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

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21 **Abstract**

22

23 This aim of this study was to assess the microbiological and anthelmintic drug residue risks

24 associated with raw milk used for cheesemaking and raw milk cheese, over an 18-month period.

25 Samples of raw milk, milk filters, curd and cheese from nine raw milk artisan cheese producers in the

26 south of Ireland were tested. Numbers of presumptive *Bacillus cereus* group, *Escherichia coli*,27 *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* were determined. The

28 determination of anthelmintic drug residues, including benzimidazoles, flukicides, macrocyclic

29 lactone (ivermectin and milbemycins), levamisole and morantel was also performed. Neither *L.*30 *monocytogenes*, nor *Salmonella* spp. were detected in any of the samples tested and no

31 anthelmintic drug residues were detected. Only one of the samples did not conform with regulatory

32 numbers for other bacteria. This survey has shown a good microbiological and residue quality (and

33 low risk) of the raw milk cheese and raw milk used for raw milk cheese produced in Ireland.

34 Moreover, it has shown the importance of frequent assessment of raw milk used for cheesemaking

35 and for raw milk cheese, as it allows the identification of potential problems facilitating resolution of

36 these issues before they cause any public health threat.

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

42

43 1. Introduction

44 In today's global market, large multinational companies have great impact on dairy commodity
45 prices. Such companies can process and deliver products at reduced costs when compared to small
46 scale producers. In order to make themselves competitive, small producers need to find market
47 niches in which they can obtain added value for their product. Raw (unpasteurized) milk cheese
48 production meets those requirements. Raw milk cheese is generally associated with being an
49 element of cultural heritage, that relies on traditional production techniques, and is marketed as
50 such. Most importantly, the use of unpasteurized milk allows for the presence enzymes and
51 microbiota that are responsible for the production of desirable flavor and aroma characteristics
52 (Yeluri Jonnala, McSweeney, Sheehan, & Cotter, 2018). However, the quality and safety of
53 unpasteurised milk used for the production of unpasteurised milk cheese is an important
54 consideration. Unpasteurised milk constitutes a major concern for regulators and small producers.
55 From a regulatory perspective, a foodborne disease outbreak is a public health issue which could
56 result from unpasteurized milk cheese. From a producers perspective, a foodborne disease outbreak
57 associate with their product could lead to a severe impact of lost markets, loss of consumer demand
58 and litigation, and ultimately could lead to the company closure (Hussain & Dawson, 2013).
59 The main sources of contamination of raw milk with foodborne pathogens are either the result of
60 infected lactating animals (*Staphylococcus aureus* being one of the most common causes of udder
61 infection), inappropriate practices during milking that may lead to the contamination of the milk
62 with animal feces, bedding materials, mud or silage (the last particularly relevant for *L.*
63 *monocytogenes* (Queiroz, Ogunade, Weinberg, & Adesogan, 2018). During processing, at the dairy,
64 inadequately maintained, improperly cleaned and sanitized equipment, the use of contaminated
65 water of improperly maintained air bleeds may cause a multitude of contamination issues with
66 different microorganisms that may cause food safety issues.
67 *Listeria monocytoges* is the causative agent of listeriosis, a disease that primarily affects pregnant
68 women and their newborns, adults older than 65, and people with a compromised immune system.

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

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

78 Among the population of generally harmless *Escherichia coli* there are some serotypes that are
79 pathogenic. These can cause severe disease, even when present in low numbers. Molecular methods
80 are increasingly being used for their detection as traditional methods are not sensitive enough and
81 rarely detect them unless they are present at relatively high numbers and are able to provide
82 relevant information for hazard characterization of the different serotypes (FAO/WHO STEC Expert
83 Group, 2019; Kagkli, Folloni, Barbau-piednoir, Eede, & Bulcke, 2012; Vallières, Saint-jean, & Rallu,
84 2013).

