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Credit author statement

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Maria Fraga: Formal alalysis, writing, review and editing.

Lorenzo De Colli: Formal alalysis, writing, review and editing.

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Kieran Jordan: Project administration, resources, supervision, writing, review and editing.

Determination of the presence of pathogens and anthelmintic drugs in raw milk and raw milk cheeses from small scale producers in Ireland

Antonio Lourenco[1], Maria Fraga,[2] Lorenzo De Colli,[2] Mary Moloney[2], Martin Danaher[2] and Kieran Jordan[1]*

[1]Food Safety Department, Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland

[2]Food Safety Department, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

* Corresponding author:

Kieran Jordan,

Food Safety Department

Teagasc Food Research Centre,

Moorepark, Fermoy, Cork, Ireland

Email: Kieran.jordan@teagasc.ie

Tel: 003532542451

Abstract

This aim of this study was to assess the microbiological and anthelmintic drug residue risks associated with raw milk used for cheesemaking and raw milk cheese, over an 18-month period. Samples of raw milk, milk filters, curd and cheese from nine raw milk artisan cheese producers in the south of Ireland were tested. Numbers of presumptive *Bacillus cereus* group, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* were determined. The determination of anthelmintic drug residues, including benzimidazoles, flukicides, macrocyclic lactone (avermectin and milbemycins), levamisole and morantel was also performed. Neither *L. monocytogenes*, nor *Salmonella* spp. were detected in any of the samples tested and no anthelmintic drug residues were detected. Only one of the samples did not conform with regulatory numbers for other bacteria. This survey has shown a good microbiological and residue quality (and low risk) of the raw milk cheese and raw milk used for raw milk cheese produced in Ireland. Moreover, it has shown the importance of frequent assessment of raw milk used for cheesemaking and for raw milk cheese, as it allows the identification of potential problems facilitating resolution of these issues before they cause any public health threat.

Keywords: Raw milk; microbiological safety; *Listeria*; *E. coli*; *Salmonella*

1. Introduction

In today's global market, large multinational companies have great impact on dairy commodity prices. Such companies can process and deliver products at reduced costs when compared to small scale producers. In order to make themselves competitive, small producers need to find market niches in which they can obtain added value for their product. Raw (unpasteurized) milk cheese production meets those requirements. Raw milk cheese is generally associated with being an element of cultural heritage, that relies on traditional production techniques, and is marketed as such. Most importantly, the use of unpasteurized milk allows for the presence enzymes and microbiota that are responsible for the production of desirable flavor and aroma characteristics (Yeluri Jonnala, McSweeney, Sheehan, & Cotter, 2018). However, the quality and safety of unpasteurised milk used for the production of unpasteurised milk cheese is an important consideration. Unpasteurised milk constitutes a major concern for regulators and small producers. From a regulatory perspective, a foodborne disease outbreak is a public health issue which could result from unpasteurized milk cheese. From a producers perspective, a foodborne disease outbreak associate with their product could lead to a severe impact of lost markets, loss of consumer demand and litigation, and ultimately could lead to the company closure (Hussain & Dawson, 2013).

The main sources of contamination of raw milk with foodborne pathogens are either the result of infected lactating animals (*Staphylococcus aureus* being one of the most common causes of udder infection), inappropriate practices during milking that may lead to the contamination of the milk with animal feces, bedding materials, mud or silage (the last particularly relevant for *L. monocytogenes* (Queiroz, Ogunade, Weinberg, & Adesogan, 2018). During processing, at the dairy, inadequately maintained, improperly cleaned and sanitized equipment, the use of contaminated water of improperly maintained air bleeds may cause a multitude of contamination issues with different microoganisms that may cause food safety issues.

Listeria monocytoges is the causative agent of listeriosis, a disease that primarily affects pregnant women and their newborns, adults older than 65, and people with a compromised immune system.

The mortality rate of listeriosis is about 24% (Maertens de Noordhout et al., 2014). It is frequently associated with cheeses and constitutes one of the major causes for product recalls of these products (Churchill, Sargeant, Farber, & O'Connor, 2019; Jackson, Gould, Hunter, Kucerova, & Jackson, 2018).

Salmonella, which has recently been associated with the contamination of low moisture foods, has also been reported as the cause of outbreaks in cheese including raw milk cheese (Guzman-Hernandez et al., 2016; Ung et al., 2019). More than 2600 different *Salmonella* serotypes have been isolated, many of them with the ability to induce gastroenteritis characterized typically by symptoms such initial nausea and vomiting that can develop to diarrhea, abdominal pain and fever.

Among the population of generally harmless *Escherichia coli* there are some serotypes that are pathogenic. These can cause severe disease, even when present in low numbers. Molecular methods are increasingly being used for their detection as traditional methods are not sensitive enough and rarely detect them unless they are present at relatively high numbers and are able to provide relevant information for hazard characterization of the different serotypes (FAO/WHO STEC Expert Group, 2019; Kagkli, Folloni, Barbau-piednoir, Eede, & Bulcke, 2012; Vallières, Saint-jean, & Rallu, 2013).

Staphylococcus aureus frequently colonizes the skin and mucous membranes of humans and many animal species as asymptomatic carriers. Intoxication by this microorganism results from ingestion of thermostable enterotoxins also resistant to gastrointestinal proteases produced during growth in contaminated food, that once consumed, lead to a rapid onset of symptoms that include nausea and violent vomiting, with or without diarrhea. This microorganism has been shown to be very common along the artisan raw milk cheese production process (Johler et al., 2018) and constitutes a major concern to dairy farmers, conditioning their attitudes and behavior (Cousin, Härdi-Landerer, Völk, & Bodmer, 2018).

Bacillus cereus is a Gram-positive, endospore-forming bacteria. Its ability to produce toxins can lead to diarrheal or emetic types of disease with an onset in a matter of hours. It is a microorganism

widespread in the environment and is often isolated from soil and vegetation but also if dairy food products (Owusu-Kwarteng, Wuni, Akabanda, Tano-Debrah, & Jespersen, 2017).

Raw milk intended for raw milk cheese production at small scale is generally the result of small, if not single, herd sizes. The risk of potential contaminants with these relevant foodborne pathogens is therefore generally higher than if milk from a larger number of herds is used, as there is no dilution with milk from other herds not containing pathogens.

As the milk for raw milk cheesemaking usually comes from smaller herds and is rarely pooled, the presence of residues and contaminants from raw milk production also needs to be assessed so that confidence in the end product can be assured in all aspects. Knowledge on toxin, contaminant and residue risks posed by unpasteurised milk cheese is limited. There is a potential that toxins, contaminants and residues may be concentrated from the milk during the cheesemaking process. This was seen with residues in milk that remained in dairy products and in some cases increased (Iezzi et al., 2014).

