from vending machines were clearly higher and showed a more heterogeneous distribution than those from pre-filled bottles (Table 1). However, a larger number of samples, especially in terms of pre-filled bottles, must be investigated for a final assessment. The microbial quality of raw milk may be influenced by a variety of factors (including hygiene during milk harvesting and milk management), but three aspects are of special interest in terms of vending machines: the chilling of the milk, the duration of milk storage, and the cleaning and disinfection (Brix and Thielke, 2016). Provided that the self-controlled chilling system of the machine works correctly and the milk is replaced as required on a daily basis, ensuring correct cleaning and disinfection according to the manufacturers' instructions can pose a challenge. Adequate procedures and frequencies must be warranted for all parts of the machine, but in particular for those parts with direct milk contact. In terms of the examined vending machines, the correct maintenance of the closed flushing system (not requiring a separate water connection) is of special importance.

Coagulase-positive staphylococci (CPS) were used as indicators for *S. aureus.* Of the 73 raw milk samples sold directly from farms to consumers, 30.1 % showed CPS results above the detection limit (1.0 x 10^1 CFU/ml), namely five bottle samples (41.7 %) and 17 vending machine samples (27.9 %). Counts of all CPS positive (≥1.0 x 10^1 CFU/ml) samples were <10^4 CFU/ml (Table 1) and growth has not to be expected in raw milk due to the competitive bacterial flora. Because of the restricted number of (CPS-positive) samples, an assessment of differences in CPS results between bottle and vending machine samples is not possible. Comparable literature data for CPS in raw milk sold to consumers are hardly available. For Switzerland, respective data have so far been lacking. But, Zweifel et al. (2006) reported that about 50 % of bulk-tank milk samples were CPS-positive with counts comparable to those reported in the present study. In England, 22.4 % of 902 raw drinking milk samples were CPS-positive with counts in the range from 2.0 x 10^1 to 1.0 x 10^4 CFU/ml and three (0.3 %) additional CPS-positive samples exceeded 1.0 x 10^4 CFU/ml (Willis et al., 2017).

Foodborne pathogens

In addition of being a commensal colonizer and being involved in infections, *S. aureus* might also cause staphylococcal food poisoning (SFP) in humans (Le Loir et al., 2003; Becker et al., 2007). SFP results from ingestion of heat-stable staphylococcal enterotoxins (SEs) and it is characterized by nausea, emesis, abdominal cramps, and diarrhea. In particular, the so-called classical enterotoxins (SEA–

TABLE 1: *Total viable counts (TVC), Escherichia (E.) coli, and coagulase-positive staphylococci (CPS) results for raw milk sold in pre-filled bottles (n = 12) and for raw milk sold from vending machines (n = 61) at farm level.*

Micro-organisms	Samples	No. (%) of results at different ranges (CFU/ml)[a]						
		<10^1	10^1–10^2	10^2–10^3	10^3–10^4	10^4–10^5	10^5–10^6	10^6–10^7
TVC	Bottle	–	–	1 (8.3 %)	8 (66.7 %)	2 (16.7 %)	1 (8.3 %)	–
	Machine	–	–	1 (1.6 %)	15 (24.6 %)	24 (39.3 %)	14 (23.0 %)	7 (11.5 %)
	Total	–	–	2 (2.7 %)	23 (31.5 %)	26 (35.6 %)	15 (20.5 %)	7 (9.6 %)
E. coli	Bottle	8 (66.7 %)	3 (25.0 %)	1 (8.3 %)	–	–	–	–
	Machine	43 (70.5 %)	12 (19.7 %)	4 (6.6 %)	2 (3.3 %)	–	–	–
	Total	51 (69.9 %)	15 (20.5 %)	5 (6.8 %)	2 (2.7 %)	–	–	–
CPS	Bottle	7 (58.3 %)	4 (33.3 %)	1 (8.3 %)	–	–	–	–
	Machine	44 (72.1 %)	5 (8.2 %)	10 (16.4 %)	2 (3.3 %)	–	–	–
	Total	51 (69.9 %)	9 (12.3 %)	11 (15.1 %)	2 (2.7 %)	–	–	–

[a]: Detection limit at 1.0 x 10^2 CFU/ml for TVC and at 1.0 x 10^1 CFU/ml for *E. coli* and CPS.

SEE) are associated with SFP outbreaks. In the present study, 22 *S. aureus* isolates obtained from raw milk sold directly from farms to consumers (five from pre-filled bottles, 17 from raw milk vending machines) were further characterized by DNA microarray profiling (Table 2). The 22 isolates were assigned to seven clonal complexes (CC), namely CC1, CC8, CC20, CC45, CC97, CC398, and CC705. The majority (54.5 %) belonged to CC705, followed by CC8 isolates (18.2 %). Eighteen isolates harbored genes for SEs and enterotoxin-like proteins. Genes for classical SEs were found in two CC8 isolates (*sea/sed* or *sed* only), two CC705 isolates *(sec),* and the CC45 isolate *(sec)*. The three *sec*-harboring isolates were also positive for *tst1* encoding the toxic shock syndrome toxin 1 (TSST-1), supporting the described combined SEC and TSST-1 production (Kenny et al., 1993; Stephan et al., 2001). Comparable literature data for enterotoxigenic *S. aureus* in raw milk sold to consumers have so far been lacking.

