FECAL CARRIAGE OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* SEROTYPES O157:H7, O26:H11, O103:H2, O145:H28 AND O111:H8 IN FRENCH CATTLE

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Abstract – This paper presents data on the prevalence in French cattle of the « five major EHEC », defined as *E. coli* strains harbouring *stx* and *eae* genes, and belonging to one of the following serotypes: O157:H7, O26:H11, O103:H2, O145:H28 and O111:H8. Fecal samples were collected in 6 French slaughterhouses from 1,318 cattle (young bull or cows). Quantitative PCR screenings showed that 17.8 % of the samples tested positive for *stx* and one (or more) combination(s) of O group markers / *eae* variant. A total of 96 isolates including 33 pEHEC (*stx*-positive isolates, potentially EHEC, because harbouring genetic markers associated with major EHEC) and 63 attaching-effacing *E. coli* (AEEC: *stx*-negative strains, but have the same genetic profile as EHEC) were recovered. Of the 33 pEHEC isolated, 18 were pEHEC O157:H7. The prevalence of pEHEC (all serotypes included) was 4.5% in young dairy bulls, 2.4% in young beef bulls, 1.8% in dairy cows and 1.0% in beef cows. The prevalence of pEHEC positive cattle was significantly highest in the young dairy bulls ($p<0.05$).

I. INTRODUCTION

The main virulence factor of enterohemorrhagic *Escherichia coli* (EHEC) contributing to pathogenicity is Shiga toxin (Stx) (1). But, not all Shiga toxin-producing *E. coli* (STEC) are able to induce illness as accessory EHEC genes may also contribute to human disease. Besides the *stx* gene, typical EHEC possess the *eae* gene, coding for the intimin protein, implicated in attaching and effacing lesions in the intestinal cells (2). Moreover, epidemiological studies have shown that, in Europe, five serotypes are frequently involved in human outbreaks (3). Therefore, the French Agency for Food Safety defined five major EHEC as STEC belonging to serotypes O157:H7, O26:H11, O145:H28, O103:H2 and O111:H8 (4). More precisely, the serotypes O157:H7 and O145:H28 are known to be associated with the *eae*-γ subtype, whereas O26:H11, O103:H2 and O111:H8 harbour the *eae*-ß, *eae*-ε and *eae*-θ subtypes, respectively. The aim of this study was (i) to evaluate the usefulness of a PCR screening detecting *eae* subtypes in order to improve the specific detection of the 5 major EHEC in cattle feces (ii) to determine the prevalence of these 'five major EHEC' in French cattle, and more particularly in the categories used for the production of ground beef.

II. MATERIAL AND METHODS

Fecal samples were collected in French slaughterhouses, selected for inclusion in this study on the basis of their geographical location (covering the French cattle production area), from 1,318 cattle including 291 young dairy bull (YDB), 296 young beef bull (YBB), 337 dairy cows (DC) and 394 beef cows (BC), from October 2010 to June 2011. Following enrichment, samples were tested for EHEC-associated genetic markers with a sequential PCR-based approach (5). An initial phase identified *stx* and *eae* positive samples, which were then screened for the presence of the O group markers and the four *eae* variants.

Immunomagnetic separation (IMS)-based isolations of EHEC were performed from samples that tested positive for *stx* and one (or more) combination(s) of O group markers / *eae* variant. Immunoconcentrated bacteria were plated onto cefixime-tellurite-sorbitol-MacConkey agar (Oxoid, Dardilly, France) for *E. coli* O157, O103 and O111, onto cefixime-tellurite-rhamnose-MacConkey agar for *E. coli* O26 and onto cefixime-tellurite-raffinose-MacConkey agar for *E. coli* O145 as previously described (5, 6). Suspect colonies were tested by slide agglutination with serogroup specific antisera (Statens Serum Institut, Copenhagen, Denmark) and were characterized for the presence of O group markers, *stx* genes, *eae* subtypes and *fliC*$_H$ alleles.

III. RESULTS AND DISCUSSION

The initial screening showed that *stx* and *eae* genes were simultaneously detected in 629 (47.7%) of the 1,318 samples (Fig. 1, Table 1).

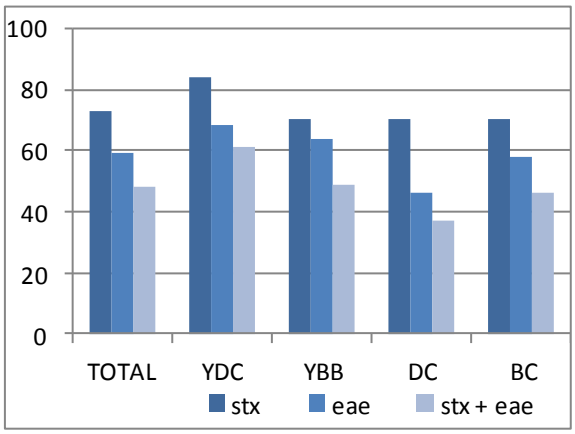

Figure 1. Percentage of *stx* and/or *eae* positive fecal samples (Total: n=1,318)

Whatever the category studied, the most frequently detected EHEC marker was *stx*. The percentage of *stx*-positive fecal samples was significantly higher in young dairy bulls (84.2%) compared to the three other categories (70.3%; 69.7% and 70.6%) ($p<0.001$). Moreover, the percentage of *eae*-positive fecal samples was significantly higher in young dairy bulls (68.4%) and in young beef bulls (64.2%) compared to dairy and beef cows (69.7% and 70.6% respectively) ($p<0.001$). Regarding the association of EHEC markers, samples positive for both *stx* and *eae* genes were more frequently detected in young dairy bulls (60.8%) compared to the three other categories ($p<0.001$).

