



Microbiological quality of raw beef imported into the European Union from third countries

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ABSTRACT

Raw beef imported into the European Union (EU) from third countries must fulfill the same legal requirements as products from EU member states. Recently, the microbiological quality of 100 raw beef samples imported from third countries into the EU was investigated. Samples were quantitatively analyzed according to DIN ISO standard methods for aerobic mesophilic colony counts (ACC), counts of *Enterobacteriaceae*, *Escherichia coli*, *Listeria monocytogenes*, coagulase-positive staphylococci and qualitatively for *L. monocytogenes* and *Salmonella* spp. Samples were also screened for the presence of extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* and Shiga toxin-producing *E. coli* (STEC). Detected STEC isolates were subjected to whole genome sequencing. An *E. coli* prevalence of 3 % was detected, with one sample exceeding the critical value of the German Society for Hygiene and Microbiology (DGHM). Coagulase-positive staphylococci were found in one sample, but at a level below the DGHM guidance value. In three samples, *L. monocytogenes* were detected quantitatively, but none of these surpassed the critical value. *Salmonella* spp. were not detected, but *Enterobacteriaceae* were present in 83 % of the samples, of which 40 % surpassed the critical value. Combined with detected high values of ACC, this may indicate hygiene deficiencies. Additionally, STEC-screening showed a prevalence of 2 % and both STEC isolates harbored *stx1* and *stx2*. Overall, the occurrence of foodborne pathogens in the samples analyzed was low, but DGHM requirements for raw beef were not completely fulfilled. In combination with the detection of STEC, this may represent a potential health hazard for consumers.

1. Introduction

The import of food, especially products of animal origin, is of immense importance for the European Union (EU). The EU is the world's largest importer of food products after the United States and China (EC, 2021a). In 2021, a total of 283,729 tons of beef were imported into the EU from third countries, representing an increase of 26 % compared to the year 2020. The main suppliers of beef were Brazil (28.8 %), Uruguay (28.3 %) and Argentina (26.7 %) (EC, 2021b). Meat imported into the EU must meet the same legal requirements as products from EU member states to ensure food hygiene standards in accordance with Regulation (EC) No 852/2004, Regulation (EC) No 853/2004, and microbiological criteria according to Regulation (EC) No 2073/2005 (EC, 2004a; 2004b, 2005). In addition, Regulation (EU) 2017/625 dictates that imported

meat must enter through an approved Border Control Post supervised by an official veterinarian (EC, 2017). Then, according to Regulation (EU) 2019/2130, each consignment containing beef imported into the EU must be subjected to documentary checks, identity checks and physical checks, with the latter being mandatory for 15 % of consignments (EC, 2019). Microbiological analyses of imports are carried out on a risk-oriented basis as part of the multi-annual national control plan (MANCP), or, in cases of suspicion, on the basis of EU protective measures or the rapid alert system for food and feed (RASFF). Consignments that do not comply with the legal requirements are rejected at the EU borders.

Due to increasing international trade in products of animal origin, the global spread of foodborne pathogens is a growing concern. It has already been shown that imported meat can serve as a vector for

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pathogenic microorganisms such as *Salmonella* spp., Shiga toxin-producing *Escherichia* (*E.*) *coli* (STEC) and *Listeria* (*L.*) *monocytogenes* (Jansen et al., 2016). Beef, in particular, is occasionally consumed raw or not fully heated. Therefore, the risk of ingesting viable pathogens either directly or indirectly through cross-contamination increases. In this context, *Salmonella* spp., *L. monocytogenes*, STEC and *Staphylococcus* spp., most notably *Staphylococcus aureus*, are some of the most important food-associated pathogens and among the major public health hazards in the EU (EFSA, 2022). In particular, STEC is primarily associated with contaminated raw beef in the EU, and it is estimated that over 52 % of STEC outbreaks are associated with bovine products (Castro et al., 2019; Wang & Salazar, 2016). In recent years, the detection of STEC has resulted in recurring border rejections of red meat consignments intended for the European market (EC, 2023; Jansen et al., 2016). Notifications in the RASFF regarding the presence of STEC in meat are predominantly associated with products originating from Argentina and Brazil; this is not unexpected given the overall large volumes of imports from these two countries (Kononiuk et al., 2017).

