Low prevalence of *Salmonella enterica* in cull dairy cattle at slaughter in Northern Italy

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Abstract

In order to evaluate *Salmonella* carrier status of cull dairy cattle at slaughter, 125 animals were randomly selected during the period February-May 2016. Dairy cows were reared in 89 farms located in two regions of Northern Italy (Lombardy and Emilia-Romagna regions), where bovine milk is primarily used for Parmigiano-Reggiano cheese and Grana Padano cheese production. Samples were collected by swabbing a 400-cm² area of the brisket hide and by rectoanal mucosal swabs. They were tested following the reference ISO 6579 method and the isolates were serotyped following the Kauffmann-White-Le Minor scheme and genotyped by XbaI PFGE. *Salmonella* was detected in 1.6% of the brisket hide samples (2/125) (95% CI: 0.4-5.6) and never found in faecal samples (95% CI: 0.3%). The positive cattle were reared in two farms located only in Emilia-Romagna region. The isolates were typed as *S. Derby* and *S. Seftenberg*. The comparison of the pulsed-field gel electrophoresis (PFGE) patterns of the bovine strains with all the PFGE patterns of the same serotypes responsible for human salmonellosis cases notified in Emilia-Romagna region in the years 2013-2015 did not find any correspondence. Therefore, the role of cull dairy cattle in transmitting *Salmonella* to humans seems to be less important than those of pigs and poultry in EU, but more data are needed for completing attribution source studies.

Introduction

*Salmonella* is an important zoonotic pathogen, often reported with high prevalence in animal production. It is the second most common foodborne pathogen in the European Union, with 88,715 confirmed laboratory cases reported in 2014. Human foodborne salmonellosis may be frequently acquired by eating raw or undercooked food of animal origin, as eggs, meat, milk and products thereof (EFSA and ECDC, 2015a).

To date, some studies on attributing foodborne salmonellosis in humans to animal reservoirs are based on the prevalence of *Salmonella* in animals and food from the EU-wide Baseline Studies conducted in the years 2006-2008 and from the European Union Summary Reports published by the European Food Safety Authority (EFSA) from 2006 to 2009, while data from human cases of salmonellosis are collected from The European Surveillance System (TESSy) administered by the European Centre for Disease Prevention and Control (ECDC) (De Knegt et al., 2015a). These attribution estimates suggest that layer hens were the most important source of salmonellosis in the EU in the study period, followed by pigs. Turkeys and broilers were found to be particularly important only in a few countries, as Denmark and Portugal respectively (De Knegt et al., 2015b).

These studies suggest that the role of cattle in transmitting *Salmonella* infection to humans by consumption of contaminated food is less important than other animal species. Nevertheless, if compared to pig and poultry data, cattle data collected by EU countries were in general poor and sometimes referred to clinical isolates only (De Knegt et al., 2015a), thus suggesting that more efforts are needed to monitor *Salmonella* contamination of food of bovine origin. For example, the prevalence of *Salmonella*-positive bovine carcasses reported by 13 EU Member States was 0.23% in 2014. In the same year, 225 *Salmonella* strong-evidence outbreaks were reported: pig meat and products thereof accounted for 9.3% outbreaks, broiler meat and products thereof for 3.6%, bovine meat and products thereof for 2.2%. Another category of meat, defined as *meat and meat products* accounted for 3.1% of the strong-evidence outbreaks (EFSA and ECDC, 2015a). To study the role of cattle in human foodborne salmonellosis, raw milk at farm and carcasses at slaughter should be more frequently investigated. As verocytotoxin-producing *Escherichia coli* (VTEC) contamination of bovine carcasses at slaughter may occur via the faecal route or contaminated hide (EFSA, 2007), the same could occur also for *Salmonella* contamination (Narváez-Bravo et al., 2013). *Salmonella*-carrier cattle may end up as contaminated carcasses at slaughter or may contribute to cross-contamination of other carcasses during processing.

The main aim of the study was to investigate the prevalence of *Salmonella* carriers among cattle at slaughter in Northern Italy and to compare the proportion of positive animals by testing cattle hide and faecal matter. The objective was also to investigate if the *Salmonella* isolates detected in cattle were responsible of notified human salmonellosis cases in the same geographic area (Emilia-Romagna region) of Northern Italy.

