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Monitoring the microbial status of raw and pasteurized milk from vending machines in Brandenburg, Germany

Überwachung des mikrobiellen Status von Rohmilch und pasteurisierter Milch aus Milchtankstellen in Brandenburg, Deutschland

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Summary

In Germany milk vending machines offering raw or pasteurized milk, are an increasingly popular form of milk marketing for direct sellers. The number of milk filling stations in the state of Brandenburg has almost tripled in 2017. Since September 2017, more than 100 raw and pasteurized milk samples from 18 milk filling stations have been examined. Varying microbial counts for total aerobic and psychrotrophic count and *Enterobacteriaceae* were observed during monitoring, indicating a wide quality spectrum of milk from vending machines. Approximately one third of all samples exceeded at least one microbial reference value. In order to assess health risks under the assumption of direct consumption, the raw milk samples were additionally tested for typical food-pathogens. Pathogens were detected by PCR in about 29 % of these samples. The dominant microorganism flora was identified by MALDI-TOF mass spectrometry from the total bacterial count and discussed with the respective operational process conditions of production as well as cleaning and disinfection. The resulting multi-stage controls showed above all hygienic weak points for the participating farms, e. g. in the milking area, by a probable interruption of the cold chain and especially in the implementation of established cleaning and disinfection procedures of the milk pipes/storage tanks and milk dispensers. Based on these results, operation-based corrective measures were proposed to optimize the organizational and hygienic working methods and processes.

Keywords: milk vending machines, quality control, consumer protection, hygiene monitoring

Zusammenfassung

In Deutschland sind Milchautomaten mit Roh- oder pasteurisierter Milch eine immer beliebtere Form der Milchvermarktung für Direktvermarkter. Die Zahl der Milchtankstellen im Land Brandenburg hat sich im Jahr 2017 fast verdreifacht. Seit September 2017 wurden mehr als 100 Roh- und pasteurisierte Milchproben von 18 Milchtankstellen untersucht. Während des Monitorings wurden sehr unterschiedliche mikrobielle Werte für die aerobe und psychrotrophe Gesamtkeimzahl und die Zahl der *Enterobacteriaceae* beobachtet, was auf ein breites Qualitätsspektrum der Automatenmilch hindeutet. Etwa ein Drittel aller Proben überschritt dabei mindestens einen mikrobiologischen Referenzwert. Um die Gesundheitsrisiken unter der Annahme eines Direktverzehrs zu beurteilen, wurden die Rohmilchproben zusätzlich auf typische lebensmittelassoziierte Krankheitserreger getestet. In 29 % dieser Proben wurden Krankheitserreger per PCR nachgewiesen. Die dominante Mikroorganismenflora wurde aus der Gesamtkeimzahl mittels MALDI-TOF-Massenspektrometrie identifiziert und in Verbindung mit den jeweiligen betrieblichen Prozessbedingungen der Herstellung sowie der Reinigung und Desinfektion diskutiert. Daraus abgeleitete Stufenkontrollen zeigten für die beteiligten Betriebe vorwiegend hygienische Schwachstellen z.B. im Melkbereich, durch eine mögliche Unterbrechung der Kühlkette und vor allem in der Umsetzung etablierter Reinigungs- und Desinfektionsmaßnahmen der Milchleitungen/-lagertanks und Milchabgabeautomaten auf. Basierend auf diesen Ergebnissen wurden betriebsbezogene Korrekturmaßnahmen zur Optimierung der organisatorischen und hygienischen Arbeitsweisen und Arbeitsabläufe vorgeschlagen.

Schlüsselwörter: Milchautomaten, Qualitätskontrolle, Verbraucherschutz, Hygieneüberwachung

Introduction

Milk and dairy products are basic components of human nutrition and a source of proteins, vitamins and minerals. Therefore, the consumption of drinking milk or dairy products contributes to a healthy diet (Huth et al., 2006). In recent years, the desire for genuine taste and regional production has become increasingly important for a growing group of buyers. In the milk sector, small farms are responding to this demand by selling raw milk and heat-treated fresh milk directly as well as by dispensing them via automatic vending systems.

The European Regulation (EC) No. 853/2004 for Hygiene rules for food of animal origin permits the use of raw milk for direct human consumption. However, member states may maintain or introduce national provisions prohibiting or restricting the placing on the market in their territory of raw milk intended for direct human consumption (Article 10(8) of Chapter IV).

In Germany, ready-to-consume milk may only be offered commercially as certified-raw or heat-treated milk (Figure 1). Certified-raw milk is subject to strict hygienic limits (BMJV, 2018) and may be sold on the market as packaged raw milk. Heat-treated milk needs to be heated for a defined temperature-time combination, as for example by pasteurization, and must not be contaminated during the process ((EC) No. 852/2004; (EC) No. 853/2004).

On the other hand, according to §17 of the Animal Food Hygiene Ordinance (BMJV, 2018), selling raw milk directly from dairy farms by self-service automatic vending machines to the consumer (Figure 1) is subject to specific conditions. In particular, the raw milk drawn from vending machines must not be older than one day. The raw milk levy has to be reported to the competent authority and an indication „Raw milk - has to be boiled before consumption“ has to be placed clearly visible and legible at the tapping point. In contrast to raw milk filling stations, which have to be located directly on the milk production holding, milk vending machines offering pasteurized milk can be installed anywhere. This considerably expands the customer base and helps to keep regional customers in times of low milk prices.

Milk vending machines are synonymous for short supply chains, because there are no intermediaries between the producer and the consumer and all actors are geographically close to each other. This is in line with the customers‘ growing desire for regional products. The sale of raw and pasteurized milk for human consumption by self-service vending machines has been increasing in Germany for a few years. In the state of Brandenburg, the number of vending machines has almost tripled in the last two years to a number of about 20. In contrast, there is no dairy farm left producing certified-raw milk.