The regulations relating to unpasteurized milk cheese vary worldwide; nevertheless, there is a general requirement that food producers place only safe food on the market (EC) No 852/2004 (European Comission, 2004). Furthermore, in the EU, Commission Regulation (EC) No 2073/2005 (European Comission, 2005) lays the specification for pathogenic bacteria and places the responsibility for their absence on the food business. In the US, the FDA requires that raw milk cheeses must be aged no less than 60 days at a temperature equal to or higher than 1.7 °C before being placed in the market (FDA, 2011), in order to reduce the risk of pathogenic bacteria as it is considered that pathogenic bacteria will decrease during the 60-day period.

Goat's and cow's milk are characterized by a distinct composition mainly due to differences in the amount and type of casein, leading to distinct types of gel and renneting times. Also, the differences in structure and composition of milk fat globules have a major impact on the volatile composition of the cheeses produced with it (Park, 2017). Most importantly, for this study, the different animal management practices, size of the herd and type of cheese produced may play a role in the type of

microorganisms and residues present. Therefore, the aim of this study was to assess microbiological and residue (anthelmintic drug residues) risks associated with unpasteurized milk used for raw milk cheese making in Ireland.

2. *Material and Methods*

2.1. Sampling

The samples, raw milk intended for raw milk cheese production, milk filters (obtained after milking), raw milk cheese curd and raw milk cheese after different ripening times, were obtained from nine raw milk artisan cheese producers in the south of Ireland (7 producing cow's milk cheese and 2 producing goat's milk cheese) over an 18-month period (Tables 1 and Table 2). The samples were collected by the producers, according to instructions provided regarding aseptic technique, and shipped to the laboratory by courier with ice packs.

A total of 234 samples, which represented seasonal production of cheese from all producers, were used in the different analyses to assess their microbiological quality (Table 1). For the residue testing, overall 147 samples were tested: sixty-eight milk samples (57 cow and 11 goat) and 79 curd/cheese samples (74 cow and 5 goat).

The processing environment samples were taken by trained laboratory staff.

2.2. Microbiological analysis

The sample were homogenised for 2 min in a stomacher (Interscience BagMixer, 400 Saint Nom, France) in the appropriate medium.

The detection and enumeration of *L. monocytogenes* was performed according to ISO 11290:2017 parts 1 and 2, respectively (ISO, 2017a, 2017b). For milk filter ½ of the filter was used (approximately 25 g). Fraser broth base and selective supplements were bought from Merck-Millipore (Darmstadt,

Germany). The Ottaviani & Agosti (ALOA) agar was bought from Biomerieux (Marcy l'Etoile, France). The detection limit for the enumeration was of 10 CFU/ml or 10 CFU/g.

Samples were tested for the presence of *Salmonella* spp. using ISO 6579-1:2017 (by enrichment) (ISO, 2017c). For milk filter ½ of the filter was used (approximately 25 g). The buffered peptone water (BPW), Modified Semi-solid Rappaport-Vassiliadis (MSRV) Agar and Xylose Lysine Deoxycholate agar (XLD agar) were bought from Oxoid (Basingstoke, Hampshire, England).

The enumeration of beta-glucuronidase-positive *Escherichia coli*, hereinafter referred to as *E. coli*, was performed according to ISO 16649-2:2001 (ISO, 2001). Samples were homogenized in BPW and plated on Tryptone Bile Glucuronic Agar (TBX Agar; Merck-Millipore). The detection limit was of 1 CFU/ml in the case of the milk samples and 10 CFU/g for the other type of samples.

The enumeration of *Staphylococcus aureus* (coagulase-positive staphylococci) was done according to ISO 6888-2:1999/Amd.1:2003 (ISO, 2003). Homogenization was done in BPW and the dilutions plated on Baird Parker-RPF agar (Biomerieux). The detection limit for this analysis was of 10 CFU/ml in the case of the milk samples and 100 CFU/g for the other type of samples.

The samples were tested for *Bacillus cereus* following the FDA Bacteriological Analytical Manual: Chapter 14 (FDA, 2012) with slight modifications. Twenty-five grams of sample, rather than 50 g, and buffered peptone water, rather than Butterfield's phosphate-buffered dilution water, were used. Appropriate dilutions were plated in duplicate in BACARA plates (Biomerieux). The typical colony morphology on BACARA is characterized by orangey colonies surrounded by an opaque halo. The detection limit for this analysis was of 10 CFU/ml in the case of the milk samples and 100 CFU/g for the other type of samples.

2.3. Polymerase chain reaction (PCR) for *L. monocytogenes* confirmation

Presumptive *L. moncytogenes* colonies from the ALOA plates were purified on TSA (Merck-Millipore) and single colonies were then used to prepare lysates to be used as PCR template. A multiplex PCR

was then performed according to Doumith, Buchrieser, Glaser, Jacquet, & Martin, (2004) using five sets of primers targeting *lmo0737*, *lmo1118*, *ORF2819*, *ORF2110* and *prs* genes. The resulting PCR products were resolved on 2 g/100 ml agarose gels (Sigma-Aldrich, St. Louis, MO, USA) in 1 × TBE buffer (Lonza AcuGENE, Rockland, ME USA).

2.4. Processing Environment sampling for *L. monocytogenes* presence

The processing environment of five small-scale dairies was sampled by trained laboratory staff. Two hundred and fourteen both food contact and non-food contact surfaces were swabbed (Sponge-Sticks, 3M™, St. Paul, MN, USA). The surfaces tested included food contact surfaces such as tanks, tables and cheese mills, and non-food contact surfaces such as drains, floors and walls. Following sample collection, the swabs were transported to the laboratory under refrigeration and processed within 18 h. according to ISO 11290:2017 part 1 (detection), as described previously. The samples were collected from dairies 3 (18.69 %, n=40), 4 (26.64 %, n=57), 5 (2.34 %, n=5), 6 (21.96 %, n=47) and 9 (30.37 %, n=65) and tested for the presence of *L. monocytogenes* by ISO 11290-1 (ISO, 2017a).