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

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

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

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

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

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

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

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

124

125 **2. Material and Methods**

126 **2.1. Sampling**

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

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

137 The processing environment samples were taken by trained laboratory staff.

138

139 **2.2. Microbiological analysis**

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

142 The detection and enumeration of *L. monocytogenes* was performed according to ISO 11290:2017
143 parts 1 and 2, respectively (ISO, 2017a, 2017b). For milk filter ½ of the filter was used (approximately
144 25 g). Fraser broth base and selective supplements were bought from Merck-Millipore (Darmstadt,

145 Germany). The Ottaviani & Agosti (ALOA) agar was bought from Biomerieux (Marcy l'Etoile, France).
146 The detection limit for the enumeration was of 10 CFU/ml or 10 CFU/g.
147 Samples were tested for the presence of *Salmonella* spp. using ISO 6579-1:2017 (by enrichment)
148 (ISO, 2017c). For milk filter ½ of the filter was used (approximately 25 g). The buffered peptone
149 water (BPW), Modified Semi-solid Rappaport-Vassiliadis (MSRV) Agar and Xylose Lysine
150 Deoxycholate agar (XLD agar) were bought from Oxoid (Basingstoke, Hampshire, England).
151 The enumeration of beta-glucuronidase-positive *Escherichia coli*, hereinafter referred to as *E. coli*,
152 was performed according to ISO 16649-2:2001 (ISO, 2001). Samples were homogenized in BPW and
153 plated on Tryptone Bile Glucuronic Agar (TBX Agar; Merck-Millipore). The detection limit was of 1
154 CFU/ml in the case of the milk samples and 10 CFU/g for the other type of samples.
155 The enumeration of *Staphylococcus aureus* (coagulase-positive staphylococci) was done according to
156 ISO 6888-2:1999/Amd.1:2003 (ISO, 2003). Homogenization was done in BPW and the dilutions
157 plated on Baird Parker-RPF agar (Biomerieux). The detection limit for this analysis was of 10 CFU/ml
158 in the case of the milk samples and 100 CFU/g for the other type of samples.
159 The samples were tested for *Bacillus cereus* following the FDA Bacteriological Analytical Manual:
160 Chapter 14 (FDA, 2012) with slight modifications. Twenty-five grams of sample, rather than 50 g, and
161 buffered peptone water, rather than Butterfield's phosphate-buffered dilution water, were used.
162 Appropriate dilutions were plated in duplicate in BACARA plates (Biomerieux). The typical colony
163 morphology on BACARA is characterized by orangey colonies surrounded by an opaque halo. The
164 detection limit for this analysis was of 10 CFU/ml in the case of the milk samples and 100 CFU/g for
165 the other type of samples.

166

167 **2.3. Polymerase chain reaction (PCR) for *L. monocytogenes* confirmation**

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

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

174

175 **2.4. Processing Environment sampling for *L. monocytogenes* presence**

176 The processing environment of five small-scale dairies was sampled by trained laboratory staff. Two
177 hundred and fourteen both food contact and non-food contact surfaces were swabbed (Sponge-
178 Sticks, 3M™, St. Paul, MN, USA). The surfaces tested included food contact surfaces such as tanks,
179 tables and cheese mills, and non-food contact surfaces such as drains, floors and walls.

180 Following sample collection, the swabs were transported to the laboratory under refrigeration and
181 processed within 18 h. according to ISO 11290:2017 part 1 (detection), as described previously.

182 The samples were collected from dairies 3 (18.69 %, n=40), 4 (26.64 %, n=57), 5 (2.34 %, n=5), 6
183 (21.96 %, n=47) and 9 (30.37 %, n=65) and tested for the presence of *L. monocytogenes* by ISO
184 11290-1 (ISO, 2017a).