In addition, imported meat can serve as a vehicle for antimicrobial resistant bacteria such as extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* (Egervärn et al., 2014; Jansen et al., 2018; Nagy et al., 2015). Infections with these resistant bacteria, especially in the case of multidrug resistance, pose a major human health concern since initial treatment may be ineffective and overall therapy options can be severely limited (Bassetti et al., 2019; Esteve-Palau et al., 2015).

While the microbiological quality and safety of imported pork and poultry meat into the EU have been described in a comprehensive study by Jansen et al. (2018), data regarding the microbiological quality of raw beef imported into the EU are very limited. Merely one previous study by Stella et al. (2013) performed a microbiological analysis focused on raw beef imported from Argentina to Italy. The aim of our project was therefore to obtain current data regarding the microbiological quality of raw beef imported into the EU from different third countries by investigating the presence of foodborne pathogens, bacterial quality criteria as well as the occurrence of ESBL-producing *Enterobacteriaceae*.

2. Material and methods

2.1. Sample collection and preparation

Samples of raw beef imported from third countries from November 2021 to May 2022 were collected at the Border Control Post of the Hamburg Harbour, Germany. In total, 100 raw beef samples imported from Brazil (n = 53), Argentina (n = 33), Uruguay (n = 12) and Paraguay (n = 2) were included in this study (Table 1). Only samples from fresh raw beef without sensory irregularities, and no further processing (e.g. seasoned, marinated or with additives), were collected. During the routine physical checks, a minimum of 100 g per individual sample was taken aseptically by authorized personnel at the Border Control Post laboratory. Samples were sealed in individual vacuum-wrapped plastic bags and stored at 2–4 °C. Within 24 h, samples were sent in a cold box with freeze packs to the Institute for Veterinary Food Science, Giessen, Germany. Upon arrival, the samples were checked for their temperature (2–4 °C) and placed in a refrigerator to maintain the cold chain. The sensory analysis of samples included a visual inspection

for color or texture irregularities as well as testing for odor deviations. Within the next 48 h, the pH value of the beef samples was measured according to DIN ISO 2917:1999 and they were subsequently prepared for microbiological analysis according to DIN ISO 6887–1:2017 and DIN ISO 6887–2:2017 (ISO, 1999; 2017a; 2017b).

2.2. Quantitative microbiological analyses

A quantitative analysis was performed for aerobic mesophilic colony count (ACC) according to DIN ISO 4833–2:2013, for *Enterobacteriaceae* according to DIN ISO 10164–1:2019–06, and for β -glucuronidase-positive *E. coli* according to the DIN ISO 16649–2:2001 standards protocols (ISO, 2001; 2013a; 2019a). The detection of coagulase-positive staphylococci was performed according to DIN ISO 6888–1:2019–06 (ISO, 2019b). From samples testing positive for coagulase-positive staphylococci, colonies were subcultured overnight on plate count agar (PC) (Merck, Darmstadt, Germany). Subsequently, DNA was isolated from the overnight cultures using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. To identify *S. aureus*, a specific PCR assay targeting the *eap* gene was performed as previously described (Hussain et al., 2008).

In order to obtain more data on the listeria load of the samples, quantitative testing of *L. monocytogenes* was added starting with the second half of the samples (n = 50) in accordance with DIN ISO 11290–2:2017 (ISO, 2017c).

As Regulation (EC) No 2073/2005 does not provide limits for raw beef at consumer level, the microbiological results were evaluated according to the guidance and critical values defined by the German Society for Hygiene and Microbiology (DGHM) for raw, seasoned or marinated beef (packed or unpacked) at consumer level, as shown in Table 2 (DGHM, 2021; EC, 2005). Samples that were below or equal to the guidance values were assessed as microbiologically satisfactory, those between the guidance and the critical value were categorized as acceptable and those that surpassed the critical value were evaluated as unsatisfactory with regard to the examined microbiological parameters (DGHM, 2018).

