INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) is one of the 6 pathotypes of diarrheagenic *E. coli* that produces potentially fatal infant diarrhea, noticeably in developing countries. The aim of this study was to detect EPEC contamination by PCR at different stages of the chicken slaughtering process. We collected swabs from chicken cloacae and washed carcasses (external and visceral cavity) during the slaughtering process in 3 sampling occasions. Unwashed eviscerated carcasses were also sampled (at the visceral cavity) in the second and third sampling occasions. Enteropathogenic *Escherichia coli* was detected in 6 to 28% of cloacal samples, 39 and 56% of unwashed eviscerated carcasses, and 4 to 58% of washed carcasses. None of the samples were positive for *bfpA*, suggesting contamination with atypical EPEC. The detection of EPEC at different stages of the chicken slaughtering process showed that the proportion of contaminated samples remained or even increased during processing. In addition, the high proportion of contaminated carcasses during chicken processing represents a risk for the consumers and a challenge to improve procedures for those working in the sanitary control service.

Key words: enteropathogenic *Escherichia coli*, chicken, slaughter, polymerase chain reaction

ABSTRACT Enteropathogenic *Escherichia coli* is a foodborne pathogen that produces potentially fatal infant diarrhea, noticeably in developing countries. The aim of this study was to detect EPEC contamination by PCR at different stages of the chicken slaughtering process. We collected swabs from chicken cloacae and washed carcasses (external and visceral cavity) during the slaughtering process in 3 sampling occasions. Unwashed eviscerated carcasses were also sampled (at the visceral cavity) in the second and third sampling occasions. Enteropathogenic *Escherichia coli* was detected in 6 to 28% of cloacal samples, 39 and 56% of unwashed eviscerated carcasses, and 4 to 58% of washed carcasses. None of the samples were positive for *bfpA*, suggesting contamination with atypical EPEC. The detection of EPEC at different stages of the chicken slaughtering process showed that the proportion of contaminated samples remained or even increased during processing. In addition, the high proportion of contaminated carcasses during chicken processing represents a risk for the consumers and a challenge to improve procedures for those working in the sanitary control service.

Enteralpathogenic *E. coli* can also present a mechanism of initial binding to host epithelial cells mediated by a type IV pilus, with a major structural subunit encoded by bundle forming pilus (*bfpA*) gene present in EPEC adherence plasmid. Depending on the presence or absence of these pili, these strains are classified as typical and atypical, respectively (Trabulsi et al., 2002).

Enteropathogenic *E. coli* has been isolated from chicken feces (Kobayashi et al., 2002; Farooq et al., 2009) and from a variety of foods, including chicken products (Nataro and Kaper, 1998; González et al., 2000; Aratújo et al., 2002; Omaye, 2004; Nzouankeu et al., 2010). Food contamination could arise during the chicken slaughtering process, which is a highly coordinated system of mechanized operations that presents numerous opportunities for contamination. Before the slaughtering process, the chickens arrive at the processing plant in cages and are hanged and killed. Then they are scalded to facilitate the plucking process, eviscerated, and washed across the internal cavity and the external surface to remove blood, fat, tissues, and fecal material. Finally, they are chilled and packaged for distribution to consumers. Because no information is available about EPEC presence along the chicken slaughtering process, the aim of this study was to de-
Detection of eae and bfpA Genes

Sample Collection

In a chicken processing plant from Argentina, samples were taken on 3 occasions. On each sampling occasion, samples (cloacal swabs) from 100 hanged chicken before slaughtering and samples from 50 washed carcasses were collected. Washed carcasses were swabbed, after the chilling step, onto external surface and visceral cavity using a different swab for each surface. On the second and third sampling occasions, samples from unwashed eviscerated carcasses were also collected (23 and 18 samples, respectively). Unwashed eviscerated carcasses were swabbed, only across the visceral cavity surface, immediately after the evisceration process. Swabs were placed in Stuart transport medium and processed at the laboratory within 24 h.