185

186 **2.5. Anthelmintic drug residue testing**

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

195 Chemical Technologies, Wexford, Ireland) containing magnesium sulphate and C18 for clean-up. The
196 extract was concentrated into dimethyl sulphoxide (Sigma-Aldrich, Dublin, Ireland), which was used
197 as a keeper to ensure analytes remained in solution. The reconstituted samples were filtered using
198 0.2 µm PTFE uniprep filter vials (Whatman plc, Maidstone, UK) prior to injection into the UHPLC-
199 MS/MS system (Waters Corp., Milford, MA, USA). Using rapid polarity switching in electrospray
200 ionisation, a single injection was capable of detecting both positively and negatively charged ions in
201 a 13 minutes run time. An injection volume of 5 µl was used.

202 The method was adapted to cheese and curd samples using the protocol outlined by Power et al.,
203 (2013). A volume of 9 ml of ultrapure water was added to 1 g of sample followed by homogenisation
204 in a water bath at 50 °C. The samples were then extracted as described above.

205

206 **2.6. Data analysis**

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

210

211 **3. Results**

212 **3.1. Microbiological**

213 *3.1.1. Milk & Milk filters*

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

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

244 3.1.3. Environmental testing for the presence of *L. monocytogenes*

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

249

250 3.2. Anthelmintic drug residues

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

252

253 4. Discussion

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

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

278 *E. coli* can arise from faecal contamination, but it can also be found in dust etc. in the general
279 environment (Jang *et al.*, 2017). In the EU regulations 2073/2005 (European Commission, 2005), there
280 is no regulation with regard to *E. coli* in raw milk used for raw milk cheese making or in raw milk
281 cheese. In the current study, the results obtained for *E. coli* in milk showed good quality. In fact, for
282 over 50 % of the samples, the *E. coli* numbers were below the detection limit (1 CFU/ml). A milk of
283 such quality, for this parameter, complies with the required quality for unpasteurized milk intended
284 for retail sale under the Australia and New Zealand legal limit (FSANZ, 2017). Gundogan & Avci
285 (2014) have observed much higher prevalence (74 %) and higher numbers (up to 10^6 CFU/ml) in the
286 positive raw milk samples, however the authors point out the importance of factors beyond hygiene
287 such geographic location and season to explain differences between studies.

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

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

299 When the microbiological results of the curd are compared to the results obtained for the milk, it
300 can be seen that, despite the variation of the results, the values obtained in the curd samples are
301 generally higher than those obtained for the milk samples. This is a consequence of the production
302 procedures that allow growth, but most importantly due to concentration of bacteria in the curd.
303 Contrary to the regulations existent in the USA where there is a requirement for 60 days of aging of
304 raw milk cheese prior to its sale (FDA, 2011), in order to allow for the elimination of pathogens that
305 may be present, the results of this study show that such a requirement is unlikely to result in
306 elimination of *E. coli* (some of which could be pathogenic), as in some cases *E. coli* actually grew
307 during ripening. In Ireland there is no specific requirement for ripening prior to sale. Dalzini et al.
308 (2014) observed a large variability of microbial concentrations (*E. coli* and coagulase-positive
309 staphylococci) in raw milk intended for goat milk cheese production. That variability was further
310 reflected in the behavior of the bacteria in the cheese throughout ripening. However, it is important
311 to keep in mind not only the initial levels of contamination but also the intrinsic characteristics of the
312 cheese. In this study, when comparing the results between dairies that must be kept in mind due to
313 the different nature of the cheeses tested that varied from hard Cheddar type to semi-soft blue
314 cheese.

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

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

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

332 Being a spore former, *B. cereus* is generally considered a problem in milk powder because it can
333 survive pasteurization and subsequently grow during powder production. It is generally associated
334 with direct contact with soil and; its presence in soil, feed, bedding and cow's faeces has been shown
335 (Heyndrickx, 2011). Some strains of *B. cereus* are pathogenic. Because of the adhesive nature of the
336 glycoproteins of *B. cereus* endospores, it is frequently the bacterium that can easily attach and form
337 biofilms on different kinds of surfaces, such as stainless steel, and become part of the 'in-house'
338 microbiota in dairy processing environments, present in milk silos or tanks (Burgess, Lindsay, & Flint,
339 2010; Kumari & Sarkar, 2016; Lequette et al., 2011). While not of direct relevance to raw milk cheese,
340 it is a bacterium of general interest to the dairy industry.