2.3. Qualitative microbiological analyses

The presence of *L. monocytogenes* was qualitatively determined for all samples according to DIN ISO 11290–1:2017, while the detection of *Salmonella* spp. was performed according to DIN ISO 6579–1:2017 (ISO, 2017d; 2017e). For the subculture to detect salmonellae, brilliant-green phenol-red lactose sucrose agar (BPLS) plates (Merck, Darmstadt, Germany) were used as the second selective culture medium, in addition to the obligatory selective culture medium xylose lysine desoxycholate agar (XLD) (Merck, Darmstadt, Germany). Presumptive *Salmonella* spp. colonies were confirmed by agglutination with a *Salmonella*-specific antiserum and by their biochemical properties using API20E™ (bioMérieux, Nürtingen, Germany).

2.3.1. Isolation and whole genome sequencing of Shiga toxin-producing *E. coli*

The detection of Shiga toxin-producing *E. coli* (STEC) was based on DIN ISO 13136:2013–04 with minor modifications: a homogenization of 25 g of each sample with 225 ml modified tryptic soy broth (Oxoid Limited, Basingstoke, UK) without antibiotic additives using a Stomacher® blender (Interscience, Saint Nom La Bretèche France) and subsequent incubation at 37 °C for 24 h (ISO, 2013b; Vimont et al., 2007). After this enrichment step, one 10 μ l loop was streaked onto CHROMagar STEC™ (Mast Diagnostica GmbH, Reinfield, Germany) and the plates were incubated at 37 °C for 24 h (Hirvonen et al., 2012). A second 10 μ l loop was streaked onto trypton bile x-glucuronide agar (TBX) (Oxoid Limited), which was incubated at 44 °C for 24 h. Based on colony morphology and color formation (CHROMagar STEC™ = mauve, TBX = blue-green or colorless), five individual colonies of presumptive STEC (if

Table 1
Origin of the raw beef samples examined in this study.

Country of Origin	Number of samples
Brazil	53
Argentina	33
Uruguay	12
Paraguay	2
Total	100

Table 2

Evaluation of microbiological criteria for raw beef imported into the EU according to guidance and critical values specified by the German Society for Hygiene and Microbiology (DGHM).

Evaluation criteria	Number of positive samples/ total samples	Colony counts (log ₁₀ cfu/g)		DGHM values ^a (log ₁₀ cfu/g)		Samples per category		
		Min. - Max.	Median	Guidance value	Critical value	> detection limit ^b ≤ guidance value	> guidance value ≤ critical value	> critical value
ACC ^c	100/100	4.18–10.04	8.06	na ^d	na	-	-	-
<i>Enterobacteriaceae</i>	83/100	2.32–8.69	4.76	4.0	5.0	19	24	40
<i>L. monocytogenes</i>	3/50 ^e	1.30–1.48	1.30	na	2.0	3	0	0
<i>E. coli</i>	3/100	1.90–5.94	2.00	2.7	3.7	2	0	1
Coagulase-positive staphylococci	1/100	2.54	-	2.7	3.7	1	0	0
<i>Salmonella</i> spp.	0/100	-	-	na	Absence in 25g	-	-	-

^a Microbial guidance and critical values for raw beef by DGHM applying to raw, seasoned or marinated beef (loose or packaged) valid at consumer level (2021).

^b According to DIN EN ISO 7218:2014–09, the detection limits for *E. coli*, *L. monocytogenes* and coagulase-positive staphylococci are 1 log₁₀ cfu/g and for *Enterobacteriaceae* 2 log₁₀ cfu/g in accordance with DIN ISO 10164–1:2019–06.

^c ACC = aerobic mesophilic colony count.

^d No values available.