Milk from vending machines, especially raw milk, is associated with original taste, regional origin, enhanced nutritional qualities and health benefits. Though scientific data to substantiate these claims are limited, it cannot be excluded that raw milk is consumed by the final customer without any heat treatment. According to EU and German regulations ((EC) No. 178/2002; BMJV, 2018) raw milk from vending machines is not intended for direct consumption and must meet food safety requirements, but concrete limit values for hygiene parameters are not mentioned. Solely, the microbial count in the delivery milk for dairies is specified ((EC) No. 853/2004). Even for pasteurized milk, according to the EU Regulation (EC) No. 2073/2005, the sole criterion applying to the process hygiene criteria for milk and dairy products is the number of *Enterobacteriaceae* at the end of the products manufacturing process (n=5; c=0; m=M=10 colony-forming units per milliliter (cfu/ml)).

In order to collect data on milk quality, a monitoring of raw and pasteurized milk from vending machines in Brandenburg was carried out with regard to the hygiene parameters. To identify hazards for consumers the presence of pathogens in raw milk was additionally determined.

Material and Methods

From September 2017 to February 2018, 133 milk samples in Brandenburg, Germany, were analyzed, consisting of 48 milk samples from seven vending machines offering pasteurized milk in e. g. supermarkets and 85 raw milk samples from eleven vending machines located directly on the dairy farms. Each sample was filled in a sterile 1-liter glass bottle. The temperature was read off at the vending machines and indicated 2 to 5°C in all instances. The samples were kept at 4°C and analyzed within 24 hours after sampling. In most cases there was an indication of the shelf life at the vending machine (3–5 days after drawing), but not of the actual filling date.

The analysis of hygiene parameters consisted of: (I) the determination of the total aerobic count (TAC) according to § 64 LFGB L 01.00-5 German Food, Commodities and Feed Code (BVL, 2005), (II) the determination of the total psychrotrophic count (TPC) according to § 64 LFGB L 01.00-42 to 01.00-52 (BVL, 2005), (III) the determination of the *Enterobacteriaceae* count according to ISO 21528-2 (ISO, 2017) and (IV) the determination of the number of coagulase-positive staphylococci according to ISO 6888-2 (ISO, 2003). In addition, all raw milk samples were tested for inhibitory substances according to § 64 LFGB L 01.01-5 (BVL; 2005).

In order to assess a health risk under the assumption of direct consumption, the raw milk samples were examined for the presence of typical food-pathogens. For detection of *Salmonella,* verotoxigenic *Escherichia coli* (VTEC), *Campylobacter* and *Listeria* spp./*Listeria monocytogenes* real-time PCR assays were carried out using qPCR kits according to the manufacturer‘s instructions on a Light Cycler 480 instrument (Roche Diagnostics GmbH, Mannheim, Germany). The analyses were done according to § 64 LFGB L 00.00-98, L 00.00-95 (V), L 00.00-96 (V) (BVL, 2005) and ISO 13136 (ISO, 2013) with minor modifications using foodproof® Kits from Biotecon Diagnostics, Potsdam, Germany (*Salmonella* Detection Kit, STEC Screening LyoKit, *Listeria* Genus Detection Kit, *Listeria monocytogenes* Detection Kit, *Campylobacter* Quantification Kit).

The colonies from the psychrotrophic bacterial count from the evaluable range were examined and the microorganisms were identified to determine the microbial flora. For identification, Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) was used. Starting from the TPC, at least ten single colonies were isolated on TSYE-Agar (Oxoid c/o Thermo Fisher Diagnostics GmbH, Wesel, Germany). A single colony of each isolate was spotted directly on a target plate, overlaid with 1 µl of 70% formic acid and air-dried (extraction-on-target). All spots were overlaid with matrix solution (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile

Journal of Food Safety and Food Quality 70, Heft 6 (2019), Seiten 157–194

TABLE 1: *Results of hygiene parameters of raw milk samples from vending machines in Brandenburg, Germany, probed between September 2017 and February 2018.*

Parameter	n (number of samples)	Samples microbial count > reference value	Mean of microbial content of all 85 samples [cfu/ml]	Median of microbial content of all 85 samples [cfu/ml]	Reference value	Reference
Total aerobic count	85	32 / 38%	3 400 000	51 000	100 000	(EC) No. 853/2004
Total psychrotrophic count	85	21 / 25%	2 700 000	21 000	100 000	internal
Enterobacteriaceae	85	13 / 15%	380 000	570	5000	internal
Coagulase-positive staphylococci	85	1 / 1%	<20	<10	500	internal

and 2.5 % trifluoroacetic acid). Each spot was measured using a microflex LT mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany). Mass spectra were analyzed using the FlexControl 3.4 software and the MALDI Biotyper databases BDAL (Revision D; 2017), Listeria (Version 1; 2007), SR Library (Version 1; 2016) and Filamentous Fungi (Version 1; 2015).

