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Hazard Analysis of *Escherichia coli* O157:H7 Contamination during Beef Slaughtering in Calvados, France

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ABSTRACT

To identify hazard points and critical points during beef slaughtering, which is a necessary first step toward developing a hazard analysis and critical control point system to control meat contamination by *Escherichia coli* O157:H7, samples (n = 192) from surfaces, work tops, worker's hands, and beef carcasses were collected from a slaughterhouse in Calvados, France. Five strains of *E. coli* O157:H7 were isolated from a footbridge and a worker's apron at the preevisceration post and from a worker's hand at the defatting post. Three isolates carried *stx*2c, *eae,* and EHEC-*hlyA* genes and showed similar molecular types by random amplified polymorphic DNA, polymerase chain reaction IS*3*, and *Xba*I pulsed-field gel electrophoresis. Thus, this study has shown that preevisceration and defatting post and associated worker's materials are critical points for carcasses contamination by *E. coli* O157:H7 during beef slaughtering.

Escherichia coli O157:H7 and O157:H- (nonmotile) can be associated with mild diarrhea, hemorrhagic colitis, and the diarrhea-associated form of the hemolytic-uremic syndrome. Humans can become infected with *E. coli* O157:H7 and O157:H- following the consumption of contaminated foods or animals *(25).* Foodborne infections have mainly implicated undercooked ground beef and raw milk *(8, 10, 20). E. coli* O157:H7 and O157:H- can be harbored by healthy domestic animals, such as cattle *(4, 11, 25, 27)*, in the feces. Therefore, cattle have been regarded as a natural reservoir of this pathogen *(4, 12).*

Several outbreaks in the United States and Scotland *(3, 23, 33, 37),* affecting hundreds of people, have shown the necessity of knowing the prevalence of *E. coli* O157:H7 in cattle and beef carcasses. Based on this knowledge, a quantitative risk assessment concerning *E. coli* O157:H7 has been developed in Europe to clarify the European situation with regard to this pathogen *(7).* Quantitative risk assessment is a method used to organize and analyze scientific information to estimate the probability and severity of an adverse event. Applied to microbial food safety, the method can also help to identify those stages in the manufacture, distribution, handling, and consumption of food that contribute to an increased risk of foodborne illness and help focus resources and efforts to most effectively reduce the risk of foodborne pathogens. In the United States, after outbreaks in 1992 and 1993, the Food Safety and Inspection Service of the U.S. Department of Agriculture issued a "zero tolerance" policy that included drastic measures to control this pathogen during slaughtering *(14, 15, 34).* In 1996, however, a new approach to meat and poultry inspection, using the concept of hazard analysis and critical control point (HACCP) systems, was published by the Food Safety and Inspection Service to ensure product safety. It included microbiological performance criteria for evaluating slaughtering facilities in respect to *E. coli* and *Salmonella (16).* HACCP principles can be found in a number of European Community directives for meat, poultry, and fish *(38).* Lack of knowledge about points that are critical to controlling microbiological contamination in meat production has impeded the development of effective HACCP systems *(9, 35).* Identification of critical points during the meat plant process, especially during beef slaughtering, is a necessary first step toward developing a fully functional HACCP system *(17, 18).*

E. coli O157 has been found in 0.28 to 7.9% of groups of cattle tested, with the highest numbers found in young animals *(10, 12, 19–21, 28, 40).* In slaughterhouses, levels of beef carcass contamination by *E. coli* O157:H7 of 0 to 0.5% have been reported *(6, 9, 12, 17, 20, 28, 40).* The generally low level of *E. coli* O157:H7 contamination in cattle and beef carcasses seems to indicate that although certain improvements in the slaughtering process are needed to reduce this bacterial hazard, drastic measures are not. Critical points during beef slaughtering should be identified, and formal hazard management procedures of the HACCP type must be established.

In this study, we evaluated the hazard of *E. coli* O157:H7 contamination during beef slaughtering at 18 critical points in a Calvados slaughterhouse.