^e The quantitative microbiological analysis for *L. monocytogenes* was only performed with n = 50 samples.

present) were picked from each agar plate, subcultured onto plate count agar (PC) (Merck) and incubated at 37 °C for 24 h. In order to confirm presumptive STEC, DNA was extracted from these colonies using the DNeasy Blood & Tissue Kit (QIAGEN) and the DNA was subjected to PCR analysis to investigate the presence of Shiga toxin genes (*stx1* and *stx2*) and the *eae* gene (Karch & Meyer, 1989; Schmidt et al., 1994). Only a single *stx1*-and/or *stx2*-positive colony from each sample was included in further investigations. The isolates were then confirmed as *E. coli* by species-specific PCR targeting the *gadA* gene as previously described (Doumith et al., 2012; Müller et al., 2021). Subsequently, confirmed STEC were subjected to whole genome sequencing (WGS) using the sequencing service and automated annotation service provided by MicrobesNG (MicrobesNG, Birmingham, UK). In order to determine the multilocus sequence type (MLST) and predicted serotype of the isolates, the genome sequences were uploaded to the Enterobase *Escherichia/Shigella* database (<https://enterobase.warwick.ac.uk>, last accessed on 18 August 2022) where they are available under accession numbers traces-0boqadF (Isolate Ha28) and traces-0rPxUql (Isolate Ha91). Further, the genome sequences obtained have been deposited in the GenBank database under accession numbers JAUALE000000000 (Ha28) and JAUALD000000000 (Ha91).

For detection of virulence-associated factors and resistance genes, Virulence Finder 2.0 (<https://cge.food.dtu.dk/services/VirulenceFinder/>, last accessed on 18 August 2022) and ResFinder 4.1 (<https://cge.food.dtu.dk/services/ResFinder/>, last accessed on 18 August 2022) were used, respectively (Bortolaia et al., 2020; Joensen et al., 2014).

2.3.2. ESBL-screening

To obtain information on the presence of ESBL producers in the samples, ESBL-screening was implemented for the remaining 50 raw beef samples. Samples were tested for the presence of ESBL-producing *Enterobacteriaceae* according to the protocol of the National Food Institute of Denmark for fresh meat with minor modifications (Hasman et al., 2015). Pre-enrichment was performed by incubating 25 g of each sample in buffered peptone water (Merck) in sterile Stomacher® blender bags (Interscience) at 37 °C for 24 h. One loopful broth was then streaked onto MacConkey agar plates containing 1 mg/l of cefotaxime (Cayman Chemical Company, Michigan, USA), which were again incubated for 24 h at 37 °C. Presumptive ESBL-producing *Enterobacteriaceae* were subjected to an ESBL-confirmatory test by disk diffusion following CLSI standards (CLSI, 2020). The panel of antibiotics for the confirmatory test included ceftazidime (CAZ, 30 µg) (Oxoid Limited),

ceftazidime-clavulanate (CAZ/CA, 30/10 µg) (Becton Dickinson, New Jersey, USA) cefotaxime (CTX, 30 µg) (Oxoid Limited), and cefotaxime-clavulanate (CTX/CA, 30/10 µg) (Becton Dickinson). The isolates were regarded as confirmed ESBL-producers if the growth inhibition zone around the CTX or CAZ disks in combination with CA was ≥5 mm larger in diameter compared to CTX or CAZ alone. As positive and negative control, *Klebsiella pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 were used, respectively.

2.4. Statistical analysis

Statistical analyses were performed using the SAS® 9.4 statistical software (Statistical Analysis System Institute Inc., Cary, NC, USA). The results of the quantitative microbiological analyses were transformed into log₁₀ scale base before evaluation. The normality of the values was tested by Shapiro-Wilk test. The non-parametric Kruskal-Wallis test was used to investigate the association between the origin of the isolates and the detected counts of ACC and *Enterobacteriaceae*. To test a correlation between high levels of contamination with ACC and *Enterobacteriaceae* in the same sample, the Spearman's rank correlation test was used (p < 0.05). Isolates originating from Paraguay were excluded from the statistical analysis because of the small sample size (n = 2). According to DIN ISO 7218:2014–09 the detection limits for *E. coli*, *L. monocytogenes* and coagulase-positive staphylococci are 1 log₁₀ colony-forming units (cfu)/g while the detection limit for *Enterobacteriaceae* is 2 log₁₀ cfu/g according to DIN ISO 10164–1:2019–06 (ISO, 2014; 2019a). As specified in both DIN ISO norms for results lower than the limit of detection, half the values of the detection limit were used for calculation (ISO, 2014; 2019a).