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

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

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

353

354 **5. Conclusion**

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

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

365 **Declaration of interest**

366 The authors have no potential conflict of interest.

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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

- 397 Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., & Martin, P. (2004). Differentiation of the Major
398 *Listeria monocytogenes* Serovars by Multiplex PCR. *Journal of Clinical Microbiology*, 42(8),
399 3819–3822. <https://doi.org/10.1128/JCM.42.8.3819>
- 400 Ehling-schulz, M., Koehler, T. M., & Lereclus, D. (2019). The *Bacillus cereus* Group: *Bacillus* species
401 with Pathogenic Potential. *Microbiol Spectrum*, 7(3), 3–32.
402 <https://doi.org/10.1128/microbiolspec.GPP3-0032-2018>
- 403 European Commission. (1996). Council Directive 96/23/EC of 29 April 1996 on measures to monitor
404 certain substances and residues thereof in live animals and animal products and repealing
405 Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. *Official*
406 *Journal of the European Communities*, L125, 10–32.
- 407 European Commission. (2004). Regulation (EC) No 853/2004 of the European Parliament and of the
408 Council of 29 April 2004 on the hygiene of foodstuffs. *Official Journal of the European*
409 *Communities*.
- 410 European Commission. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on
411 microbiological criteria for foodstuffs. *Official Journal of the European Union*, L 338/1.
- 412 FAO/WHO STEC Expert Group. (2019). Hazard identification and characterization: Criteria for
413 categorizing shiga toxin-producing *Escherichia coli* on a risk basis. *Journal of Food Protection*,
414 82(1), 7–21. <https://doi.org/10.4315/0362-028X.JFP-18-291>
- 415 FDA. (2011). Code of Federal Regulations, Title 21 e Food and Drugs, Part 133 Cheeses and Related
416 Cheese Products. Washington, DC.: Food and Drug Administration.
- 417 FDA. (2012). Chapter 14 - *Bacillus cereus*. In *FDA's Bacteriological Analytical Manual*. Food and Drug
418 Administration. Retrieved from [https://www.fda.gov/food/laboratory-methods-](https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam)
419 [food/bacteriological-analytical-manual-bam](https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam)
- 420 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-](https://doi.org/10.1111/1471-0307.12149)
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., Devleeschauwer, 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-](https://doi.org/10.1016/S1473-3099(14)70870-9)
489 [3099\(14\)70870-9](https://doi.org/10.1016/S1473-3099(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., Koprak, 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	<i>S. aureus</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>S. enterica</i>
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	<i>S. aureus</i>					<i>E. coli</i>					<i>B. cereus</i>					
			min	Q1	Median	Q3	max	min	Q1	Median	Q3	max	min	Q1	Median	Q3	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	< 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

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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