2.5. Anthelmintic drug residue testing

The samples were collected and frozen at -20°C and transported frozen to Teagasc Food Research Centre, Ashtown (TRFCA) where they were kept frozen at -20 °C prior to analysis. The samples were analysed for anthelmintic drug residues including benzimidazoles, flukicides, macrocyclic lactone (avermectin and milbemycins), levamisole and morantel by applying the method that was previously reported for the analysis of milk samples (Whelan et al., 2010). Briefly, anthelmintic residues were isolated from milk samples into acetonitrile (Romil Ltd, Cambridge, UK) using magnesium sulphate (United Chemical Technologies, Wexford, Ireland) and sodium chloride (Applichem, Darmstadt, Germany), followed by centrifugation. The supernatant was poured into a d-SPE tube (United

Chemical Technologies, Wexford, Ireland) containing magnesium sulphate and C18 for clean-up. The extract was concentrated into dimethyl sulphoxide (Sigma-Aldrich, Dublin, Ireland), which was used as a keeper to ensure analytes remained in solution. The reconstituted samples were filtered using 0.2 µm PTFE uniprep filter vials (Whatman plc, Maidstone, UK) prior to injection into the UHPLC-MS/MS system (Waters Corp., Milford, MA, USA). Using rapid polarity switching in electrospray ionisation, a single injection was capable of detecting both positively and negatively charged ions in a 13 minutes run time. An injection volume of 5 µl was used.

The method was adapted to cheese and curd samples using the protocol outlined by Power et al., (2013). A volume of 9 ml of ultrapure water was added to 1 g of sample followed by homogenisation in a water bath at 50 °C. The samples were then extracted as described above.

2.6. Data analysis

Statistica version 7.0 (Statsoft, Tulsa, OK, USA) was used to perform the descriptive statistical analysis as well as the Box whisker-plots with mean, quartiles and range to assess the data dispersion.

3. *Results*

3.1. Microbiological

3.1.1. Milk & Milk filters

For all the samples tested both for milk and milk filters, no *L. monocytogenes* (by enumeration or detection methods) or *Salmonella* spp. were found (Table 1).

For the majority of the other analysis performed, the results obtained were below the detection limit of the various tests (Figure 1). The highest microbiological counts obtained, within all types of sample, for *S. aureus*, *E. coli* and *B. cereus* were obtained in milk filter samples. When compared to the milk samples, the milk filter results were generally one to two log CFU/g or ml higher. The range of values obtained is shown in Figure 1B.

221 *3.1.2.Curd & Cheese*

222 For all of the samples tested, *L. monocytogenes* and *Salmonella* spp. were below the detection limit
223 of the tests.
224 For *S. aureus* and *B. cereus*, many of the samples were below the detection limit of the tests. The
225 highest value observed for *S. aureus* on curd was 5.28 log CFU/g (Table 2). This value was obtained in
226 a sample from a producer that also presented high *S. aureus* counts in the milk in an isolated event.
227 The highest count for *B. cereus* was recorded in a sample from a producer that in a short period was
228 also dealing with high counts of other spore forming bacteria (data not shown). As is shown on
229 Figure 1C for curd and Figure 1D for cheese, the variation of the results obtained was high for both *S.*
230 *aureus* and *B. cereus.* The values for *S. aureus* range from below the detection limit to above 5 log
231 CFU/g in curd (Figure 1 C) and slightly less in cheese (Figure 1 D). The results obtained for *B. cereus*
232 were generally below the detection limit and when that was not the case varied enough to be
233 considered statistically as extremes (Figure 1).
234 The results obtained for *E. coli* show higher variably in the curd samples where 50% of the samples
235 ranged from below the detection limit to approximately 3.5 log CFU/g. In the cheese samples, 50%
236 of the counts were below the detection limit.
237 For *E. coli*, the milk, curd and cheese (made from the milk) from one manufacturer were analysed
238 from five independent batches after about 60 days of ripening. In these five batches the initial
239 contamination of the milk was always below 1 log CFU/ml. These values increased in the respective
240 curd by as much as 2.5 log CFU/g, representing growth and concentration of the bacteria in the curd.
241 For these five batches, the *E. coli* levels increased during ripening for two batches. For the other
242 three batches, a decrease in the level of *E. coli* was observed. The greatest reduction was observed
243 in the cheese batch with the longest ripening time (Figure 2).

3.1.3.Environmental testing for the presence of L. monocytogenes

A total of 214 processing environment swabs were taken from 5 different production facilities. *L. monocytogenes* was not found in any of the environments tested. Two dairies were tested once (numbers 3 and 5) two dairies were tested twice (numbers 6 and 9) and one dairy was tested on 8 different occasions throughout a period of a month.

3.2. Anthelmintic drug residues

Anthelmintic drug residues were not detected in any of the milk, curd or cheese samples analysed.

4. Discussion

The results of this study demonstrate the good microbiological and residue quality raw milk for raw milk for cheesemaking and of raw milk cheese in Ireland. No *L. monocytogenes*, *Salmonella* spp. or anthelmintic drug residues were detected in any of the samples tested. Generally, *L. monocytogenes* is detected in about 5 to 12 % of these type of samples (FSAI, 2015) however a meta-analysis on the incidence within different types of cheese shows a wide variability (Martinez-Rios & Dalgaard, 2018). *L. monocytogenes* and *Salmonella* spp. are primarily environmental contaminants of milk. The absence of *L. monocytogenes* and *Salmonella* spp. in the dairy samples and the absence of *L. monocytogenes* in the processing environment (including non-food contact surfaces), indicates that the hygiene procedures of the raw milk cheesemakers are very good. A study in 2009 on the occurrence of foodborne pathogens in Irish farmhouse cheese in Ireland showed a prevalence *of L. monocytogens* of 6 % in the 330 chesses analyzed (O'Brien, Hunt, Mcsweeney, & Jordan, 2009). However, in that study not only raw but also pasteurized milk farmhouse cheeses were analyzed. The fact that the raw milk and raw milk cheese surveyed in this study was intended for raw milk cheese production may be an important fact towards explaining the results obtained. The producers were aware of the potential food safety risks with raw milk products and particular awareness and

care was taken for that reason. In fact, it may also be relevant that the production facilities surveyed have been collaborating in research studies for several years and are therefore particularly aware to food safety issues. Sonnier *et al*. (2018) have, in a large study to access the prevalence of pathogens in US dairy operations, observed a statistically significant effect of herd size on the prevalence of *L. monocytogenes* and *S. enterica* in the dairy operations. The authors observed higher prevalence of both pathogens in operations with large (≥500 cows) and medium (100–499) herds than in small herds (30–99). This too may be relevant in explaining the results obtained as the average size of the Irish dairy herd is 63 (Donnellan, Hennessy, & Thorne, 2015) and the farmhouses surveyed in this study are below that.