TABLE 2: *Results of pathogen parameters of raw milk samples from vending machines in Brandenburg, Germany, probed between September 2017 and February 2018.*

Parameter	n (number of samples)	Positive results (number of samples / in %)	Reference
Campylobacter	85	9 / 11%	§ 64 LFGB L 00.00-96 (V)
Listeria spp.	85	19 / 22%	§ 64 LFGB L 00.00-95 (V)
Listeria monocytogenes	85	2 / 2%	§ 64 LFGB L 00.00-95 (V)
Salmonella	85	1 / 1%	§ 64 LFGB L 00.00-98
VTEC	85	13 / 15%	ISO 13136

Results

Raw milk vending machines

In this study, 85 raw milk samples from eleven vending machines were analyzed with seven to eight sample collections per machine. For the total aerobic count (TAC), an assessment value of 1 * 10^5 cfu/ml was determined ((EC) No. 853/2004), analogous to the standards for the milk quality of delivered milk. The same value was set for the TPC. These values provide information on milk quality, milking hygiene, cold chain and process hygiene as well as the psychrotrophic potential. The assessment values for the number of *Enterobacteriaceae* and the number of coagulase-positive staphylococci were set to 5000 and 500 cfu/ml, respectively, as a criterion for the hygiene of milking, process and staff, and an indicator of successful cleaning procedures, disinfection and animal health.

In 32 of 85 samples, the reference value of the TAC was exceeded. The median microbial content of all samples was 5.1 * 10^4 cfu/ml for the TAC with a sample range between 4 * 10^3 and 3 * 10^8 cfu/ml (Table 1). In the TPC, a quarter of all samples exceeded the reference value of 1 * 10^5 cfu/ml, but the median value was 2.1 * 10^4 cfu/ml. The value of *Enterobacteriaceae* was conspicuous in 15 % of the samples, with a median of 570 cfu/ml. Only one sample exceeded the reference value for coagulase-positive staphylococci. All raw milk samples were negative for inhibitory substances.

Five out of eleven raw milk vending machines showed lower values than pre-defined assessment levels for the tested parameters. Seven vending machines were below reference values for *Enterobacteriaceae* (Figure 2). While five of the vending machines exceeded the reference values for the TAC and TPC by one order of magnitude, one even exceeded them by about three orders of magnitudes.

In order to assess health risks assuming direct consumption, raw milk samples were additionally tested for typical food-pathogens by PCR. *Campylobacter* was detected in nine samples. In 13 samples, VTEC-specific gene sequences *(stx1, stx2)* were detected. *Listeria* spp. was detected in 19 of the tested raw milk samples. Among the known *Listeria* species, *L. monocytogenes* is by far the most important human pathogenic species (Robert-Koch-Institut, 2015). Therefore, the samples were additionally tested for *Listeria monocytogenes*. In two samples, positive results for this pathogen were obtained. Salmonella was positively tested in only one sample (Table 2).

Pasteurized milk vending machines

In this study, 48 pasteurized milk samples from seven vending machines were analyzed with six to seven collections per machine. For the TAC an assessment value of 3 * 10^4 cfu/ml was determined, for the TPC a value of 5 * 10^4 cfu/ml, due to the former German Milk Ordinance (BMG, 2000). The maximum value for the number of *Enterobacteriaceae* ((EC) No. 2073/2005) and the number of coagulase-positive staphylococci were both set to 10 cfu/ml, as criterion for process and staff hygiene.

In 17 of 48 samples, the TAC exceeded the reference value and in twelve samples, the reference value for the TPC was surpassed (Table 3). The median values of these

