Prevalence of *Salmonella, Yersinia, Aeromonas, Campylobacter,* and Cold-Growing *Escherichia coli* on Freshly Dressed Lamb Carcasses

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(MS# 95-27: Received 6 February 1995/Accepted 8 May 1995)

**ABSTRACT**

Thirty lamb carcasses were analyzed for selected bacteria just after slaughter. The frequency of isolation was *Salmonella* (10%), motile aeromonads (33%), *Yersinia enterocolitica* (20%) and cold tolerant *Escherichia coli* (56.6%). *Campylobacter* was not detected. Seven isolates of *Salmonella* (identified as *S. typhimurium*) grew at 7°C. The 16 isolates of motile aeromonads were identified as *A. hydrophila* (8 strains), *A. caviae* (7 strains), and unidentified (1 strain). The percentage of carcasses carrying presumptively virulent aeromonads was 13.3%. None of the 21 strains of *Yersinia enterocolitica* were found to be presumptively virulent. Among them, 3 belonged to biovar 1 and 4 to biovar 3, 3 lacked one property of biovar 3, and 11 had properties of both biovars. Of the 85 cold-growing strains of *E. coli*, 31 were sorbitol negative. The O157 antigen was not detected among these isolates. The structure of abattoirs and slaughtering practices influenced the contamination of carcasses with certain organisms (i.e., *salmonellae* and *E. coli*).

Key words: Foodborne pathogens, lamb carcasses, psychrophaths, abattoir hygiene

The microbiology of raw meats is highly dependent on the conditions under which meat animals are slaughtered. Surfaces of carcasses are easily contaminated with microorganisms during skinning and evisceration. A variable percentage of these organisms are psychrophaths, which are potential spoilage organisms of chilled meats, and/or foodborne pathogens. Included in this latter group are gram-negative rod-shaped bacteria that are capable of both respiratory and fermentative metabolism of glucose, such as *Aeromonas hydrophila, Yersinia enterocolitica,* and several types of diarrhea-producing *Escherichia coli.* In addition, certain pathogenic bacteria (e.g., *Campylobacter*) survive better at refrigeration temperatures, and there are strains and serovars of *Salmonella* with the ability to grow at low temperatures (27).

Taking into account that chilled raw meats are frequently temperature abused, that is, stored between 5 and 10°C, we considered it of interest to investigate the incidence of selected gram-negative bacteria (capable of growth at low temperatures) on lamb carcasses obtained at commercial abattoirs with different hygienic statuses.

**MATERIALS AND METHODS**

**Abattoirs**

Three commercial abattoirs located at three different towns in the province of León (Spain) were selected. On the basis of their technical structure and slaughtering practices, they were classed as A (excellent), B (acceptable), and C (poor).

**Sampling**

The abattoirs were visited twice and 30 lamb carcasses were examined. On each visit, 5 carcasses were removed immediately after final washing and hung from a detention rail adjacent to the main line. Surface areas (50 cm² on the neck, leg, and flank) were sampled using the double-swab technique (25) and dipped into maximal recovery diluent (MRD)(BBL). Samples were carried in re-frigerated containers to the laboratory within 3 h of collection and cultured on the same day. Swabs from the three sampled sites were blended (Colworth 400 Stomacher, Lab Blender, London) for 2 min in MRD.

**Isolation, identification, typing and virulence of Yersinia**

Two milliliters of the homogenate were placed into flasks with 18 ml of three enrichment media (phosphate-buffered saline[PBS], pH 7.6; PBS-sorbitol-bile salts, pH 7.6; and bile oxalate sorbose broth[BOS] [23]) and incubated at 4°C for 21 days.

Following enrichment, a loopful of each broth was plated on cefsulodin-Irgasan-novobiocin medium (C1N) (Oxoid) with postenrichment alkali treatment (24). After incubation (2 days at 28°C), suspect colonies (3 per plate) were inoculated onto triple sugar iron (TSI) (Oxoid) and Christensen's urea medium (Oxoid) (28). Isolates showing typical characteristics of *yersinias* were tested for motility (25°C), ly-sine and ornithine decarboxylase, gelatinase, citrate (25°C) and acid production from cellobiose, rhamnose, sucrose, melibiose, sorbose, sorbitol, trehalose, raffinose and -methyl-D-glucoside (1, 2, 9).
Biotyping of *Yersinia* isolates was carried out according to Bercover et al. (3). Additional tests were for DNase, lipase (Tween 80), indole, xylose, and nitrate reduction (9).

The markers of virulence investigated were calcium dependence (2, 10), autoagglutination (14), pyrazinamidase activity (13), salicin and esculin fermentation (13), and type of growth on Congo red-magnesium oxalate agar medium (CRMOX) (22). *Y. enterocolitica* ATCC 9610 was included as a control.

**Isolation, identification and serotyping of Salmonella**

Twenty-five milliliters of the homogenate were inoculated into 225 ml of selenite cystine broth (SC) (Oxoid) and tetrathionate broth (T) (Oxoid) and incubated at 37°C for 48 h, and at 41°C for 24 h, respectively.

The SC and T broth cultures were streaked onto brilliant green agar, bismuth sulphite agar, and xylene-lysine-deoxycholate agar after both 18 to 24 h and 48 h incubations and incubated at 37°C for 24 h. Typical colonies were picked onto slants of TSI agar and Lysine agar. Suspect colonies were subjected to biochemical testing (6). Confirmation and serotyping of isolates were kindly done by the Virology and Microbiology National Centre, Majadahonda, Madrid.