MATERIALS AND METHODS

Meat and surface sampling. Samples of beef carcasses (n = 48) and surfaces (n = 144) were collected during an 8-week period from a French slaughterhouse. The slaughtered cattle, aged 2 to 7 years, had been bred and calved on various farms of the Calvados region. Sampling of beef carcasses followed the procedures of sterile excision described in the Association Française de Normalisation standard *(1).* Briefly, after delimitation of an area

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TABLE 1. *Work stations and sites (workers and equipment) from which samples were taken to detect* E. coli *O157:H7*

Work stations	Sampling sites (no. of samples)
Bleeding	Knife (8)
Preevisceration	Footbridge (8)
	Apron (8) and metallic glove (8)
Oesophagus tying	Metallic glove (8) and cuff (8)
Evisceration	Metallic glove (8) and cuff (8)
Defatting	Metallic glove (8) and cuff (8)
	Disposable latex glove (8)
	Knife edge (8)
	Operator's hand before (8) and after (8) washing
Carcass weighed	Operator's hand before (8) and after (8) washing
Cold room	Cold room door (8)
Floor	Surface (8)

of 25 cm^2 with a disinfected metal frame, thick meat samples (2 to 3 mm) were removed from the carcass with a sterile scalpel. Samples, representing a total area of 225 cm^2, were collected from outside and inside neck and flank, middle of shoulder, brisket center line, and posterior hocks. Since the carcass conveyor belt in this slaughterhouse is not automated from the weighing station to the cold room, samples were taken from the contact point between the worker's hand and the carcass where he pushes the carcass into the cold room. Surface samples were taken by swabbing (sterile wipes, ATL, Humeau Laboratories, La Chapelle-sur-Erdre, France) at 18 different critical points of the slaughtering environment as shown in Table 1.

Detection and characterization of *E. coli* O157. Samples of tissue were diluted 10 times in buffered peptone water. Wipes were homogenized in 100 ml of buffered peptone water. These inoculated broths were incubated 6 h at 37 ± 1°C. An immunomagnetic separation method (Dynabeads anti–*E. coli* O157, Dynal, Compiègne, France) was used according to manufacturer's instructions *(39, 42)*. The beads-bacteria complex was isolated on sorbitol MacConkey agar (Oxoïd, Dardilly, France) and sorbitol MacConkey agar supplemented with cefixime tellurite (Oxoïd, Dardilly, France). Plates were incubated at 37 ± 1°C for 18 to 24 h.

Typical colonies of *E. coli* O157:H7 were biochemically identified by the API 20E gallery (bioMérieux, Marcy l'Etoile, France) and serotyped with latex test *E. coli* O157 (Oxoïd, Basingstoke, England). Isolates were streaked on Columbia agar with 5% sheep's blood (bioMérieux) and on enterohemorrhagic agar with $CaCl_2$-washed sheep erythrocytes (Oxoïd) and incubated at 37 ± 1°C. α-Hemolysin was read after 22 h on Columbia agar with 5% sheep's blood and confirmed by the read of enterohemorrhagic agar after 3 h. Enterohemolyin was read after 22 h on enterohemorrhagic agar. Isolates were finally sent to the French Reference Center at Institut Pasteur of Paris (France) for confirmation of isolates identification.

After purification of these isolates, three polymerase chain reaction (PCR) assays on cells suspended in water were performed to detect (i) rfb_{O157}, marker of the O157 serogroup *(29)*; (ii) *eae (29)* and EHEC-*hlyA (29)* simultaneously; and (iii) the *stx* genes *(2, 26)*. PCR products (15 μl) were analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide. An endonuclease restriction of *stx*-Lin PCR products was carried out as described by Bastian et al. *(2)*. We used a video gel analysis system (Photo-Capt, Bioblock Scientific, Illkirch, France) to estimate molecular weight.

Molecular typing by RAPD and PCR IS*3*. Isolates were streaked on horse blood agar (bioMérieux) and cultured at 37 ± 1°C overnight. A single colony was inoculated into casein-peptone soya broth (Merck, Darmstadt, Germany), which was incubated for 18 h at 37 ± 1°C without shaking to the stationary growth phase. DNA was extracted with the Wizard genomic DNA purification kit (Promega, Madison, Wis.), except that cell pellets were washed with water before cell lysis. This washing of cell pellets is important because it prevents the appearance of weaker and less reproducible banding patterns. Each sample was analyzed twice, and each experiment included a negative control reaction in which DNA was replaced by distilled water.