E. coli can arise from faecal contamination, but it can also be found in dust etc. in the general environment (Jang et al., 2017). In the EU regulations 2073/2005 (European Comission, 2005), there is no regulation with regard to *E. coli* in raw milk used for raw milk cheese making or in raw milk cheese. In the current study, the results obtained for *E. coli* in milk showed good quality. In fact, for over 50 % of the samples, the *E. coli* numbers were below the detection limit (1 CFU/ml). A milk of such quality, for this parameter, complies with the required quality for unpasteurized milk intended for retail sale under the Australia and New Zealand legal limit (FSANZ, 2017). Gundogan & Avci (2014) have observed much higher prevalence (74 %) and higher numbers (up to 10^6 CFU/ml) in the positive raw milk samples, however the authors point out the importance of factors beyond hygiene such geographic location and season to explain differences between studies.

Milk filters were tested with the purpose of accessing variation within each dairy over time. With a pore size of 100 - 150 µm, milk filters only have a purpose of filtering of large debris such as soil or feces that might have come in contact with the milk during the milking process. They do not necessarily have a function in bacterial removal, but because the same filter can be used during milking of the entire herd, they can concentrate bacteria. A survey carried out by the Food Safety Authority of Ireland on raw milk and raw milk filters obtained higher incidence of pathogens in milk filters than in milk. A similar result was observed in the USA (FSAI, 2015; Sonnier et al., 2018). By

accessing the microbiological quality of the filter, it is possible to obtain a glimpse of the hygienic conditions in which milking was performed. While they are some indication of pathogen occurrence, they are of little value for enumeration of other bacteria, although the numbers of other bacteria could be used as an indication of the need to change the filter more frequently.

When the microbiological results of the curd are compared to the results obtained for the milk, it can be seen that, despite the variation of the results, the values obtained in the curd samples are generally higher that those obtained for the milk samples. This is a consequence of the production procedures that allow growth, but most importantly due to concentration of bacteria in the curd. Contrary to the regulations existent in the USA where there is a requirement for 60 days of aging of raw milk cheese prior to its sale (FDA, 2011), in order to allow for the elimination of pathogens that may be present, the results of this study show that such a requirement is unlikely to result in elimination of *E. coli* (some of which could be pathogenic), as in some cases *E. coli* actually grew during ripening. In Ireland there is no specific requirement for ripening prior to sale. Dalzini et al. (2014) observed a large variability of microbial concentrations (*E. coli* and coagulase-positive staphylococci) in raw milk intended for goat milk cheese production. That variability was further reflected in the behavior of the bacteria in the cheese throughout ripening. However, it is important to keep in mind not only the initial levels of contamination but also the intrinsic characteristics of the cheese. In this study, when comparing the results between dairies that must be kept in mind due to the different nature of the cheeses tested that varied from hard Cheddar type to semi-soft blue cheese.

The number of *S. aureus* in cheese made from raw milk is regulated in the EU. The maximum number permitted is 10^5 CFU/g in two of 5 samples with a maximum number of 10^4 CFU/g in the other 3 samples of the batch analysed (European Comission, 2005). In the current study, only one sample was taken on each occasion, although 128 milk, curd and cheese samples were tested during the study. Of the samples tested, only one sample (curd) was > 10^5 CFU/g (5.28 log CFU/g). The sample size was too small to use for an enterotoxin test, but subsequent samples showed compliant

levels of *S. aureus*. Brooks et al., (2012) in a survey of the microbiological quality raw milk cheeses in the market, detected the presence of only 3 samples with *S. aureus* contamination in a total of 41 samples tested. In this study, a higher prevalence of *S. aureus* was detected for the cheese, contrary to the study by Brooks et al., (2012)**.** The numbers of *S. aureus* have been shown to decrease with ripening (Hunt, Schelin, Rådström, Butler, & Jordan, 2012).

Bacillus cereus is one of the most relevant spore-forming pathogens encountered in raw milk and subsequent dairy products (Gopal et al., 2015). The pathogenicity of *B. cereus* group is associated with tissue-destructive/reactive exoenzyme production (Bottone, 2010). Among these secreted toxins are hemolysins, phospholipases, emesis-inducing toxins and pore-forming enterotoxins whose properties differ due to plasmid content or gene expression among *B. cereus sensu lato* (Ehling-schulz, Koehler, & Lereclus, 2019).

Being a spore former, *B. cereus* is generally considered a problem in milk powder because it can survive pasteurization and subsequently grow during powder production. It is generally associated with direct contact with soil and; its presence in soil, feed, bedding and cow's faeces has been shown (Heyndrickx, 2011). Some strains of *B. cereus* are pathogenic. Because of the adhesive nature of the glycoproteins of *B. cereus* endospores, it is frequently the bacterium that can easily attach and form biofilms on different kinds of surfaces, such as stainless steel, and become part of the 'in-house' microbiota in dairy processing environments, present in milk silos or tanks (Burgess, Lindsay, & Flint, 2010; Kumari & Sarkar, 2016; Lequette et al., 2011).While not of direct relevance to raw milk cheese, it is a bacterium of general interest to the dairy industry.

In this study, the majority of the microbiological results obtained for both milk and milk filters were below the detection limit (10 and 100 CFU/ml, respectively). These values can be considered of good quality since both spore and vegetative cells are being quantified. The high variability of the results for curd and cheese, most of the time below the detection limit, is likely to be a reflection not only of the differences between dairies but most importantly of the variability associated to the samples independently of the dairy. Despite the good results obtained over the period of the study, there is a

need for continuous analysis of raw milk in order that any problems can be detected at an early stage, thus avoiding potential public health issues.

None of the analysed residues were above the regulatory limits in any of the samples tested. Under Directive 96/23/EC (European Comission, 1996) the food industry is required to have self-monitoring programs in place to monitor for residues in food of animal origin. The absence of residues indicates that herd management practices were followed and indicate compliance with EU legislation.

5. Conclusion

This study has shown a good microbiological and residue quality (and low risk) of raw milk cheese and raw milk used for raw milk cheese produced in Ireland. It has shown the importance of frequent assessment of raw milk used for cheesemaking and for raw milk cheese, as it allows the identification of potential problems facilitating resolution of these issues before they cause any public health threat.

Promptly informing the cheesemakers of the results of their samples during the 18-month period of analysis allowed them to perform corrective measures on their procedures every time the results were not satisfactory. This study further shows good on-farm hygiene and animal health of Irish farms and stresses the importance of maintaining high standards of quality of raw milk and raw milk cheese to guarantee food safety.