On the other hand, all 73 raw milk samples sold directly from farms to consumers tested negative for *Campylobacter, L. monocytogenes,* and Shiga toxin genes. However, it must be considered that these and other foodborne pathogens have been isolated from raw milk sold to consumers in other countries, even though detection rates were generally low. In particular, an Italian study comprising 60'907 raw milk samples from 1'239 vending machines in seven regions found *C. jejuni, E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* in 53, 24 83, and 18 samples, respectively (Giacometti et al., 2013). Similar results for raw milk from vending machines were also reported in other Italian studies, albeit on a smaller scale and mainly for certain regions (Giacometti et al., 2012a; Bianchi et al., 2013; Bertasi et al., 2016). *Campylobacter, L. monocytogenes,* and Shiga toxin-producing *E. coli* were occasionally also present in raw drinking milk on retail sale in England (Willis et al., 2017) and in raw milk from vending machines in Lower Saxony (Brix and Thielke, 2016). Furthermore, *L. monocytogenes* were also found in raw milk sold to consumers in Poland (Pyz-Łukasik al., 2015) and bottled raw milk in Finland (Castro et al., 2017).

Antibiotic resistance

With regard to antibiotic resistance, *S. aureus* isolates (DNA microarray assay) and *E. coli* isolates (disk diffusion tests) were further investigated. Of the 22 *S. aureus* isolates, 72.7 % were negative for the examined resistance-associated genes. The distribution of resistance-associated genes among the six positive isolates is shown in Table 2. Interestingly, none of the 22 *S. aureus* isolates was positive for *mecA* (or *mecC*) encoding methicillin resistance. Of the 22 *E. coli* isolates, 77.3 % were sensitive to the tested antibiotics. However, one isolate showed resistance to ampicillin, kanamycin, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline, one isolate to kanamycin, streptomycin, and tetracycline, one isolate to ampicillin, and two isolates to streptomycin. A current challenge involving not only the health care system but also the general community, the environment, animals, and food products are ESBL-producing Enterobacteriaceae (Guenther et al., 2011; Seiffert et al., 2013). Applying selective methods, no ESBL-producing Enterobacteriaceae were detected among the 73 examined raw milk samples sold directly from farms to consumers, which is in agreement with Swiss data from bulk-tank milk (Geser et al., 2012).

TABLE 2: *Characterization of 22 Staphylococcus aureus isolates obtained from raw milk sold directly from farms to consumers: assigned clonal complexes, presence of genes encoding staphylococcal enterotoxins (SEs), enterotoxin-like proteins, toxic shock syndrome toxin (TST), and antimicrobial resistance.*

Clonal complex	No. of isolates	Genes encoding SEs, enterotoxin-like proteins, and TST[a,b]	Resistance-associated genes[a,c]
CC1	1	*seh* (1)	–
CC8	4	*sea/sed/sej/ser* (1), *sed/sej/ser* (1)	blaZ/I/R/cat/ccrA1/B2 (1), ccrA2/B2 (3)
CC20	2	*egc* (2)	–
CC45	1	*sec/sel/egc/tst1* (1)	–
CC97	1	–	ermB (1)
CC398	1	–	tetM (1)
CC705	12	*sec/sel/egc*/ORF CM14/*tst*1 (2), *egc*/ORF CM14 (10)	–

a: The number of positive isolates is indicated in brackets.
b: *sea, sec,* and *sed* encoding classical SEA, SEC, and SED; *egc,* enterotoxin gene cluster; ORF CM14, enterotoxin-like protein; *tst1,* toxic shock syndrome toxin 1 (CC45: human variant; CC705: bovine variant).
c: *blaZ/R/I,* ß-lactamse gene/repressor/regulatory protein; *cat,* chloramphenicol acetyltransferase; *ccrA1/A2/B2,* cassette chromosome recombinase genes A1/A2/B2; *ermB,* rRNA methyltransferase associated with macrolide/ lincosamide resistance; *tetM,* ribosomal protection protein associated with tetracycline resistance.

Conclusions

This study generated initial baseline data on the microbiological quality of raw cow milk sold directly from farms to consumers in Switzerland. Results showed that there is potential for improvement with regard to TVC (considerable proportion $>10^5$ CFU/ml), *E. coli* (detection rate: 30.1 %) and CPS (detection rate: 30.1 %). TVC results for raw milk from vending machines (about a third $>10^5$ CFU/ml) were thereby clearly higher than those from pre-filled bottles. In this context, the importance of ensuring correct cleaning and disinfection procedures of vending machines must be emphasized. Moreover, the implementation of an official monitoring program assessing the microbiological quality and safety of raw milk from vending machines is recommended. With regard to foodborne pathogens in raw milk sold directly from farms to consumers, *S. aureus* harboring SE genes (including *sea, sec,* and *sed*) were detected. A favorable situation seems currently given in Switzerland in view of *Campylobacter, L. monocytogenes,* Shiga toxin-producing *E. coli,* and antibiotic-resistant bacteria in raw milk marketed at farm level. But because the occurrence of foodborne pathogens can never be ruled out, raw milk should always be properly heated before consumption.

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Conflict of interest

The authors declare no potential conflict of interest.

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Address of corresponding author:
Prof. Dr. Dr. h.c. Roger Stephan
Institute for Food Safety and Hygiene
Vetsuisse Faculty University of Zurich
Winterthurerstrasse 272
CH-8057 Zurich
Switzerland
roger.stephan@uzh.ch