Materials and Methods

Sampling

From February to May 2016, a total of 125 cull dairy cattle at slaughter was randomly selected to be tested for *Salmonella*. Sampling collection was performed during eight visits, spaced one to two weeks. The number of animals tested per visit ranged from 10 to 20 (average number: 15.6). Dairy cattle were reared in 89 farms located in the provinces of Cremona, Mantua, Parma, Piacenza and Reggio Emilia (Lombardy and Emilia-Romagna regions, of Northern Italy). In those provinces bovine milk is primarily used for hard cheese production, as Parmesan cheese and Grana Padano cheese. Cattle were tested by swabbing a 400 cm²...
area of the brisket hide, which is considered at risk because the brisket area of the hide is cut during dehiding and is thus a likely source of carcass contamination (EFSA, 2007). Swabbing was performed by using sterile sponges moistened with Buffered Peptone Water (BPW) (3M Health Care, S. Paul, MN, USA). Faecal samples were collected by using sterile cotton-tipped swabs and swabbing an area (3 to 5 cm) inside the anal canal of each animal prior to slaughter. This kind of sampling is called rectoanal mucosal swabs (RAMS) and was used in similar studies (Agga et al., 2016; Beach et al., 2002). Swabs were immediately placed in sterile tubes containing 1 mL of Buffered Peptone Water (BPW; Oxoid, Basingstoke, UK). All samples were refrigerated after collection, transported to the laboratory within two hours and analysed on the same day. Confidence intervals in the observed prevalence were estimated by using the Wilson binomial approximation (Brown et al., 2001).

**Salmonella detection and typing**

A total of 250 samples (125 hide samples and 125 faecal samples) were tested following the ISO 6579:2002 method (hide samples) and the ISO 6579:2007 Amd.1 (faecal samples) (ISO, 2002, 2007). All samples were previously pre-enriched in BPW suspending each hide sponge in 90 mL and each RAMS sample in 9 mL of the liquid medium. The sponge suspensions were manually shaken for 2 min. The cultures were then incubated at 37±1°C for 18±2 h. Following the ISO 6579:2002 method, after incubation the sponge cultures were inoculated into 10 mL of Mueller-Kauffmann tetraionate broth (MKTT; Oxoid) and 10 mL of Rappaport-Vassiliadis soy broth (RVSB; Oxoid), with dilution ratios of 1:10 and 1:100, respectively. The MKTT and RVSB cultures were incubated at 37±1°C and 41.5±1°C for 24 h, respectively. After incubation, 10-µL of each culture was streaked onto Xylose Lysine Deoxycholate agar (XLD; Oxoid) and Chromogenic Salmonella agar (Oxoid) plates, incubated at 37±1°C for 24 h. Suspect colonies were seeded into Triple Sugar Iron Agar (Biolife, Milan, Italy), Lysine Iron Agar (Oxoid) and Christensen’s Urea Agar (Biolife) and incubated at 37±1°C for 20-24 h. Typical Salmonella colonies were tested by slide agglutination with an O-omnivalent Vassiliadis serum (Denka Seiken, Tokyo, Japan). Biochemical identification to the genus level was performed by using the API 20 E® microsubstrate system (bioMérieux, Marcy l’Étoile, France). Following the ISO 6579:2007 Amd.1 method, after incubation 100-µL of each faecal culture in BPW was seeded onto plates of Modified semisolid Rappaport Vassiliadis (MSRV; Oxoid) following ISO recommendations (3 drops placed separately on the plate surface). After incubation at 41.5±1°C for 24 ±3 h, the plates showing a grey-white zone extending out of the inoculum were further tested by streaking a 10-µL loopful of the bacterial growth onto XLD (Oxoid) agar plates. Plates not showing bacterial growth were incubated for further 24±3 h, after that they were considered negative if no grey-white growth has developed. XLD agar plates were incubated at 37±1°C for 24 h and suspect Salmonella colonies were selected and subjected to ISO 6579 confirmation tests as previously described. The reference Salmonella strain ATCC 14028 was used as positive controls.

Salmonella serotyping was performed according to the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen specific sera (Bio-Rad, Marnes-La Coquette, France; Denka Seiken, Tokyo, Japan; Sifin, Berlin, Germany). Salmonella pulsed-field gel electrophoresis (PFGE) typing was performed according to standard methods (PulseNet, 2010) with XbaI restriction of DNA.

**Results**

Hide sponges: Salmonella was detected in 1.6% of the brisket hide samples (2/125) (95% CI: 0.4-5.6). The positive animals were reared in two-arms located in Emilia-Romagna region. The two isolates were serotyped as S. Dublin and S. Senftenberg. The comparison of the PFGE patterns of the bovine strains to all the PFGE patterns of the same serotypes that were identified among human salmonellosis cases notified in Emilia-Romagna region in the years 2013-2015, did not find any correspondence. The human Salmonella isolates were sero-typed and PFGE-typed within the regular food-borne diseases surveillance activity by the Institute for Experimental Veterinary Medicine of Lombardy and Emilia-Romagna (Italy). The comparison of the pulsortypes of the two isolates of S. Dublin and S. Senftenberg with the PFGE profiles of the same serovars detected in cattle during the period 2011-2015, demonstrated that the PFGE type of S. Dublin detected in the present study was rather common among bovine population in Italy, while the pulsortype of S. Senftenberg was not frequently identified. RAMS samples: Salmonella was never detected in the faecal samples tested (95% CI: 0-3%).