365 **Declaration of interest**

366 The authors have no potential conflict of interest.

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372

373 ***References***

374 Bottone, E. J. (2010). *Bacillus cereus*, a volatile human pathogen. *Clinical Microbiology Reviews*,
375 *23*(2), 382–398. https://doi.org/10.1128/CMR.00073-09

376 Brooks, J. C., Martinez, B., Stratton, J., Bianchini, A., Krokstrom, R., & Hutkins, R. (2012). Survey of
377 raw milk cheeses for microbiological quality and prevalence of foodborne pathogens. *Food*
378 *Microbiology*, *31*(2), 154–158. https://doi.org/10.1016/j.fm.2012.03.013

379 Burgess, S. A., Lindsay, D., & Flint, S. H. (2010). Thermophilic bacilli and their importance in dairy
380 processing. *International Journal of Food Microbiology*, *144*(2), 215–225.
381 https://doi.org/10.1016/j.ijfoodmicro.2010.09.027

382 Churchill, K. J., Sargeant, J. M., Farber, J. M., & O'Connor, A. M. (2019). Prevalence of *Listeria*
383 *monocytogenes* in Select Ready-to-Eat Foods—Deli Meat, Soft Cheese, and Packaged Salad: A
384 Systematic Review and Meta-Analysis. *Journal of Food Protection*, *82*(2), 344–357.
385 https://doi.org/10.4315/0362-028x.jfp-18-158

386 Cousin, M. E., Härdi-Landerer, M. C., Völk, V., & Bodmer, M. (2018). Control of *Staphylococcus*
387 *aureus* in dairy herds in a region with raw milk cheese production: Farmers' attitudes,
388 knowledge, behaviour and belief in self-efficacy. *BMC Veterinary Research*, *14*(1), 1–13.
389 https://doi.org/10.1186/s12917-018-1352-0

390 Dalzini, E., Cosciani-cunico, E., Sfameni, C., Monastero, P., Daminelli, P., Losio, M. N., & Varisco, G.
391 (2014). Microbiological and Physico-Chemical Changes During Manufacture of an Italian Goat
392 Cheese Made from Raw Milk. *Italian Journal of Food Safety*, *3*, 4586.
393 https://doi.org/https://doi.org/10.4081/ijfs.2014.4586

394 Donnellan, T., Hennessy, T., & Thorne, F. (2015). The End of the Quota Era: A History of the Irish
395 Dairy Sector and Its Future Prospects. *Teagasc , Athenry Co, Galway, Ireland*. Retrieved from
396 https://www.teagasc.ie/media/website/publications/2015/End_of_the_Quota_Era_final.pdf

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., & Martin, P. (2004). Differentiation of the Major *Listeria monocytogenes* Serovars by Multiplex PCR. *Journal of Clinical Microbiology*, *42*(8), 3819–3822. https://doi.org/10.1128/JCM.42.8.3819

Ehling-schulz, M., Koehler, T. M., & Lereclus, D. (2019). The Bacillus cereus Group: *Bacillus* species with Pathogenic Potential. *Microbiol Spectrum*, *7*(3), 3–32. https://doi.org/10.1128/microbiolspec.GPP3-0032-2018

European Comission. (1996). Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. *Official Journal of the European Communities*, *L125*, 10–32.

European Comission. (2004). Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. *Official Journal of the European Communities*.

European Comission. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, *L 338/1*.

FAO/WHO STEC Expert Group. (2019). Hazard identification and characterization: Criteria for categorizing shiga toxin–producing *Escherichia coli* on a risk basis. *Journal of Food Protection*, *82*(1), 7–21. https://doi.org/10.4315/0362-028X.JFP-18-291

FDA. (2011). Code of Federal Regulations, Title 21 e Food and Drugs, Part 133 Cheeses and Related Cheese Products. Washington, DC.: Food and Drug Administration.

FDA. (2012). Chapter 14 - Bacillus cereus. In *FDA's Bacteriological Analytical Manual*. Food and Drug Administration. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

FSAI. (2015). Raw Milk and Raw Milk Filter Microbiological Surveillance Programme (12NS2).

421 *Monitoring and Surveillance Series - Microbiology*, (March), 1–26.

422 FSANZ. (2017). Microbiological Limits for Food. In *Australia New Zealand Food Standards Code -*
423 *Standard*. Commonwealth of Australia, Canberra.

424 Gopal, N., Hill, C., Ross, P. R., Beresford, T. P., Fenelon, M. A., & Cotter, P. D. (2015). The Prevalence
425 and Control of *Bacillus* and Related Spore-Forming Bacteria in the Dairy Industry, *6*(December),
426 1–18. https://doi.org/10.3389/fmicb.2015.01418

427 Gundogan, N., & Avci, E. (2014). Occurrence and antibiotic resistance of *Escherichia coli* ,
428 *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey.
429 *International Journal of Dairy Technology*, *67*(4), 562–569.https://doi.org/10.1111/1471-
430 0307.12149

431 Guzman-Hernandez, R., Contreras-Rodriguez, A., Hernandez-Velez, R., Perez-Martinez, I., Lopez-
432 Merino, A., Zaidi, M. B., & Estrada-Garcia, T. (2016). Mexican unpasteurised fresh cheeses are
433 contaminated with *Salmonella* spp., non-O157 Shiga toxin producing *Escherichia coli* and
434 potential uropathogenic *E. coli* strains: A public health risk. *International Journal of Food*
435 *Microbiology*, *237*, 10–16. https://doi.org/10.1016/j.ijfoodmicro.2016.08.018

436 Heyndrickx, M. (2011). The Importance of Endospore-Forming Bacteria Originating from Soil for
437 Contamination of Industrial Food Processing. *Applied and Environmental Soil Science*, *2011*, 1–
438 11. https://doi.org/10.1155/2011/561975

439 Hunt, K., Schelin, J., Rådström, P., Butler, F., & Jordan, K. (2012). Classical enterotoxins of coagulase-
440 positive *Staphylococcus aureus* isolates from raw milk and products for raw milk cheese
441 production in Ireland. *Dairy Science & Technology*, *92*(5), 487–499.
442 https://doi.org/10.1007/s13594-012-0067-4

443 Hussain, M., & Dawson, C. (2013). Economic Impact of Food Safety Outbreaks on Food Businesses.
444 *Foods*, *2*(4), 585–589. https://doi.org/10.3390/foods2040585

445 Iezzi, S., Lifschitz, A., Sallovitz, J., Nejamkin, P., Lloberas, M., Manazza, J., … Imperiale, F. (2014).
446 Closantel plasma and milk disposition in dairy goats: Assessment of drug residues in cheese and
447 ricotta. *Journal of Veterinary Pharmacology and Therapeutics*, *37*(6), 589–594.
448 https://doi.org/10.1111/jvp.12135

449 ISO. (2001). ISO 16649-2:2001. Microbiology of food and animal feeding stuffs -- Horizontal method
450 for the enumeration of beta-glucuronidase-positive *Escherichia coli* -- part 2: Colony-count
451 technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-d-glucuronide. Geneva,
452 Switzerland: International Organization for Standardization.