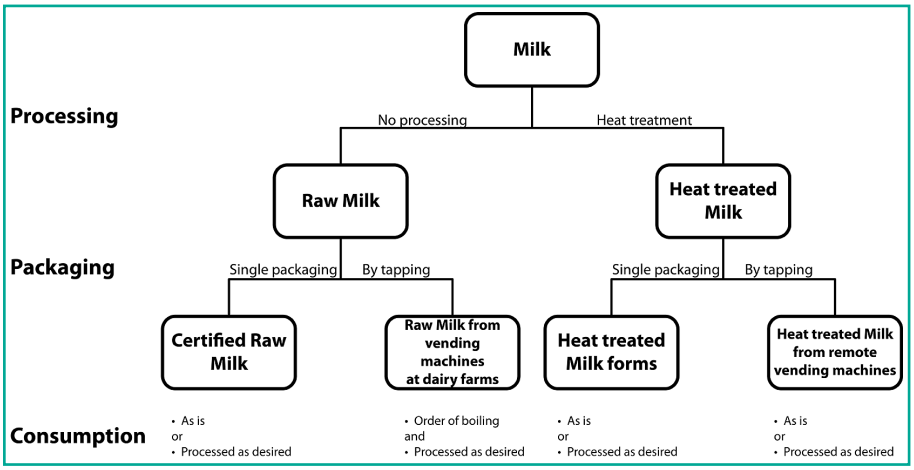

FIGURE 1: *Schematic overview of the forms of distributed milk in Germany.*

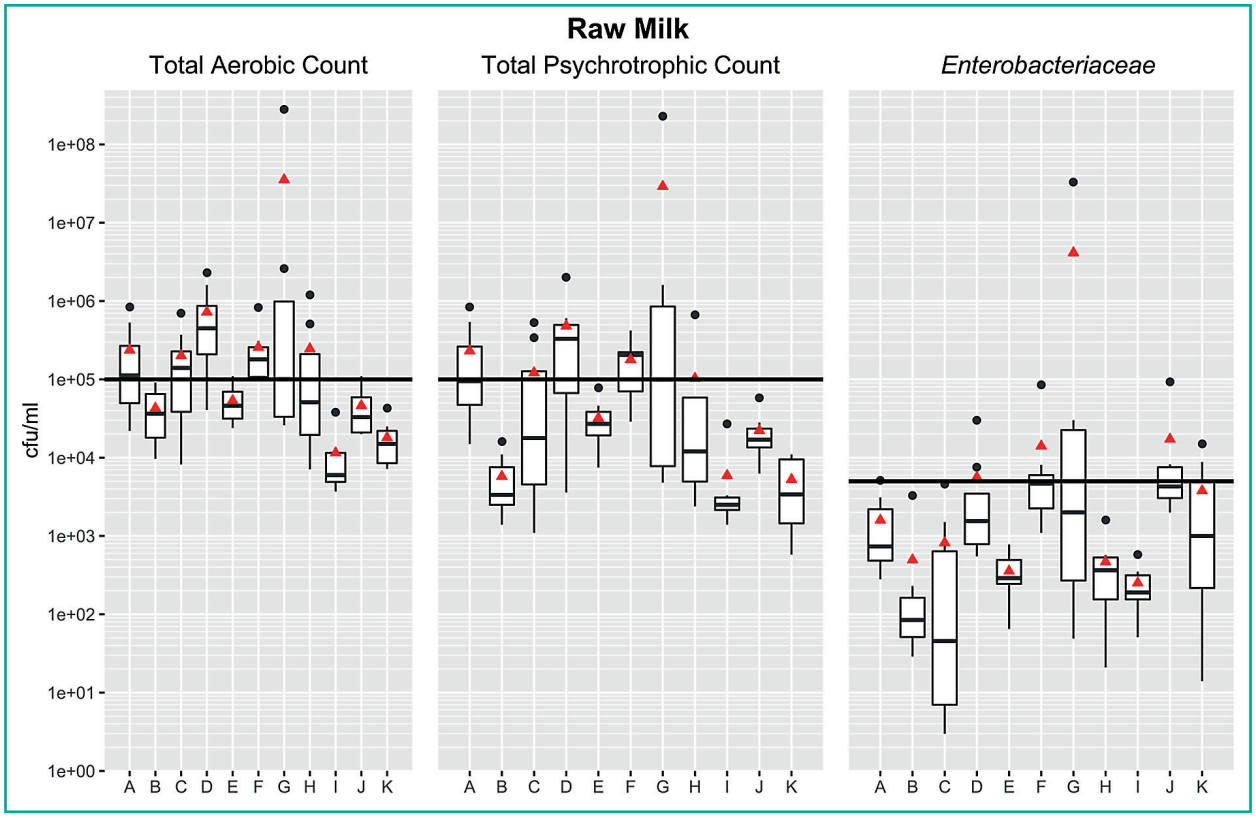

FIGURE 2: *Boxplots of the hygiene parameter data of eleven vending machines offering raw milk in Brandenburg, Germany. For each machine individual boxplots for values of total aerobic count, total psychrotrophic count and the number of Enterobactericeae are displayed. Red triangles represent mean values. Black lines indicate reference values (1 * 10⁵ cfu/ml for total aerobic and psychrotrophic count; 5000 cfu/ml for Enterobacteriaceae).*

two parameters were around 1 * 10^4 and 6500 cfu/ml, respectively, but the range for lowest and highest cell counts was between 100 and 1 * 10^7 cfu/ml. Two thirds of all pasteurized milk samples showed high values for *Enterobacteriaceae,* but all samples were below the assessment value regarding the number of coagulase-positive staphylococci (Table 3). Only nine of the 48 samples were below the target values for all hygiene parameters at the same time.

Analyzing the individual vending machines revealed that only three out of seven machines were below the assessment value of 3 * 10^4 cfu/ml in the TAC and five below the limit value of 5 * 10^4 cfu/ml for the total psychrotrophic count (Figure 3). None of the vending machines remained averagely below the limit for *Enterobacteriaceae* of 10 cfu/ml. Two vending machines were above the value for Enterobacteria in each sample. However, value fluctuations in all vending machines were quite high and included several orders of magnitude (Figure 3).

Identification of the dominant microbiological flora

Exemplarily, the identifications of the microbiological flora from five selected vending machines by MALDI-TOF MS are shown in Table 4 (remaining data not shown). Raw milk vending machine B showed a low TPC (see also Figure 2) and a broad spectrum of different genera at identification level. These include besides Gram-positive microorganisms such as staphylococci or lactic acid bacteria also yeasts and Gram-negative germs. The value for the TPC of vending machine F was increased compared to the reference value, with a dominant flora containing lactic acid bacteria, *Enterobacteriaceae, Pseudomonas* and a broad spectrum of other genera. The raw milk vending machine G showed a significant, considerable exceedance in the mean value of the TPC (Figure 2). Here, most commonly lactic acid bacteria and enterobacteria were identified. Vending machine M, offering pasteurized milk, showed a 20-fold increase of the mean psychrotrophic count

TABLE 3: *Results of hygiene parameters of pasteurized milk samples from vending machines in Brandenburg, Germany, probed between September 2017 and February 2018.*

Parameter	n (number of samples)	Samples microbial count > reference value	Mean of microbial content of all 48 samples [cfu/ml]	Median of microbial content of all 48 samples [cfu/ml]	Reference value	Reference
Total aerobic count	48	17 / 35%	250 000	9900	30 000	former German Milk Ordinance
Total psychrotrophic count	48	12 / 25%	235 000	6450	50 000	former German Milk Ordinance
Enterobacteriaceae	48	31 / 65%	1900	125	10	(EC) No. 2073/2005
Coagulase-positive staphylococci	48	0 / 0%	0	0	10	internal

Journal of Food Safety and Food Quality 70, Heft 6 (2019), Seiten 157–194

compared to the reference value. Almost exclusively cells of the genus *Pseudomonas* were identified here. Pasteurized milk vending machine Q showed a mean value just below the reference value and a main spectrum of lactic acid bacteria, *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* (Table 4).

Discussion

The concept of regional production, sales and purchase and the demand of customers for natural, unprocessed und unpacked food result in an increased request for raw or pasteurized milk from vending machines.