Random amplified polymorphic DNA (RAPD) analyses were carried out using a method derived from that of Birch et al. *(5)*. Each 25-μl reaction contained one Ready-To-Go RAPD analysis bead (Pharmacia Biotech, Uppsala, Sweden), a 20-ng template DNA, 25 pmol of RAPD M13 primers *(5)*, and distilled water. Each Ready-To-Go bead contains a unknown amount of Amplitaq DNA polymerase and Stoeffel fragment, 0.4 mM deoxynucleoside triphosphates, 2.5 μg of bovine serum albumin, 3 mM $MgCl_2$, 30 mM KCl, and 10 mM Tris (pH 8.3). The PCR cycles consisted of an initial 5-min denaturation at 94°C followed by 40 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min.

IS*3* PCR was carried out using a method derived from that of Thompson et al. *(36)*. Each reaction contained one Ready-To-Go RAPD analysis bead (Pharmacia Biotech), a 20-ng template DNA, 25 pmol of PCR IS*3* primers *(36)*, and distilled water to a final volume of 25 μl. The PCR cycles consisted of an initial 5-min denaturation at 94°C followed by 50 cycles at 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min and a final elongation at 72°C for 7 min.

PCR products (10 μl) were resolved by electrophoresis in a 1.4% agarose gel stained with ethidium bromide. The amplified fragments were photographed using a Kappa CCD CF 8/1 camera (Pharmacia Biotech). Profile interpretation was standardized using Taxotron software (Institut Pasteur, Paris, France).

Molecular typing by PFGE *Xba*I. Pulsed-field gel electrophoresis (PFGE) was performed by a modification of the method described by Heuvelink et al. *(22)* with a contour-clamped homogeneous electric field DR-II apparatus (BioRad Laboratories, Richmond, Calif.). Classic plugs were replaced by drops deposited on a sterile petri dish for solidification of 50 μl of the mix (washed cells in 300 μl of 100 mM Tris [pH 7.5]–100 mM EDTA [pH 7.5]–150 mM NaCl carefully mixed with an equal volume of 2% agarose [Ultra-Pure Low Melting Point Agarose, Gibco BRL, Life Technologies Ltd., Paisley, England]). These drops, called frog eggs, permit a better cell lysis and DNA digestion. One frog egg from the sample, cut in half, was used to establish genomic profiles by PFGE.

The genomic patterns were analyzed with Taxotron software (Institut Pasteur) with cubic Spline algorithm *(30)* and compared with our laboratory PFGE database. To determine the relationship among different strains, we compared the number and size of fragments.

RESULTS AND DISCUSSION

E. coli are not naturally present on or in red meat, but they are present as the direct result of feces deposited on the carcass at one or several points between slaughter and packaging of meat. Some level of fecal contamination of a

TABLE 2. E. coli *O157:H7 isolates from the slaughterhouse characterized by PCR and serogrouping*

Strain no.	Sampling week	Sampling site	Presence of[a]: *stx*	*eae*	EHEC-*hlyA*[b]	Shiga toxins typing[c]	Serotyping[d]
1	1	Footbridge	−	−	−	−	O157:H7
2	2	Footbridge	−	+	−	−	O157:H7
3	7	Footbridge	+	+	+	*stx*2c	O157:H7
4	7	Apron	+	+	+	*stx*2c	O157:H7
5	7	Hand before washing	+	+	+	*stx*2c	O157:H7

[a] +, present; −, absence of PCR products *(26, 29)*.
[b] Gene detection was confirmed on enterohemorrhagic agar (bioMérieux).
[c] Typing of Shiga toxins by digestion of *stx*-Lin PCR products *(26)* by *Hinc*II according to the method of Bastian et al. *(2)*.
[d] Serotyped by the French Reference Center at Institut Pasteur of Paris (France) and confirmed for O serogroup by rfb_{O157} PCR *(29)*.

beef carcass during these operations is considered largely unavoidable, but control measures at different critical points could prevent this from spreading.