453 ISO. (2003). ISO 6888-2:1999/Amd.1:2003. Microbiology of food and animal feeding stuffs —
454 Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus
455 aureus and other species) - Part 2: Technique using rabbit plasma fibrinogen agar medium.
456 Geneva, Switzerland: International Organization for Standardization.

457 ISO. (2017a). ISO 11290-1:2017. Microbiology of the food chain -- Horizontal method for the
458 detection and enumeration of Listeria monocytogenes and of Listeria spp. -- Part 1: Detection
459 method. Geneva, Switzerland: International Organization for Standardization.

460 ISO. (2017b). ISO 11290-2:2017. Microbiology of the food chain -- Horizontal method for the
461 detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. -- Part 2:
462 Enumeration method. Geneva, Switzerland: International Organization for Standardization.

463 ISO. (2017c). ISO 6579-1:2017. Microbiology of the food chain -- Horizontal method for the
464 detection, enumeration and serotyping of *Salmonella* -- Part 1: Detection of *Salmonella* spp.
465 Geneva, Switzerland: International Organization for Standardization.

466 Jackson, K. A., Gould, L. H., Hunter, J. C., Kucerova, Z., & Jackson, B. (2018). Listeriosis outbreaks
467 associated with soft cheeses, United States, 1998–2014. *Emerging Infectious Diseases*, *24*(6),
468 1116–1118. https://doi.org/10.3201/eid2406.171051

469 Jang, J., Hur, H., Sadowsky, M. J., Byappanahalli, M. N., Yan, T., & Ishii, S. (2017). Environmental
470 *Escherichia coli*: ecology and public health implications — a review.
471 https://doi.org/10.1111/jam.13468

472 Johler, S., Macori, G., Bellio, A., Acutis, P. L., Gallina, S., & Decastelli, L. (2018). Short communication:
473 Characterization of *Staphylococcus aureus* isolated along the raw milk cheese production
474 process in artisan dairies in Italy. *Journal of Dairy Science*, *101*(4), 2915–2920.
475 https://doi.org/10.3168/jds.2017-13815

476 Kagkli, D., Folloni, S., Barbau-piednoir, E., Eede, G. Van Den, & Bulcke, M. Van Den. (2012). Towards a
477 Pathogenic *Escherichia coli* Detection Platform Using Multiplex SYBR H Green Real-Time PCR
478 Methods and High Resolution Melting Analysis. *PLoS ONE*, *7*(6), e39287.
479 https://doi.org/10.1371/journal.pone.0039287

480 Kumari, S., & Sarkar, P. K. (2016). *Bacillus cereus* hazard and control in industrial dairy processing
481 environment. *Food Control*, *69*, 20–29. https://doi.org/10.1016/j.foodcont.2016.04.012

482 Lequette, Y., Garénaux, E., Tauveron, G., Dumez, S., Perchat, S., Slomianny, C., … Faille, C. (2011).
483 Role played by exosporium glycoproteins in the surface properties of *Bacillus cereus* spores and
484 in their adhesion to stainless steel. *Applied and Environmental Microbiology*, *77*(14), 4905–
485 4911. https://doi.org/10.1128/AEM.02872-10

486 Maertens de Noordhout, C., Devleesschauwer, B., Angulo, F. J., Verbeke, G., Haagsma, J., Kirk, M., &
487 Havelaar, A. (2014). The global burden of listeriosis : a systematic review and meta-analysis.
488 *The Lancet Infectious Diseases*, *14*(11), 1073–1082. https://doi.org/10.1016/S1473-
489 3099(14)70870-9

490 Martinez-Rios, V., & Dalgaard, P. (2018). Prevalence of *Listeria monocytogenes* in European cheeses:
491 A systematic review and meta-analysis. *Food Control*, *84*, 205–214.
492 https://doi.org/10.1016/J.FOODCONT.2017.07.020

493 O'Brien, M., Hunt, K., Mcsweeney, S., & Jordan, K. (2009). Occurrence of foodborne pathogens in
494 Irish farmhouse cheese. *Food Microbiology*, *26*(8), 910–914.
495 https://doi.org/10.1016/j.fm.2009.06.009

496 Owusu-Kwarteng, J., Wuni, A., Akabanda, F., Tano-Debrah, K., & Jespersen, L. (2017). Prevalence,
497 virulence factor genes and antibiotic resistance of *Bacillus cereus* sensu lato isolated from dairy
498 farms and traditional dairy products. *BMC Microbiology*, *17*(1), 9–16.
499 https://doi.org/10.1186/s12866-017-0975-9

500 Park, Y. W. (2006). Goat Milk – Chemistry and Nutrition. In Y. Park, H. George, & W. Wendorf (Eds.),
501 *Handbook of Milk of Non-Bovine Mammals* (Second edi, pp. 34–58). Blackwell Publishing Ltd.

502 Power, C., Danaher, M., Sayers, R., O'Brien, B., Clancy, C., Furey, A., & Jordan, K. (2013). Investigation
503 of the migration of triclabendazole residues to milk products manufactured from bovine milk,
504 and stability therein, following lactating cow treatment. *Journal of Dairy Science*, *96*(10), 6223–
505 6232. https://doi.org/10.3168/jds.2013-7016

506 Queiroz, O. C. M., Ogunade, I. M., Weinberg, Z., & Adesogan, A. T. (2018). Silage review : Foodborne
507 pathogens in silage and their mitigation by silage additives. *Journal of Dairy Science*, *101*(5),
508 4132–4142. https://doi.org/10.3168/jds.2017-13901

509 Sonnier, J. L., Karns, J. S., Lombard, J. E., Kopral, C. A., Haley, B. J., Kim, S., & Kessel, J. A. S. Van.
510 (2018). *Escherichia coli* in bulk tank milk and milk filters from US dairy operations in the
511 National Animal Health Monitoring System Dairy 2014 study. *Journal of Dairy Science*, *101*(3),
512 1943–1956. https://doi.org/10.3168/jds.2017-13546