Raw milk vending machines must be labelled with „Raw milk, boil before consumption". However, reports of outbreaks of food infections associated with raw milk consumption suggest that consumers do not always follow this advice. The Federal Institute for Risk Assessment points out that the consumption of raw milk is associated with health risks, as raw milk can be contaminated with pathogenic germs (BfR, 2016a). Focusing on *Campylobacter, Listeria monocytogenes,* VTEC and *Salmonella,* we tested all raw milk samples and detected pathogens in 29 % of it by PCR (Table 2). Depending on the state of health, immune status, age, etc., the risk of infection may vary. Nevertheless, there seems to be a correlation between raw milk filling stations and the number of *Campylobacter* infections in Germany (BfR, 2016b). This assumption is supported by other European studies, which also indicate a link between raw milk for direct consumption and campylobacteriosis or VTEC infections (Marek & Pászto, 2017; Bertasi et al., 2016; Giacometti et al., 2015, Giacometti et al., 2017). In our study, in the vending machines with a positive detection of Campylobacter (overall 11 %) and VTEC (15 %), these pathogens were repeatedly detected in the raw milk during the sampling period. One can assume an increased risk of infection by direct consumption of raw milk.

About a third of the analyzed raw milk samples exceeded the reference value for the TAC and TPC, indicating problems with milking, process hygiene or the cold chain. In a study by Godic Torkar et al., 47 % of the collected raw milk samples from vending machines in Slovenia did not met the requirements set out in EU Regulation (EC) No. 853/2004 (Godic Torkar et al., 2017). Psychrotrophic microorganisms in particular are able to show high growth rates under cold storage conditions and develop into the dominant bacterial flora. They are able to produce heat-stable enzymes, which can spoil milk even when the microorganisms are inactivated. Thus, psychrotrophic microorganisms become the limiting factor for shelf life (Oliveira et al., 2015; Moyer & Morita, 2007). With a bacterial count of $1 * 10^5$ cfu/ml, an incipient spoilage cannot be excluded (Ledenbach & Marshall, 2009). This number was exceeded in app. 25 % of all tested raw milk samples. Furthermore, bacterial counts regarding *Enterobacteriaceae* were detectable in high values in 13 samples. This group is a process hygiene indicator and is clearly related to milking process hygiene. Coagulase-positive staphylococci exceeded the reference value only once, which indicates good animal health and personal hygiene in general.

A hygienically impeccable raw milk contains a low total bacterial count and this consists mainly of Gram-positive microorganisms such as micrococci, streptococci and other lactic acid bacteria, etc. and typically 10–20 % Gram-negative germs. These often have a cold-tolerant potential and limit shelf life even under refrigerating conditions. The identification of the dominant microorganism flora from the evaluable range of the TPC showed very diverse results, depending on the vending machine. Three raw milk vending machines will be discussed here exemplarily, pointing out various situations.

Vending machine B had a low TPC throughout the entire sampling period and contained a broad spectrum of different genera identified, including Gram-positive microorganisms, yeast and Gram-negatives (Figure 2, Table 4). This machine is clearly a positive example and shows that the quality of the milk offered can be very good, even over a longer sampling period. The psychrotrophic value of vending machine F exceeded the amount of the reference value by two-fold. Mainly lactic acid bacteria, *Enterobacteriaceae, Pseudomonas* but also a broad spectrum of other genera were identified (Figure 2, Table 4). These results indicate a lack of process hygiene and defects in the established cleaning and disinfection processes carried out on the farm or the vending machine. Vending machine G shows a considerable exceedance over two orders of magnitude in the TPC (Figure 2). Here, mainly lactic acid bacteria and Enterobacteria were identified (Table 4). This indicates inadequate milking hygiene and also an interruption of the cold chain, as many lactic acid bacteria could still be identified in the evaluable range of these high bacterial counts, and usually lactic acid bacteria do

TABLE 4: *Results of identification via MALDI-TOF-MS. Isolates were taken from the total psychrotrophic count of selected vending machines B, F, G, M, Q. Results in % for Gram-positives, Gram-negatives, yeasts and filamentous fungi. As examples of these groups, only Genera are listed. Remaining 11% for vending machine B were identified as yeast and filamentous fungi.*

Vending machine	Type of milk offered	rounded mean of total psychrotropic count [cfu/ml]	Gram-positive Micro-organisms	Examples	Gram-negative Micro-organisms	Examples
B	Raw milk	5700	45%	Aerococcus, Enterococcus, Lactococcus, Leuconostoc, Staphylococcus, Streptococcus	44%	Acinetobacter, Chryseobacterium, Escherichia, Pseudomonas, Serratia, Yersinia
F	Raw milk	179 000	16%	Carnobacterium, Lactococcus, Leuconostoc, Streptococcus	84%	Acinetobacter, Chryseobacterium, Delftia, Klebsiella, Pseudomonas, Serratia
G	Raw milk	29 000 000	39%	Lactobacillus, Lactococcus, Staphylococcus, Streptococcus	61%	Acinetobacter, Citrobacter, Enterobacter, Klebsiella, Pseudomonas
M	Pasteurized milk	1 140 000	1%	Lactococcus	99%	Acinetobacter, Pseudomonas, Serratia
Q	Pasteurized milk	41 400	28%	*Carnobacterium, Lactobacillus, Lactococcus, Leuconostoc*	72%	*Acinetobacter, Hafnia, Pseudomonas, Serratia*