On 18 critical points of beef slaughtering evaluated during 8 sampling weeks in this study, four *E. coli* O157:H7 were isolated from material sampled (footbridge and apron) at the preevisceration post (Table 2).

One *E. coli* O157:H7 strain from the footbridge had the gene *eae* encoding intimin associated with enteropathogenic *E. coli* but not the hemolysin and Shiga toxin genes (Table 2). Because of the extrachromosomal situation of these genes, this strain could have been present and then lost during subculture *(24)*. Two other strains, from footbridge and apron, carried all the pathogenicity genes: *stx, eae*, and EHEC-*hlyA*. The digestion of *stx*-Lin PCR products by *Hinc*II shows two fragments with molecular weights of about 565 and 325 bp, characteristics of Stx2c toxin variants. This result reflects a level of carcass contamination that was assumed to be proportional to the prevalence of healthy carriers or animals shedding the pathogen *(13, 31)*. On the one hand, these steps in slaughtering correspond to the opening of the carcass with a knife to eviscerate it before going further. The skill of the given worker counts for a lot in preventing feces contamination due to accidental perforation of parts of the digestive tract. Decontamination of workers' aprons could be investigated as a control measure, but decontamination of the footbridge would be difficult because of the speed of the slaughter chain (one carcass per min) and would also create a safety hazard. The footbridge enables workers to reach the top of the carcass. Carcasses come in contact with the footbridge, and this could favor the cross contamination of the meat. Nonetheless, no meat samples from carcasses have been found to be contaminated during this study. At the same slaughterhouse, we found one positive sample in 255 carcasses analyzed. This isolate is an *E. coli* O157:H7–carrying *eae* gene, though not verotoxigenic (data not shown). These results from dairy cows do not lead one to expect a high risk of cross contamination by contact with the footbridge. On the other hand, this contamination could result from the skinning-dressing step. Fecal material residing on the hide of the carcass may come in contact with the newly exposed meat at that stage. This fecal material or soil could have come primarily from the slaughtered animal but also from cross contamination with fecal material from several other animals in the slaughterhouse yard pens.

Only one *E. coli* O157:H7 isolate carrying all the pathogenicity genes was detected on the operator's hand at the defatting post before washing and not after decontamination (Table 2). Hand washing with bactericidal soap seems to be an efficient control measure to avoid cross contamination between carcasses. However, Wilson et al. *(41)* reported cases of symptom-free carrier status with regard to this bacterium among slaughterhouse operators, which allowed not only person-to-person contamination but also the contamination of meat. In our case, the operator could have been such a carrier; however, this possibility was not investigated. Contamination could also result during the evisceration process. There is a possibility of significant contamination occurring if the gut is nicked during evisceration. Excessive handling during evisceration and trimming is likely to spread filth more evenly over the carcass surface. Failure to wash carcasses between evisceration and defatting, which could reduce the pathogen load, obviously cannot be used to explain why no contaminated carcasses were found during this study.

Molecular fingerprinting analyses of enterohemorrhagic *E. coli* O157:H7 isolates by RAPD, IS*3*-based PCR, and *Xba*I PFGE suggest that the three strains isolated the same week are similar. The isolates number 4 (Table 2) presents only a difference of one band rather than other fingerprints using IS*3*-based PCR. This may imply that the slaughterhouse was contaminated by a single strain or a clone.

This study has shown that preevisceration and defatting post and associated worker's materials are critical points for carcass contamination by *E. coli* O157:H7. These two steps in the slaughtering process have not usually been described in the literature, which identifies the skinning and evisceration steps as critical points *(12, 18),* even after Schnell et al. *(32)* showed that the microbial load on the carcass after evisceration is not significantly different than just after skinning. Improvement of hazard management *(9)* in this Calvados slaughterhouse through simple control measures should significantly reduce even the current low level of contaminated beef carcasses.

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