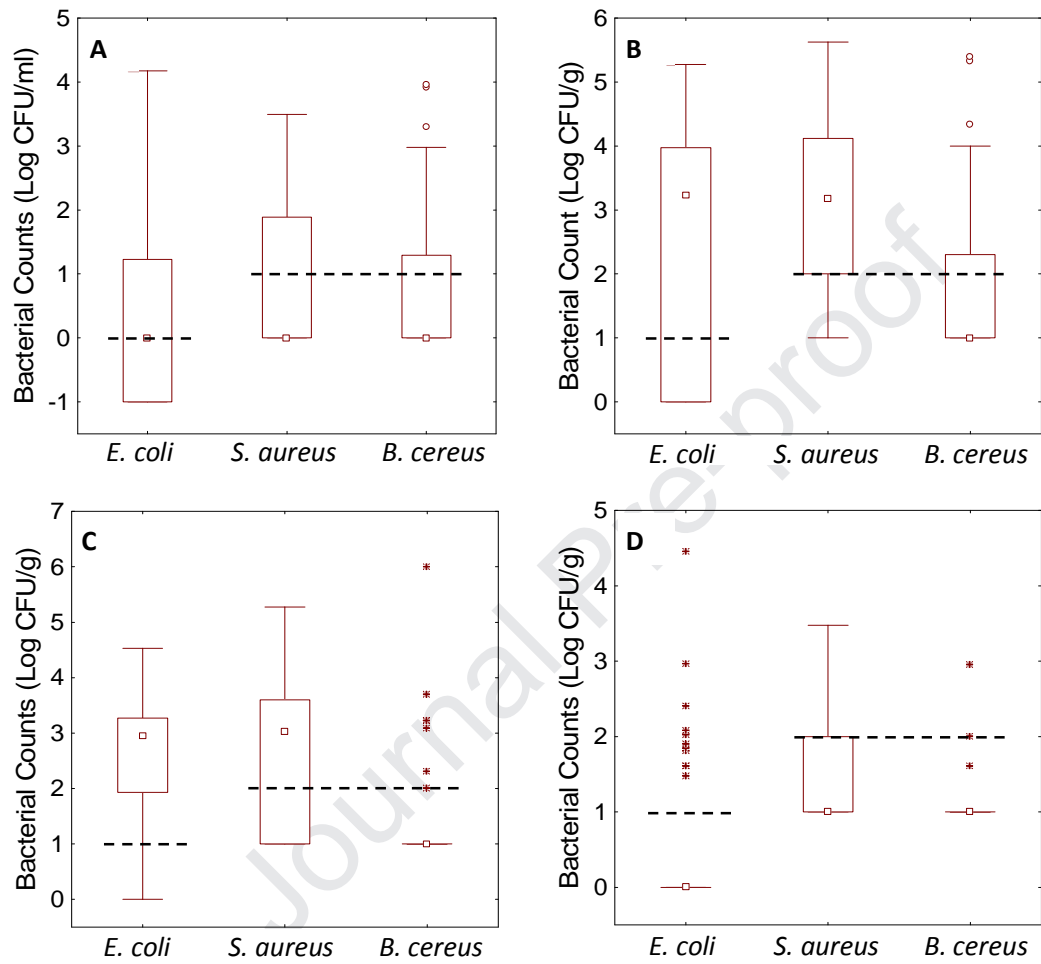
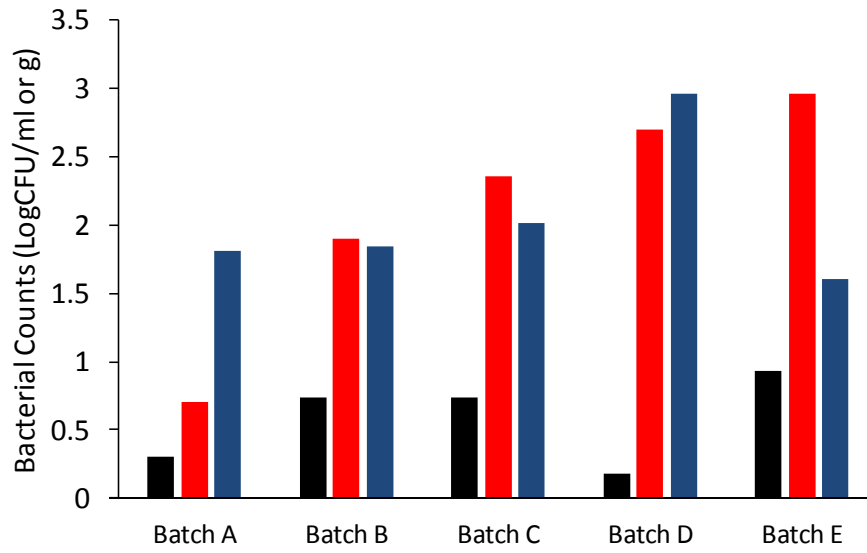


FIGURE 2



- 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.

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Conflict of interest statement

The authors have no conflict of interest to declare

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