513 Ung, A., Baidjoe, A. Y., Van Cauteren, D., Fawal, N., Fabre, L., Guerrisi, C., … Le Hello, S. (2019).
514 Disentangling a complex nationwide *Salmonella Dublin* outbreak associated with raw-milk
515 cheese consumption, France, 2015 to 2016. *Euro Surveillance*, *24*(3), 1–10.
516 https://doi.org/10.2807/1560-7917.es.2019.24.3.1700703

517 Vallières, E., Saint-jean, M., & Rallu, F. (2013). Comparison of Three Different Methods for Detection
518 of Shiga Toxin- Producing *Escherichia coli* in a Tertiary Pediatric Care Center. *Journal of Clinical*
519 *Microbiology*, *51*(2), 481–486. https://doi.org/10.1128/JCM.02219-12

520 Whelan, M., Kinsella, B., Furey, A., Moloney, M., Cantwell, H., Lehotay, S. J., & Danaher, M. (2010).
521 Determination of anthelmintic drug residues in milk using ultra high performance liquid
522 chromatography–tandem mass spectrometry with rapid polarity switching. *Journal of*
523 *Chromatography A*, *1217*(27), 4612–4622. https://doi.org/10.1016/J.CHROMA.2010.05.007

524 Yeluri Jonnala, B. R., McSweeney, P. L. H., Sheehan, J. J., & Cotter, P. D. (2018). Sequencing of the
525 Cheese Microbiome and Its Relevance to Industry. *Frontiers in Microbiology*, *9*(May), 1020.
526 https://doi.org/10.3389/fmicb.2018.01020

527

Tables

Table 1. Number of samples tested: according to sample type, origin and each microorganism

Sample	Total samples	Origin	*S. aureus*	*E. coli*	*B. cereus*	*L. monocytogenes*	*S. enterica*
Milk	68	cow	*38*	*54*	*53*	*57*	*57*
		goat	9	11	11	11	11
Milk Filter	58	cow	36	45	43	47	47
		goat	9	11	11	11	11
Curd	47	cow	28	42	40	45	45
		goat	1	4	4	2	2
Cheese	61	cow	47	47	48	50	47
		goat	5	9	9	11	9

Table 2. Boxplot of bacterial counts (Log CFU/g) of dairy samples (milk, cheese milk filters), obtained from nine raw milk artisan cheese producers in the south of Ireland, for enumeration of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*. [min] – "minimum": lowest value of the data set; [Q1] – "first quartile": middle number between the smallest number and the median of the data set; "median": value separating the higher half from the lower half of the data set; [Q3] – "third quartile": middle value between the median and the highest value of the data set; [max] – "maximum"- highest value of the data set). nd – not done. <DL – Below detection limit. The green highlight emphasizes a value <DL. When only one sample was tested and the result was <DL, that was displayed in [max] column; when the value was >DL it was displayed only on the [min] column.

Dairy	Sample	Number of samples	*S. aureus* min	*S. aureus* Q1	*S. aureus* Median	*S. aureus* Q3	*S. aureus* max	*E. coli* min	*E. coli* Q1	*E. coli* Median	*E. coli* Q3	*E. coli* max	*B. cereus* min	*B. cereus* Q1	*B. cereus* Median	*B. cereus* Q3	*B. cereus* max
1	Milk	1	nd	-	-	-	-	< DL	< DL	< DL	< DL	< DL	-	-	-	-	< DL
	Cheese	4	nd	-	-	-	-	1.48	-	1.54	-	1.6	-	-	-	-	< DL
	Filter	1	nd	-	-	-	-	1.18	-	-	-	-	-	-	-	-	< DL
3	Milk	10	< DL	< DL	1.65	3.04	3.5	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	2.67	2.7
	Curd	2	5.28					< DL	-	-	-	2.09	< DL	-	-	-	< DL
	Cheese	7	< DL	< DL	2.88	3.04	3.13	< DL	< DL	< DL	< DL	4.45	< DL	< DL	< DL	< DL	< DL
	Filter	10	< DL	< DL	3.42	4.49	5.63	< DL	< DL	< DL	1.31	3.65	< DL	< DL	< DL	2.65	5.32
4	Milk	3	< DL	< DL	1.83	2.00	2.00	< DL	< DL	< DL	0	0	-	-	-	-	< DL
	Curd	4	< DL	< DL	3.33	4.57	4.95	2.32	2.37	2.73	3.16	3.24	-	-	-	-	< DL
	Cheese	12	< DL	< DL	< DL	3.29	3.48	< DL	< DL	< DL	1.04	2.4	< DL	< DL	< DL	1.25	2.95
5	Milk	9	nd				-	< DL	< DL	< DL	1.28	2.07	< DL	< DL	< DL	3.10	3.91
	Curd	9	< DL	< DL	< DL	2.82	2.96	1.94	2.91	3.07	3.65	4.05	< DL	< DL	< DL	3.23	6.00
	Cheese	1		-	-	-	< DL	-	-	-	-	< DL	-	-	-	-	< DL
	Filter	8	< DL	< DL	< DL	2.05	2.40	3.07	3.23	3.45	4.02	5.07	< DL	< DL	< DL	3.18	4.34
6	Milk	25	< DL	< DL	< DL	1.69	2.36	< DL	< DL	0.51	1.38	2.20	< DL	< DL	< DL	1.74	2.98
	Curd	12	2.00	2.51	3.11	3.63	3.95	< DL	< DL	3.20	4.21	4.53	< DL	< DL	< DL	< DL	< DL
	Filter	26	< DL	< DL	2.13	4.41	5.44	< DL	1.20	3.65	4.13	5.27	< DL	< DL	< DL	2.13	3.51
7	Milk	2	-	-	-	-	< DL	< DL	-	-	-	1	-	-	-	-	< DL
	Curd	1	-	-	-	-	< DL	2.16	-	-	-	-	nd	-	-	-	-
	Filter	1	-	-	-	-	< DL	>3	-	-	-	-	-	-	-	-	< DL

8	curd	1	-	-	-	-	< DL	1.93	-	-	-	-	-	-	-	-	< DL
	Filter	1	-	-	-	-	< DL	1.94	-	-	-	-	-	-	-	-	< DL
9	Milk	7	nd	-	-	-	-	< DL	< DL	0.30	0.74	0.94	< DL	< DL	< DL	1.78	2.00
	Curd	7	nd	-	-	-	-	< DL	< DL	1.90	2.70	2.95	< DL	< DL	< DL	< DL	3.08
	Cheese	32	< DL	< DL	< DL	< DL	3.28	< DL	< DL	< DL	< DL	2.96	-	-	-	-	< DL
10	Milk	11	< DL	< DL	< DL	2	2.16	< DL	< DL	1.44	2.25	4.17	< DL	< DL	< DL	<DL	3.96
	Curd	11	< DL	< DL	3.15	4.24	4.29	1.13	2.60	3.45	3.88	4.26	< DL	< DL	< DL	2.30	3.70
	Cheese	5	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
	Filter	11	< DL	< DL	2.60	3.57	4.12	< DL	< DL	2.93	4.00	5.23	< DL	< DL	< DL	3.56	5.40