**Discussion**

In the present study, Salmonella was detected from hide samples only thus confirming the role of cattle hide as likely source of Salmonella onto carcasses during slaughtering. Other studies support the role of hide in beef contamination at slaughter, even if a wide variation in prevalence has been reported. It ranged from 36.25% in Venezuela (Narváez-Bravo et al., 2013), 15.4% in the USA (Bacon et al., 2002) to 0.75% in Ireland (Ken et al., 2014). Very high prevalence values were reported by Brichta-Harhaj et al. (2008), which detected Salmonella in 89.6% of cull cattle hides in the USA, and by Fegan et al. (2005) which found 68% hide prevalence in Australia. These differences can be attributable to epidemiological factors, breeding conditions, management practices, transportation and lairage hygiene, analytical methods or sampling strategies.

In our study, all faecal samples were negative for Salmonella. This result can be attributable to different factors: i) Salmonella shedding by cattle is commonly intermittent (Wray and Sojka, 1977) and therefore faecal samples can test negative at slaughter; ii) hide contamination could have been previously acquired at farm, during transportation or at lairage from other shedding cattle or contaminated environment. As shown in a study by Beach et al. (2002), hide contamination with Salmonella increased in adult cattle prior and after transport from farm to slaughter from 20% to 56% and thus contaminated trucks and holding pens could play a role in Salmonella transmission to hides before slaughter. Regarding the use of RAMS instead of manual collection of faeces from the rectum (faecal grab; FG), RAMS is considered a more practical and reliable alternative to FG for assessing Salmonella fecal shedding status among cattle (Agga et al., 2016).

Considering that the prevalence on culled cows hides was very low, the negativity of rectal samples was a reliable finding. As a matter of fact, in other studies Salmonella prevalence on cattle hides was higher than in faeces as shown by Fegan et al. (2005), which detected 16% of positive faeces vs 68% of positive hides and by Narváez-Bravo et al. (2013), which found 13.75% of positive faeces vs 36.25% of positive hides.

In our study, the isolates detected on hides belonged to serovars frequently found in cattle. Infections by S. enterica subsp. enterica serovar Dublin (S. Dublin) is of concern in cattle industries in several countries because of economic losses and health injuries as high mortality, abortion and...
reduction in milk production (Carrique-Mas et al., 2010; Lewerin et al., 2011). In cattle, Dublin was the second most frequently detected serovar (29.4%) after Typhimurium (38.6%) in 2013 at EU level (EFSA and ECDC, 2015b). S. Dublin may be persistent in dairy farms, with an average duration of infection of approximately 2 years (Nielsen and Dohoo, 2013) due to its environmental survival (Findlay, 1972) and persistent infection in some animals (House et al., 1993). Uncommon but serious human infections are reported and are mainly attributed to the consumption of raw or undercooked beef or unpasteurised milk products (Helms et al., 2003; Maguire et al., 1992). As expected, S. Dublin is frequently reported among serovars detected in bovine meat in the European countries (EFSA and ECDC, 2015b).

*S. enterica* subsp. *enterica* serovar Senftenberg (*S. Senftenberg*) is not frequently reported from cattle, but in some studies conducted in the USA it was one of the most prevalent serovars (Fitzgerald et al., 2003). Noteworthy, if S. Dublin is closely related to cattle, S. Senftenberg can be transmitted to humans by a variety of foods, including poultry (Pedersen et al., 2008) and vegetables like basil and pistachios (CDC, 2016; Pezzoli et al., 2008). The isolation from cattle at slaughter in Italy strengthen the hypotheses that S. Senftenberg could survive on cattle hide and be transmitted from bovines to the slaughterhouse environment and, potentially, to beef carcasses.

### Conclusions

In this study a very low prevalence of *Salmonella* carriers among culled dairy cows at slaughter was detected. Nevertheless, since beef may be responsible for transmission of *Salmonella* to consumers and outbreaks from raw or undercooked beef products (as roast-beef and ground beef) can occur (Friesema et al., 2012; Laufer et al., 2016), bacteriological monitoring of cattle presented at slaughter is important for both epidemiological and attribution estimates. A recent attribution source study of human *Salmonella* infection routes of *Salmonella* in cattle at slaughter was detected. Nevertheless, to enable more detailed conclusions regarding contamination routes of *Salmonella* at slaughter, future studies involving higher numbers of cattle are needed. The present study can be thus considered a preliminary research to investigate the role of culled dairy cows as likely source of *Salmonella* to beef during slaughter operations.

### References


CDC, 2016. Multistate outbreak of *Salmonella* Montevideo and *Salmonella* Senftenberg infections linked to Wonderful Pistachios (final update). Centers for Disease Control and Prevention www.cdc.gov/salmonella/montevideo/03-16/