The second screening showed that approximately one third (*i.e.* 235) of the 629 *stx* and *eae*-positive samples were also positive for at least one O group marker / eae variant combination. As several samples contained more than one combination, 363 IMS assays were performed.

A total of 96 isolates including 33 pEHEC and 63 Attaching-effacing *E. coli* (AEEC) were recovered, corresponding to an overall isolation rate of 26.4%, with differences between serotypes.

Table 1. Number of combinations of the 'top five' EHEC- associated genetic markers detected in 1,318 bovine fecal samples

Combinations of EHEC markers	No of combinations (% of positive samples)
stx/eae/rfbE$_{0157}$	216 (16.4%)
*stx/eae-*γ1*/rfbE*$_{0157}$	47 (3.6%)
stx/eae/wzx$_{026}$	202 (15.3%)
*stx/eae-*β1*/wzx*$_{026}$	129 (9.8%)
stx/eae/wzx$_{0103}$	262 (19.9%)
*stx/eae-*ε*/wzx*$_{0103}$	93 (7.1%)
stx/eae/wbdl$_{0111}$	27 (2.0%)
*stx/eae-*θ*/wbdl*$_{0111}$	14 (1.1%)
stx/eae/ihpl$_{0145}$	510 (38.7%)
*stx/eae-*γ1*/ihpl*$_{0145}$	80 (6.1%)

Of the 33 EHEC isolated, 18 were EHEC O157, 8 EHEC O103, 3 EHEC O26, 2 EHEC O145 and 2 EHEC O111. EHEC O157 was identified in the four bovine categories, whereas EHEC O103 was mainly identified in young bulls. The results showed that 2.4% of the 1,318 tested animals harboured EHEC. The proportion of EHEC positive animals in the dairy young bulls was 4.5%. This proportion was 2.4, 1.8 and 1.0 % in the beef young bulls, dairy cows and beef cows respectively (Fig 2).

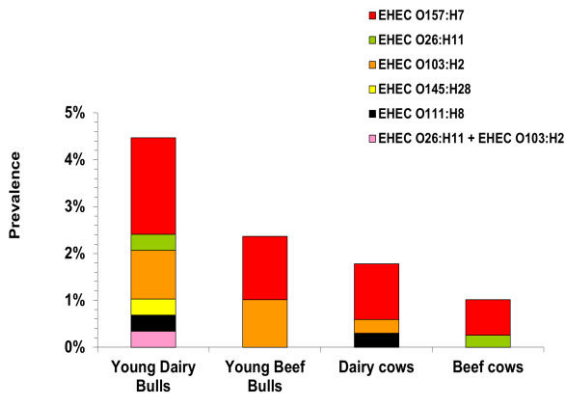

Fig. 2. Prevalence of bovine harbouring the "top five" EHEC per category.

The prevalence of EHEC O157:H7 was also significantly higher in the dairy young bulls. These results are in agreement with the results of PCR screenings showing that the simultaneous presence of *stx* and *eae* genes was significantly more frequently detected in feces from young dairy bulls compared to other categories. Overall, these results are consistent with the results of previous studies evaluating the influence of age of animal and farm type on STEC fecal shedding. A Scotland investigation on 14,856 cattle fecal samples showed that an increased probability of a sampling group containing a STEC O157:H7 shedding animal was associated with larger numbers of 12-30 months finish cattle (7). They also showed that a higher maximum age of animals in the sampling group was significantly associated with a lower prevalence of STEC O157:H7. Moreover, a review of published on farm prevalence surveys had already shown that 0.5 – 1% of sampled animals were *E. coli* O157:H7 carriers, and this percentage raised to 5% for latter weaned calves and heifers (8).

When taking into account all animals, EHEC O157:H7, the most prevalent serotype, was detected in the four cattle categories with prevalence ranging from 0.8% to 2.1%. These values are in agreement with previous results of European prevalence studies. The average proportion of STEC O157 positive samples, based on the investigation of a high number of feces or hides from animals sampled either at farm or slaughter, ranged from 0.2% to 2.3% for the 2009-2011 period (9, 10).

Concerning the non-O157:H7 EHEC serotypes, prevalence data directly comparable to our results are lacking in the literature. Recent studies focused on the detection of the top five STEC in cattle feces; but none of them led to the estimation of their prevalence, due either to a limited number of serogroup-specific strain isolation performed (11, 12). In our study, the four non-O157:H7 EHEC serotypes were detected in slaughtered categories at low prevalence, ranging from 0.0% to 1.0%.

Concerning the 63 AEEC, they included 34 AEEC O26, 19 AEEC O103, 8 AEEC O145 and 2 AEEC O157. Among the 1,318 tested animals, 4.6% harboured at least one AEEC.Lastly, the prevalence per category was weighted by the number of slaughtered bovine within each category, and the prevalence of STEC, all five serotypes combined, was estimated to 1.8% in French adult cattle slaughtered. To our knowledge, this is the first prevalence French cattle study taking into account the proportion of bovine slaughtered per category.

IV. CONCLUSION

The sequential PCR-based approach used in this study represents an interesting and valuable strategy for rapid screening of a large amount of bovine fecal samples.

When applied to 1318 fecal samples, this approach showed that the 'five major EHEC' were detected in French cattle with a low prevalence. EHEC O157:H7 remained the main isolated serotype whereas amongst EPEC, O26:H11 was the main isolated serotype.

Finally, the prevalence of STEC, all five serotypes combined, was low and estimated to 1.8% in French adult cattle slaughtered.

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