3. Results

3.1. Analyses of pH values

The pH values of the majority of the samples (n = 90), were in the expected range for raw beef of pH 5.4–6.2 with a median pH value of 5.59 (Ferguson et al., 2001; Vidal Junior et al., 2020). In the remaining 10 % of the samples, pH values were below 5.4 (5.11–5.36).

3.2. Quantitative analyses of the microbiological quality

The values of ACC of all raw beef samples (n = 100) examined in this study ranged from 4.18 log₁₀ cfu/g to 10.04 log₁₀ cfu/g, with a median

value of 8.06 log₁₀ cfu/g. Taking into account the origin of the samples, the ACC values of the samples from Brazil (n = 53) ranged from 4.18 to 10.04 log₁₀ cfu/g (median: 8.04 log₁₀ cfu/g), while those from Argentina (n = 33) ranged from 5.15 to 9.63 log₁₀ cfu/g (median: 7.95 log₁₀ cfu/g) and those from Uruguay ranged from 6.89 to 8.99 log₁₀ cfu/g (median: 8.06 log₁₀ cfu/g). There was no statistically significant difference in the ACC counts (p = 0.394) when considering the different origins of the isolates. The ACC values of the two samples originating from Paraguay were 8.36 and 8.66 log₁₀ cfu/g, respectively.

Enterobacteriaceae were detected with a frequency of 83 % in the samples, with values for individual samples ranging from 2.32 log₁₀ cfu/g to 8.69 log₁₀ cfu/g, with a median value of 4.76 log₁₀ cfu/g (Table 2). In total, 40 % of the samples surpassed the critical value for *Enterobacteriaceae* defined by the DGHM, 24 % were in the range between the guidance value and the critical value, and 19 % were below the guidance value (Table 2). In the remaining 17 samples, no *Enterobacteriaceae* were detected. Considering the origin of the samples, the *Enterobacteriaceae* colony counts for samples from Brazil were between 2.32 and 8.69 log₁₀ cfu/g (median: 4.34 log₁₀ cfu/g), for samples from Argentina between 2.83 and 7.11 log₁₀ cfu/g (median: 4.97 log₁₀ cfu/g) and for samples from Uruguay between 2.83 and 6.81 log₁₀ cfu/g (median: 4.81 log₁₀ cfu/g). In one of the two samples originating from Paraguay, *Enterobacteriaceae* were detected with a value of 4.71 log₁₀ cfu/g. Again, no statistically significant differences in the values were observed in relation to the origin of the samples. Correlation analysis revealed only a weak association between high levels of ACC counts and high counts of *Enterobacteriaceae* (Spearman's correlation, r_s = 0.27, p = 0.007).

Escherichia coli were detected quantitatively in three samples (n = 2 from Brazil, n = 1 from Argentina). Of these, one sample from Brazil surpassed the critical value defined by DGHM with 5.94 log₁₀ cfu/g (Table 2).

Also, coagulase-positive staphylococci were detected in only a single sample from Argentina. The detected colony count of 2.54 log₁₀ cfu/g was below the guidance value defined by DGHM (Table 2). The isolate was confirmed as *S. aureus* by PCR.

In 5 % of all samples (n = 3 from Argentina, n = 1 from Uruguay and n = 1 from Paraguay), *L. monocytogenes* was detected. Of these, two Argentinian samples and the sample originating from Uruguay, were tested quantitatively for *L. monocytogenes* with none of the samples surpassing the critical value defined by DGHM (Table 2).