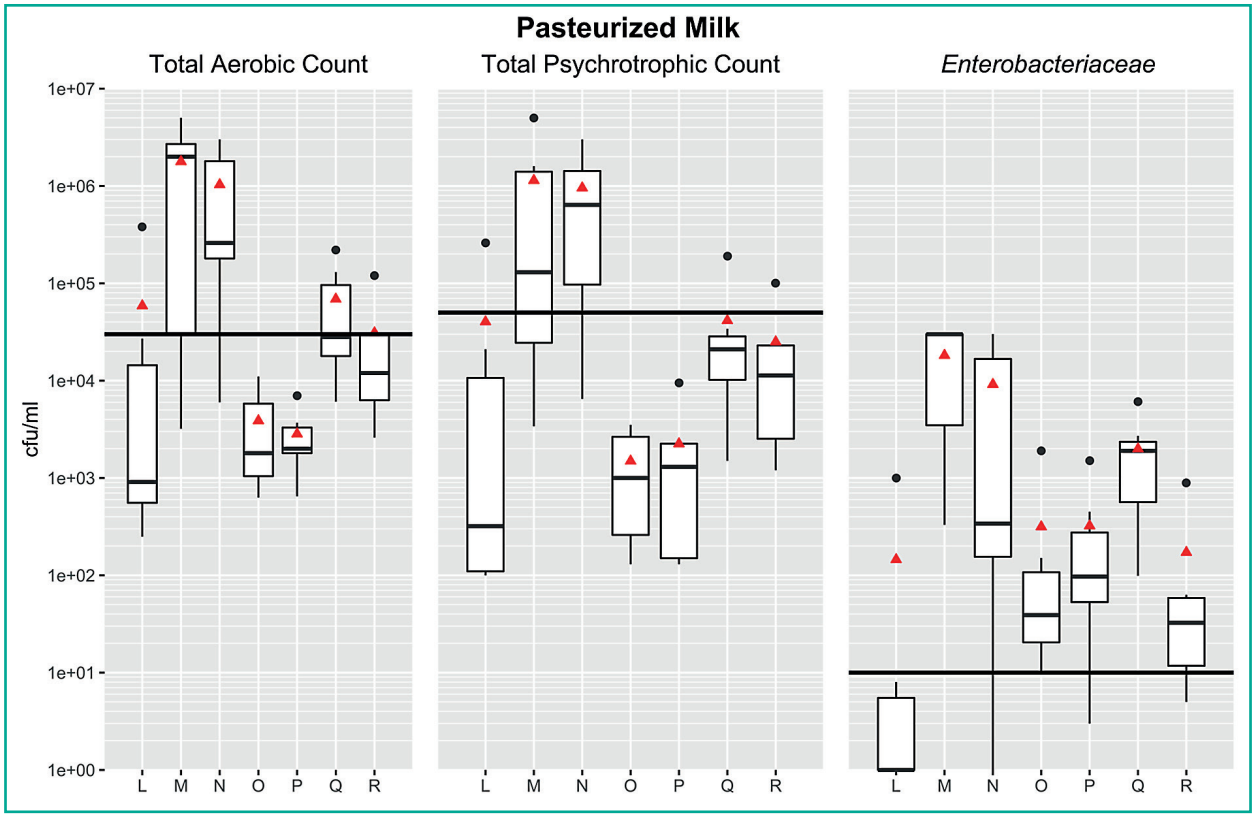

FIGURE 3: *Boxplots of the hygiene parameter data of seven vending machines offering pasteurized milk in Brandenburg, Germany. For each machine individual boxplots for values of total aerobic count, total psychrotrophic count and the number of Enterobactericeae are displayed. Red triangles represent mean values. Black lines indicate reference values (3 * 10⁴ cfu/ml for total aerobic count, 5 * 10⁴ cfu/ml for total psychrotrophic count, 10 cfu/ml for Enterobacteriaceae).*

not multiply at chilled temperatures (Müller, 1986). Remaining stocks of older milk, e. g. in the bulk tank, could also lead to this result.

In this analysis, 25–35 % of the pasteurized milk samples had high values for the TAC and TPC and two thirds of all samples showed a too high content of *Enterobacteriaceae* (Table 3, Figure 3). For vending machine Q delivering pasteurized milk, the TAC and TPC are around the reference values and comprise a main spectrum of lactic acid bacteria and Gram-negatives, with a threefold exceed in the value of *Enterobacteriaceae* (Table 4, Figure 3). This indicates either insufficient heating during pasteurization or recontamination. However, the results of the alkaline phosphatase measurements exclude a lack of heating, so that the pasteurized milk was presumably recontaminated. A similar situation was observed at vending machine M, with very high levels of TAC, TPC and *Enterobactericeae* (Figure 3). Almost exclusively cells of the genus *Pseudomonas* were identified. This indicates insufficient cleaning and disinfection or exceeded milk storage time. *Pseudomonas* are considered the most common recontaminants in water-based systems and are capable of forming persistent biofilms (Oliveira et al., 2015). A study carried out by Angelidis et al. in 2016 showed that all examined pasteurized milk samples from vending machines in Greece met the quality standards of pasteurized milk (Angelidis et al., 2016).

A mean bacterial count of 3.4 * 10^6 cfu/ml of the raw milk samples and a mean count of *Enterobacteriaceae* of 3.8 * 10^5 cfu/ml led to step controls in cooperation with two dairy farms in Brandenburg. At both farms, the bacterial count of the milk increased by several orders of magnitude directly after pasteurization to filling from the vending machine. Therefore all steps from milking to the output of the raw and pasteurized milk in the corresponding vending machines were probed in the process and the following critical points have been uncovered:

It was shown that even with an acceptable initial situation and production hygiene a change in the composition of the dominant bacterial flora could be found. Inadequate cleaning of the milking equipment led to fat and protein residues on surfaces in contact with milk, while the milk sugar was rinsed out. This provides a nutrient enrichment for fat- and protein-splitting bacteria. In combination with a psychrotrophic potential, this leads to the enrichment of cold-tolerant contaminants on the milk-conducting surfaces, but also in the immediate environment. Primarily a Gram-negative flora with a tendency for biofilm formation and a psychrotrophic potential is manifested.