**Campylobacter**

A most-probable-number procedure was used (20). *Campylobacter*-like colonies were selected from each selective agar medium (Butzler's agar and Skirrow agar) (Oxoid) and examined for Gram stain, cell morphology, motility, catalase, hyphurate hydrolysis and other biochemical characteristics (26).

**Aeromonas**

Aliquots of 1 ml of an MRD homogenate and its decimal dilutions were distributed on three plates (0.3, 0.3, and 0.4 ml) of starch-ampicillin agar (19). After incubation overnight at 28°C, the plates were flooded with 5 ml of Lugol's iodine solution (18). Gram-negative, oxidase-positive isolates were biochemically tested with the schemes of Popoff (21), Palumbo et al. (18), and Joseph et al. (12). Elastase production (29) and hemolytic activity (7) were also investigated. *Aeromonas hydrophila* ATCC 7966 was included as a control.

**Psychrotrophic coliforms and E. coli**

The *E. coli* "completed test" (11) was performed. Incubation of the second broth at 35°C allowed recovery of most of servars. Suspect colonies from plates of Levine eosin-methylene blue (LEMB) agar (BBL) were inoculated into nutrient broth (Oxoid) and incubated at 7°C for 10 days. The population was identified using the API20E System (Vercieu, France) and tests suggested by Hitchins et al. (11). *E. coli* isolates were subjected to the EC O157 latex agglutination test (Oxoid) (17).

**RESULTS**

*Campylobacter* was not detected. A total of 6 carcasses yielded *Yersinia*. The presence of *Aeromonas* and *Salmonella* was detected on 10 and 3 carcasses, respectively. The number of isolates and the species found are presented in Table 1. Of the 21 *Y. enterocolitica* strains, 3 belonged to biovar 1 and 4 to biovar 3, 3 lacked one property of biovar 3, and 11 had properties of both biovars. None were found to be presumptively virulent using autoagglutination, calcium dependency, and type of growth on CRMOX. A total of 16 isolates fermented salicin and esculin.

All motile aeromonads were able to grow at 7°C. They were identified as *A. hydrophila* (5 strains) and *A. caviae* (3).

**DISCUSSION**

According to this study, cold-growing salmonellae and motile aeromonads are probably the most prevalent psychrotrophic pathogens on freshly dressed lamb carcasses. In addition, the rate of detection of the organisms studied was strongly dependent on the abattoir characteristics. The percentage of lamb carcasses carrying *Salmonella* just after slaughter was 10%. Seven strains of *Salmonella* (identified as *S. typhimurium*) grew at 7°C. They were detected on carcasses sampled at abattoir C (poor hygienic conditions) from lambs belonging to different flocks, and slaughtered on different days. It appears that the abattoir conditions can influence the contamination of meat by this organism.

Motile *Aeromonas* species were isolated from 33.3% of the lamb carcasses. The percentage for presumptively virulent strains was 13.3%. Our results and those of Majed et al. (15, 16) suggest that the incidence of motile aeromonads on lamb is lower than that found on other red meats. The ability of motile aeromonads to grow at low temperatures likely contributes to the higher percentages of contamination in ground meat and other products sampled at the retail level. Six carcasses carrying motile aeromonads were slaughtered at abattoir B (acceptable hygienic conditions), 3 at abattoir C, and only 1 at abattoir A (excellent hygienic conditions). Since intestinal carriage cannot totally explain the incidence of *Aeromonas* in red meat (4), water used in washing could be a source of contamination.
The 23 strains of *Yersinia* identified to species level correlated in all characteristics with the descriptions of *Y. enterocolitica* (21 isolates) and *Y. kristensenii* (2 isolates). Only 2 isolates could not be assigned to known species. One of the strains correlated best with *Y. enterocolitica* biovar 5 and the other shared properties of *Y. frederiksenii* and *Y. pseudotuberculosis*. *Y. enterocolitica* was detected on 20% of the lamb carcasses but none of the isolates were found to be presumptively virulent.

The frequency of isolation of psychrotrophic *E. coli* was 56.6%. Contamination of the lamb carcasses ranged from 10% (abattoir A) to 100% (abattoir C), the figure for abattoir B being 60%. These data and those obtained with salmonellae and *Enterobacter* (only detected at abattoirs B and C) suggest that the structure of abattoirs and slaughtering practices may influence contamination with certain Enterobacteriaceae.

The O157 antigen was not detected among the 31 sorbitol-negative *E. coli* isolates. It must be noted that the incidence of this organism on lamb is very low (5). Nevertheless, lamb carcasses carrying *Salmonella*, *Enterobacter* and *Y. enterocolitica* were also contaminated with cold-growing *E. coli*. On the other hand, this organism was not found on the majority of carcasses sampled at abattoir A, where carcasses being 60%. These data and those obtained with salmonellae of the lamb carcasses but none of the isolates were found to be due to the low incidence of the organism in sheep (8) as well as to difficulties in isolating it in the presence of competitive flora.

The structure of abattoirs and slaughtering practices apparently influenced the contamination of carcasses with certain organisms (i.e., salmonellae and *E. coli*). Nonetheless, more research is needed before drawing definite conclusions.

**ACKNOWLEDGMENTS**

The authors acknowledge the financial support of CICYT (Project ALI-88-405) and Diputación Provincial de León.

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