3.3. Qualitative microbiological analyses and whole genome sequencing results

In two samples originating from Argentina, STEC was detected. Visible growth of the bacteria was obtained from both positive samples on TBX agar (Oxoid), with the colonies from sample H91 showing a blue-green color, while those from sample H28 formed colorless colonies. In contrast, merely sample H28 showed visible growth on CHROMagar STEC™ (Mast Diagnostica GmbH). From sample H28, all colonies tested by PCR were positive for the *stx1* and *stx2* genes, whereas only two of the five colonies tested for sample H91, showed a positive PCR result for the *stx1* and *stx2* genes. From both beef samples, one *stx1* and *stx2*-positive colony was included in further investigations. Both STEC isolates were then tested for the presence of the *eae* gene, resulting negative. In order to further investigate the characteristics of the isolates, they were subjected to whole genome sequencing. Analysis of the sequences revealed that isolate Ha28 belonged to serotype O178:H7, sequence type ST278 and harbored *stx1a* and *stx2c* (Table 3). In contrast, isolate Ha91

belonged to ST6661, was determined as serotype B17:H19 and harbored *stx1a* and *stx2a*. Both isolates belonged to phylogenetic group B1. No resistance genes were detected in either of the isolates via the ResFinder software, but other fitness and virulence-associated factors were present, such as the adhesion encoding *iha* gene in isolate Ha28 (Table 3).

From five samples, visible growth on ESBL-screening plates was detected, showing a pink color formation and exhibiting very small colonies (<0.5 mm diameter). From each agar plate one colony was picked and subjected to the ESBL-confirmatory test. All isolates showed large zone diameters of ≥27 mm and ≥22 mm for cefotaxime and ceftazidime, respectively. The diameters did not increase 5 mm or more in the presence of clavulanic acid. Thus, none of these isolates was confirmed as ESBL-producer.

Furthermore, no *Salmonella* spp. was detected in any sample of this study.

4. Discussion

In this study, we assessed the microbiological quality of a total of 100 raw beef samples imported into the EU from third countries. Samples originated from Brazil (53 %), Argentina (33 %), Uruguay (12 %) and a small percentage from Paraguay (2 %). This is an approximation to the distribution of the predominant beef importers into the EU (EC, 2021). Results indicate that the majority of the samples were within the expected pH range for raw beef, in which the typical characteristics, nutrition properties and texture of beef, are maintained (Ferguson et al., 2001; Vidal Junior et al., 2020). Low pH values (<5.4) are typically related to the growth of lactic acid bacteria and are linked with a higher water loss in the further course (Crichton et al., 2017). However, samples in our study with pH values below 5.4 showed no apparent sensory irregularities and no increased ACC values compared to the samples within the expected pH range.

In general, microbiological analysis indicated that the occurrence of the major relevant foodborne pathogens in the samples is low and the critical values for those defined by the DGHM were not surpassed. However, criteria by DGHM for hygienic indicator bacteria were not completely fulfilled and a considerable number of samples exceeded the critical value.

The ACC is used as an indicator of appropriate processing conditions, for maintaining the cold chain, and for predicting the shelf life of raw meat (González-Gutiérrez et al., 2020). No values for ACC are defined by the DGHM for raw beef, while for other types of meat, such as raw lamb, poultry and pork meat, a guidance value of 6.7 log₁₀ cfu/g is specified by the DGHM (DGHM, 2021), which is well below the median value of 8.06 log₁₀ cfu/g we observed in our samples. In general, for raw meat, it is reported that high detection frequencies of ACC between 7 log₁₀ cfu/g and 8 log₁₀ cfu/g are associated with poorer quality, an earlier onset of changes in texture and smell, and a generally reduced shelf life (González-Gutiérrez et al., 2020; McCain et al., 2015). However, as mentioned, none of the samples in the present study showed deviations in sensory parameters even when ACC values exceeded 7 log₁₀ cfu/g. Overall, high values of ACC in raw meat, as found in our study, combined with high detection frequencies of other hygienic indicator bacteria such as *Enterobacteriaceae*, indicate unhygienic conditions in the production chain or inadequate temperature during transport and storage (Jaja et al., 2018). Indeed, our results demonstrated a high prevalence of *Enterobacteriaceae* within our samples and merely 36 % of all samples were below the guidance values defined by the DGHM. In almost half of the samples, the DGHM critical value for

Table 3

Results of whole genome sequencing of STEC isolates from raw beef imported in the EU from third countries.