In the field of milk production, already the milking area must be sufficiently hygienic. Milking cups, milking installations, collecting hoses, pipeworks and tanks need to be cleaned sufficiently between successive milking operations. Inadequately implemented measures for teat pre-cleaning, intermediate cluster disinfection and general milking hygiene lead to a high entry of microorganisms into the raw milk. Step controls showed e. g. that despite of an intermediate disinfection in the teat cups with a peracetic acid-containing disinfectant, after 6 rounds of milking a total bacterial count of >3 * 10^5 cfu/swab was achieved, with a dominant Gram-positive flora (initial situation: TAC 260 cfu/swab). Influencing factors such as pulsation settings and the age of the teat liners also have a significant influence on the number of bacteria per swab, but were not recorded during the stage control.

Journal of Food Safety and Food Quality 70, Heft 6 (2019), Seiten 157–194

Furthermore, the flow paths must be considered. Long flow paths, unsuitable components for connections or incorrect installation of seals increase the risk of contamination and inadequate cleaning. One should pay attention to e. g. T-pieces, connecting pipes or O-rings of valves, as bacteria may settle there and biofilms may emerge. In the step controls, parts of the outlet valve of the raw milk tanks in contact with the product were unobjectionable in terms of microbial contamination, but the immediate surroundings of the connections (e. g. seals: TAC: >3 * 10^5 cfu/swab, *Enterobacteriaceae:* 1.8 * 10^4 cfu/swab; connecting threads: TVC: >3 * 10^5 cfu/swab, *Enterobacteriaceae:* 1.8 * 10^5 cfu/swab) form a very high contamination risk. These areas urgently need to be included in cleaning and disinfection. Remaining residual water after the cleaning process in the vending machine tanks for raw and pasteurized milk (e. g. TVC: >3 * 10^5 cfu/ml with 60 % Gram-negative bacteria, dominant *Pseudomonas* spp., *Enterobacteriaceae:* 2.6 * 10^5 cfu/ml) is conspicuous and very critical. Milk would also cause a significant increase in these germs during cold storage and significantly limit shelf life. Complete emptying and drying after cleaning and disinfection of the containers must be ensured. In the pasteurizer tank, the outlet was not the lowest point due to slight inclination of the tank, and therefore residual water could also be detected in the interior after cleaning. The tank wall of the middle ground area at the level of the stirrer showed a load of Gram-negative bacteria (TAC: 3700 cfu/ml, most notably *Pseudomonas* spp.).

Referred to the example just mentioned, in which a small production of pasteurized milk (300 L) is filled in a 5000 L collection tank in one of the probed dairy farms, another important point to state is the dimension of the used tanks. If these are significantly too large for a rather small quantity of product, problems can arise with tank cooling, since depending on the type of cooling, it can only be switched on after a certain amount of milk is added in order to avoid freezing. The time elapsed before the cooling system is switched on promotes the growth of the microorganisms present in the milk. In the example, the concentration of Gram-negative bacteria is sufficient to ensure a germ number enrichment of cold-adapted spoilage germs of 2–3 orders of magnitude in the pasteurized milk. A levelling of the tank to ensure the complete emptying of the rinsing water is a recommended measure. The correct execution of the cleaning procedure (temperature, time, concentration of cleaner) must also be ensured.

Additional components such as pumps and connecting tubes, which are temporarily installed for refilling/filling, prove to be essential influencing factors in recontamination. One major problem in this step check was a pump with its connecting tube (TAC: 6.3 * 10^4 cfu/swab, *Enterobacteriaceae:* 1.3 * 10^4 cfu/swab). Even with successful cleaning and disinfection after filling, the result of this measure is nullified by improper placement and storage of the pump and the tubes (e. g. tube ends lie on the floor, partly in puddles of water while the pump with the connectors is located in an environment highly contaminated with Enterobacteria (TAC: >3 * 10^5 cfu/ml, *Enterobacteriaceae:* 2.9 * 10^5 cfu/ml).

Another point is the type of milk vending machine. There are on-site cleaning systems designed for raw milk, as raw milk vending machines must be installed directly on the dairy farm. On the other hand, there are systems where the tank of the vending machine has to be taken to the dairy farm to be cleaned. These are intended for heat-treated milk, as the pasteurized milk vending machines do not necessarily have to be located on the milk production farm, but can also be installed in supermarkets, etc., where suitable cleaning can be difficult. Only in rare cases vending machine operators have access to hot water (or similar) directly on site in supermarkets, i.e. they have to bring hot water or tempered detergents to the vending machine. With a certain distance to the dairy farm, it can be difficult to keep the detergents at the recommended temperatures, e. g. when placed next to the new vending machine tank with the cooled milk during transport. Most vending machine manufacturers offer both systems, but vending machines with on-site cleaning are often used for pasteurized milk because they are cheaper to buy. This can lead to problems with proper cleaning and disinfection, which is another important point. Regardless of which system is used, the downstream storage and filling is likewise decisive for recontamination. Exemplarily, in a system rinsing water remains in the dispensing tube after cleaning. This rinsing water was highly contaminated in the step controls (TVC: >3 * 10^4 cfu/ml; 70 % Gram-negative bacteria) and causes recontamination despite repeated rinsing after milk filling. The passage of the raw milk through the vending machine thus led to an increase of microbial count by more than one order of magnitude. However, the manufacturer has already eliminated this source of contamination and no rinsing water remains in the filling pipe. It is important to distinguish between technical difficulties and those of staff. One of the technical difficulties in cleaning and disinfection is the type of machine mentioned above. The errors brought in by staff during cleaning and disinfection can be much more diverse. The wrong dosage of cleaning agents and the resulting incomplete cleaning, forgetting to clean small parts such as the filling spout, or the placement of parts of the machine on the floor that can later recontaminate the milk to a high degree. This can also be caused by remaining residual water in the rinsed vending machine tanks or the repeated use of a wiping cloth to clean parts of the machine.