Figure Captions

Figure 1. Box plot of data obtained from the bacterial counts of *E. coli*, *S. aureus* and *B. cereus* on milk (A), milk filters (B), curd (C) and cheese (D) samples, showing the variation obtained for each organism in the different sample matrices. The dotted line represents the detection limit of the method. For results below the detection limit, an arbitrary value of 1 log below detection limit was given to the sample. □ - Median, the value separating the higher half from the lower half of the data set; ▭ - 25%-75%, first quartile to third quartile; ⊥ - Non-Outlier Range; ○ - Outliers; ✳ - Extremes

Figure 2. Bacterial counts of *E. coli* in five different batches in milk and its respective curd and ripened cheese. The ripened cheese was tested with different times for each batch. Batch A- 48 days, Batch B- 50 days, Batch C- 54 days, Batch D- 55 days, Batch E- 61 days. ■ - Milk, ■ - Curd, ■ - Cheese

FIGURE 1

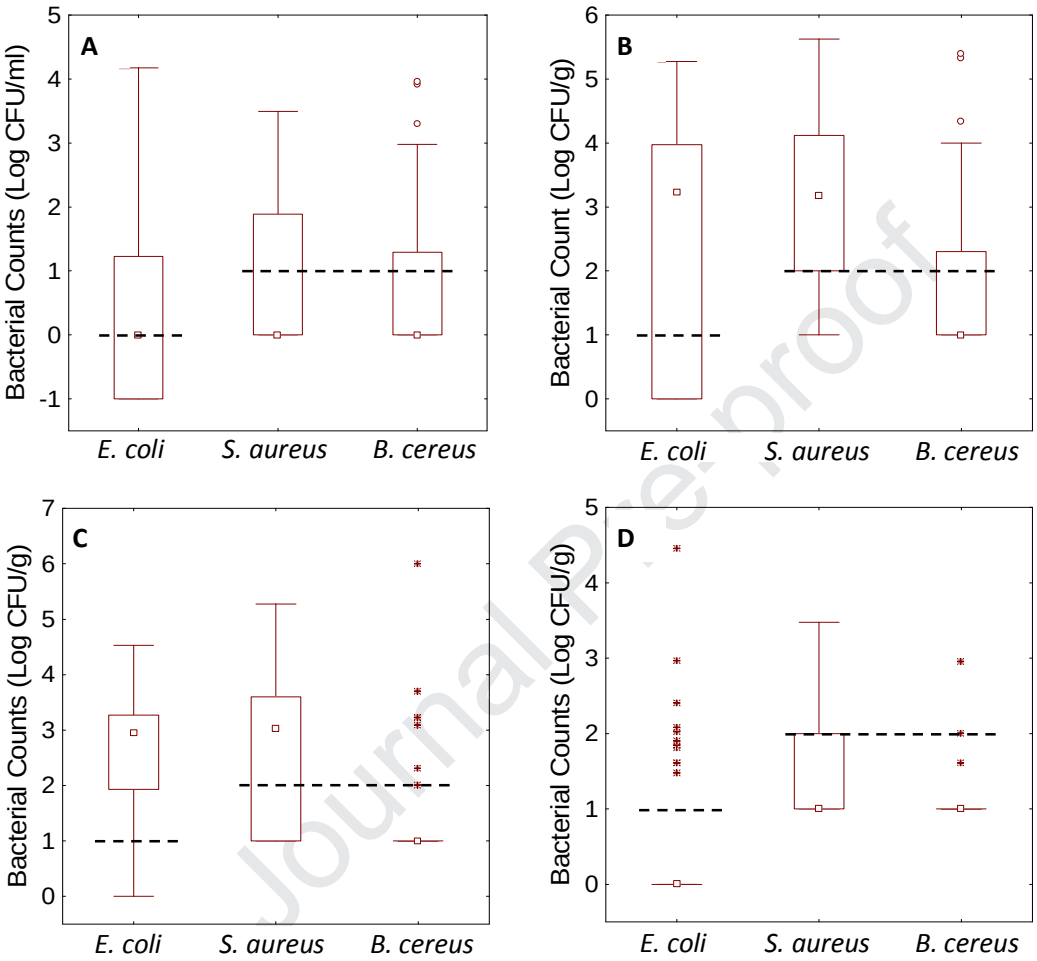
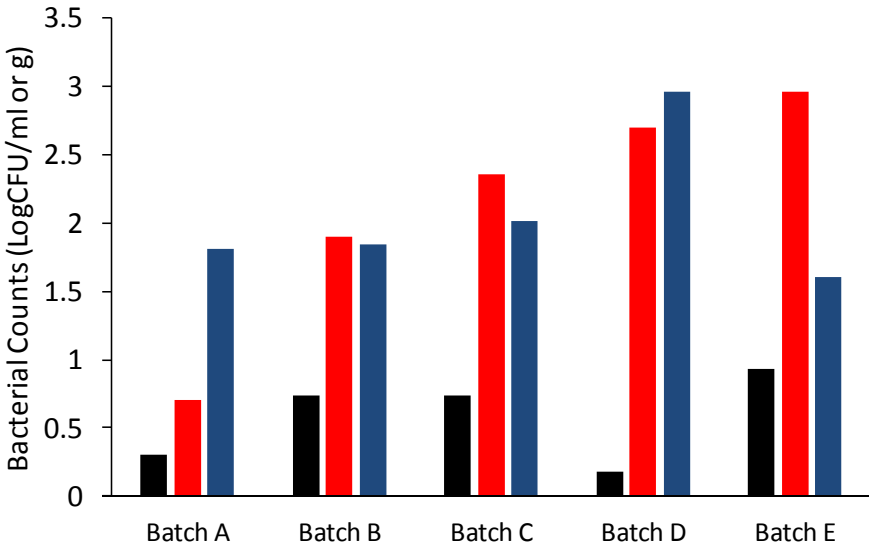


FIGURE 2



- Residues of anthelmintic drug and bacteria were analysed in the same 234 samples
- No anthelmintic drug residues above the reporting limit were found
- No. *L. monocytogenes* or *Salmonella* spp. were detected in the milk or cheese.

Conflict of interest statement

The authors have no conflict of interest to declare