Isolate	Origin	Predicted serotype	Phylogenetic group	MLST	<i>stx</i> -subtypes	<i>eae</i> gene	Additional Virulence-associated factors
Ha28	Argentina	O178:H7	B1	ST278	<i>stx1a</i> , <i>stx2c</i>	–	<i>astA</i> , <i>fimH</i> , <i>gad</i> , <i>iha</i> , <i>iss</i> , <i>lplfA</i> , <i>ompT</i> , <i>terC</i>
Ha91	Argentina	B17:H19	B1	ST6661	<i>stx1a</i> , <i>stx2a</i>	–	<i>celb</i> , <i>cia</i> , <i>fimH</i> , <i>ehxA</i> , <i>espP</i> , <i>gad</i> , <i>ompT</i> , <i>terC</i>

Enterobacteriaceae was surpassed and they were consequently evaluated as microbiologically unsatisfactory (DGHM, 2018). There was a weak correlation between high ACC values and high values of *Enterobacteriaceae* within the same sample. Notably, despite the high *Enterobacteriaceae* counts, *Escherichia coli* was not prominent in our samples.

To date, there are no comparable studies available focusing on the detection frequencies of *Enterobacteriaceae* in raw beef imported into the EU. Only one study has examined the microbiological metrics of frozen poultry and pork meat imported into the EU from regions of similar origin as our samples, also following ISO standard methods (Jansen et al., 2018). This research detected *Enterobacteriaceae* in almost all samples, but in contrast to our results, the majority of the samples was below the guidance value by DGHM and less than 1 % of poultry meat samples surpassed the critical value for *Enterobacteriaceae* (Jansen et al., 2018). In a previous study examining raw beef imported into the USA from Uruguay, the same country of origin as some of our samples, their results were different from ours. The authors observed a lower frequency of contamination (31.3 %) and also a much lower mean *Enterobacteriaceae* count of 2.0 log₁₀ cfu/g than we obtained (4.76 log₁₀ cfu/g). One other study by Stella et al. (2013) examined raw beef imported from Argentina to Italy. However, as the authors used swab sampling and determined cfu/cm², quantitative results from that study are not directly comparable to our results. The authors reported a low level of bacterial contamination in their 24 samples, and *E. coli* was not detected in any sample (Stella et al., 2013). Overall, the comparability of these previous studies with our results is somewhat limited, as frozen meat was examined in those studies, which may have resulted in lower numbers of culturable bacteria. In addition, methodological differences compared to the standard methods used in our study may have also affected detection rates and numbers.

Apart from their role as hygiene indicators, certain *Enterobacteriaceae*, such as strains of *E. coli*, can also be pathogenic to humans (Kotloff et al., 2013; Woodward et al., 2019). Shiga toxin-producing *E. coli* in particular are of significance to public health because they can cause conditions like severe gastroenteritis, hemolytic uremic syndrome (HUS), thrombocytopenic purpura and hemorrhagic colitis (Gyles, 2007). Unlike in the EU, HUS is endemic in Argentina, with an incidence of 0.65 per 100,000 inhabitants and in 2019, a total of 290 HUS cases were reported (MSAL, 2020). The two STEC isolates in this study did not belong to the major serogroups (O26, O103, O111, O121, O145, and O157) that are primarily involved in human disease outbreaks in the EU (EFSA, 2022). Isolate Ha28 belongs to serotype O178:H7, which is a common serotype on beef in Argentina, whereas relatively few cases of human illness were associated with O178 infections and O178:H7 STEC in particular were only isolated from patients with uncomplicated disease (Masana et al., 2011; Miko et al., 2014). For isolate Ha91, WGS analysis predicted antigen sequence type B17, which was first described in *Shigella boydii* and some B17 STEC have been detected in food since then (Lacher et al., 2014, 2016). However, there are no reports on the detection of B17 STEC in raw beef to date and the detection of this serotype underlines the fact that raw meat products imported into the EU can be a source of unusual genotypes of *E. coli*, as previously reported by Müller et al. (2018). Both STEC isolates were negative for the *eae* gene, which encodes intimin, a relevant binding protein of pathogenic STEC (Minh et al., 2015). Nevertheless, it is known that *eae*-negative strains can also be involved in causing severe human diseases, such as HUS (EFSA, 2022; Newton et al., 2009). Both isolates carried more than one other adherence factor (*iha*, *lpfA*, *ompT*, *fimH*) and isolate Ha91 further harbored the enterohemolysin-encoding gene, *ehxA*, which is considered a critical factor in STEC pathogenesis (Hua et al., 2021). In this work, the detection frequency of STEC with 2 % is comparable to the official data of the EU One Health Zoonoses Report with a prevalence of STEC in bovine meat in the EU of 1.7 % in 2021 (EFSA, 2022). Comparable data on the overall prevalence of STEC in Argentina and Brazil in raw beef is limited. Recent studies in Brazil reported detection frequencies of 0 %, 2.8 % and 10 % in beef samples, respectively, and STEC