Many of these critical points are now subject to DIN 10541, in which the general hygiene requirements for the design, materials and operation of the concerned equipment is laid down (DIN Deutsches Institut für Normung e. V., 2019). For example, all system components that are cleaned in a continuous process must be designed in a way that they can be cleaned and disinfected with suitable means. Dead volumes are to be avoided. All components of the system that cannot to be cleaned by the continuous cleaning process must be dismantled easily for cleaning and disinfection purposes.

Likely, this DIN will be the assessment standard for the official food control, but is currently not legally binding. Other European countries already implemented special regulations that are legally anchored. For example, Austria‘s Raw Milk Ordinance stipulates that raw milk may only be distributed via tested milk vending machines that ensure that raw milk does not undergo any hygienic impairment through the milk vending machine or its location (BMGF, 2006).

In 2007 the Italian Ministry of Health approved the sale and distribution of unpackaged raw milk via self-service vending machines. This distribution is regulated by an agreement between the state and the regions (GI, 2007; Bianchi et al., 2013), which also specifies set up and management of vending machines. For example, the machines must have a filling valve that interrupts the sale

of raw milk when the milk temperature exceeds 4 °C (Bianchi et al., 2013).

Due to the growing demand for unprocessed food, EU Regulation (EC) No. 853/2004 initially permits the sale of raw milk in the EU member states, but allows the possibility of national regulation. With the national implementation of this regulation, Germany created the „certified-raw milk" levy model. This enables sale of closed packaged ready-to-consume raw milk by certified farms. Furthermore, these farms as well as certified-raw milk are subject to stricter controls. With regard to the certified-raw milk this means a higher frequency of controls, more examination parameters and stricter limit values. As a result of the legal and economic costs only a few milk producers established certified-raw milk. The levy of raw milk directly from dairy farms by self-service automatic vending machines is permitted for registered farms and appears to be much more lucrative. This raw milk is not subject to any separate or stricter controls and can be distributed in bulk. In this case, farmers largely pass on their responsibility to minimize the risk of pathogens to the consumer. From a legal point of view, raw milk is not ready for human consumption and must therefore always be labeled „boil before consumption". With the establishment of automatic vending machines in Germany, the economic significance of this marketing has increased, as sales can be made around the clock.

In general, the distribution of ready-to-consume raw milk is a sensitive issue in European countries due to the pathogenic risk. In the national legislations of most Western European countries there are quite uniform orientations: In most of these countries it is not possible to sell ready-to-consume raw milk or only under special conditions. In Switzerland, only pasteurized milk may be distributed via milk vending machines (EDI, 2016). Since December 2008, vending machines in Italy need to indicate that raw milk must be boiled before consumption (GI, 2008; Giacometti et al., 2013). The sale of raw milk is banned in Scotland after a series of deaths in 1983 (FSS, 2007). In England, Wales and Northern Ireland sales via registered producers are permitted. These must undergo an extensive certification process and meet significantly higher hygiene standards (FSA, 2006a; FSA, 2006b; FSA, 2013; FSA, 2017). In order to communicate the risk of the presence of pathogens in raw milk, each bottle must be labeled with the warning „This product has not been heat-treated and may contain harmful organisms". However, retail trade is not possible. In Eastern European countries, the distribution of ready-to-consume raw milk is less regulated. In Estonia, sales on farmers' markets have been permitted for many years. The sale of raw milk via vending machines in grocery stores started there in 2012. Already in April 2013 there were 32 dairy farms with veterinary certificates which allowed the sale of raw milk in large quantities via retail outlets, e.g. vending machines, markets, grocery stores and other points of sale (Kalmus et al., 2015). Slovenia was one of the first countries to establish a large number of raw milk dispensers. Raw milk distribution via vending machines has been possible throughout the country since 2010. From 2018, ready-to-consume raw milk will also be available on the market (Godic Torkar et al., 2017).

Conclusion

The large number of studies throughout Europe which address both health benefits and microbiological hazard potentials of raw milk consumption underline the topicality of this discussion and the uncertainties that still exist. The focus of these considerations is mainly on distribution via milk vending machines and the associated hygienic risks. Some European countries began to define specifications for milk vending machines years ago. In Germany, this marketing trend was slow to take hold, so these considerations have only begun in recent years. However, the results of our study show how important such requirements are.

The quality of raw and pasteurized milk from vending machines in Brandenburg were of varying quality in our analysis. There were milk vending machines that continuously delivered very good milk with a low bacterial count, but also others where the germ content was far too high. In cooperation with the dairy farms, multi-step quality controls were performed and suggestions were made for improving process hygiene. Milking hygiene, flow paths and tank dimensions are important influences. The respective machine type and the associated cleaning and disinfection processes are of great importance.

The implementation of DIN 10541 will impose stricter requirements in Germany. More frequent controls associated with consumer education are also required to guarantee consistently good quality in vending machine milk, to ensure quality during and after transport by the consumer and to prevent raw milk associated outbreaks of disease.

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Conflict of interest

The authors of this article acknowledge that they have no connection with or interest in any organizational unit with a financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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Journal of Food Safety and Food Quality 70, Heft 6 (2019), Seiten 157–194

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