isolates in these studies do not belong to the major serogroups (O157, O45, O103, O111, O121, O145), either (Peresi et al., 2016; Ristori et al., 2017; Santos et al., 2018). In Argentina, previous studies with a focus on the detection of non-O157 STEC demonstrated a prevalence of up to 25 % in retail beef cuts from nine beef exporting abattoirs and 9 % in beef carcasses, respectively (Etcheverría et al., 2010; Masana et al., 2011). In contrast, a lower prevalence of 5.8 % in raw beef cuts produced for export and local markets was detected in 2014 by Brusa et al. (2017). These lower detection frequencies may be due to the improvement of intervention measures to reduce STEC on carcasses by The National Service of Agrifood Health and Quality of Argentina in 2014 (Brusa et al., 2019; SENASA, 2014).

Data regarding the prevalence of *Salmonella* spp. in imported raw beef into the EU is rare. In the previously mentioned study by Bosilevac et al. (2007), a very low *Salmonella* spp. prevalence of 0.4 % in raw beef imported into the USA from Uruguay was reported. Also taking into account that none of the samples in the present studies tested positive for *Salmonella* spp., imported beef does not appear to be a significant source of exposure for consumers.

As imported raw beef is stored refrigerated for extended periods during transportation, the growth of psychotropic bacteria such as *L. monocytogenes* may be an additional concern (Jones & D'Orazio, 2013). However, in our study the prevalence of *L. monocytogenes* was low and none of the samples examined quantitatively surpassed the critical value defined by DGHM. Previous studies investigating the prevalence of *L. monocytogenes* in meat intended for import into the EU reported variable results. Stella et al. (2013) reported the absence of *L. monocytogenes* in Argentinian frozen raw beef, while Teixeira et al. (2019) observed a *L. monocytogenes* prevalence of 12 % in raw beef from export-authorized processing plants in Mato Grosso, the state with the highest beef-export activity in Brazil.

5. Conclusion

Overall, the presence of foodborne pathogens, also including *S. aureus* and ESBL-producing *Enterobacteriaceae*, was low and no increased consumer exposure is expected from raw beef imported from third countries compared to raw beef produced in the EU (EFSA, 2022). Nevertheless, an uncommon STEC serotype was detected indicating that imported raw beef constitutes a potential source for the dissemination of STEC serotypes that differ from those commonly reported in the EU. Most notably, high ACC values and the DGHM critical value exceedances for *Enterobacteriaceae* in a considerable number of samples are of concern, indicating potential hygiene deficiencies. This was observed independently of the countries of origin. Due to the limited sample size of the samples examined in this study, the findings need to be confirmed by further studies, preferably with larger sample sizes. Nevertheless, our findings highlight the importance of adhering to hygienic conditions throughout the meat production and transport chain in order to ensure a hygienically sound product even after long transportation and storage times.

CRedit authorship contribution statement

Nicola Rinn: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ann-Sophie Braun:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Anja Müller:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Katharina Wadepohl:** Writing – review & editing, Resources, Investigation, Formal analysis. **Bettina Gerulat:** Writing – review & editing, Validation, Resources, Formal analysis. **Franziska Kumm:** Writing – review & editing, Validation, Methodology, Formal analysis. **Min Yue:** Writing – review & editing, Validation, Methodology, Formal analysis. **Corinna Kehrenberg:** Writing – review & editing, Writing – original draft,

Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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