Detection of eae and bfpA Genes

Cloacal and carcass swabs were processed according to the method described by Fernández et al. (2009) and Etcheverría et al. (2010) for samples of bovine origin, with modifications. Briefly, the swabs were cultured in Luria Bertani broth (cloacal samples) or buffered peptone water (carcass samples), with shaking (100 rpm) at 37°C for 18 h. An aliquot of these cultures was diluted in sterile double-distilled water and boiled for 10 min to obtain a crude DNA template. All samples were analyzed by a multiplex PCR that detects eae gene simultaneously with shiga toxin (stx) genes (Paton and Paton, 2002). Amplification products were separated by agarose gel electrophoresis in the presence of ethidium bromide and visualized with a UV transilluminator. In this study, all eae-positive but stx-negative samples were considered to be contaminated with EPEC and, consequently, were tested for the presence of bfpA by a monoplex PCR, described by Gunzburg et al. (1995). Escherichia coli O157:H7 strain EDL933 (eae+; supplied by J. Blanco, Reference Laboratory for E. coli, Lugo, Spain) and E. coli O157:H45 (bfpA+; supplied by A. Bentancor, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina) were used as positive controls. Double-distilled water was used as negative control. The detection of eae gene without the corresponding bacterial isolation is incomplete and is regarded as a presumptive diagnosis, but is valid as an indicator of EPEC contamination in stx-negative samples and is useful for the screening of this type of contamination.

RESULTS

Enteropathogenic Escherichia coli was detected by PCR in 19% of cloacal swabs, 49% of unwashed eviscerated carcasses, and 33% of washed carcasses considering all sampling occasions. All the eae-positive samples were negative for bfpA gene.

On the first sampling occasion, EPEC contamination was detected in a similar proportion in both cloacal and washed carcasses. However, on the second and third sampling occasions, contamination increased during the chicken slaughtering process from cloacal samples (before slaughter) to carcasses (both unwashed and washed; Table 1).

Among washed carcasses, EPEC contamination was detected in a similar proportion at external (9%), visceral cavity (13%), or both (11%) surfaces, considering an average from all sampling occasions. However, in the second sampling occasion, EPEC predominated at the visceral cavity surface of sampled carcasses (Table 1).

DISCUSSION

Samples from cloacae and carcasses were contaminated with EPEC at different stages of the chicken slaughtering process. It is important to clarify that eae-positive samples were stx negative (data not shown), ruling out the possibility of contamination with Shiga toxin-producing E. coli harboring stx and eae genes. None of the samples were positive for bfpA, suggesting that chicken samples harbored atypical EPEC, in agreement with results reported by Kobayashi et al. (2002), Krause et al. (2005), and Farooq et al. (2009) in this type of samples. This finding is also consistent with Trabulsi et al. (2002), who found that atypical

Table 1. Percentages of samples contaminated with enteropathogenic Escherichia coli at different stages of the chicken slaughtering process

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Cloacae</th>
<th>Unwashed carcasses</th>
<th>Washed carcasses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>E and V</td>
</tr>
<tr>
<td>1</td>
<td>6 (6/100)</td>
<td>ND&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2 (1/50)</td>
</tr>
<tr>
<td>2</td>
<td>28 (28/100)</td>
<td>56 (13/23)</td>
<td>12 (6/50)</td>
</tr>
<tr>
<td>3</td>
<td>22 (22/100)</td>
<td>39 (7/18)</td>
<td>12 (6/50)</td>
</tr>
<tr>
<td>Total</td>
<td>19 (56/300)</td>
<td>49 (20/41)</td>
<td>9 (13/150)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values in parentheses represent positive samples/samples collected.
<sup>2</sup>E: carcasses positive only at the external surface; V: carcasses positive only at the visceral cavity surface; E and V: carcasses positive at both the external and visceral cavity surfaces.; total: E + V + E and V.
<sup>3</sup>ND: not done.
EPEC serotypes were strongly associated with animal hosts. However, other researchers have isolated atypical EPEC from humans (Beatin et al., 2003).

Enteropathogenic *Escherichia coli* contamination differed among sampling occasions. On the first sampling occasion, the proportion of contaminated samples remained similar through different stages of chicken slaughtering whereas on the other sampling occasions the contamination increased. The first sampling was performed in autumn and the others in spring; therefore, the differences could be attributable to season-related factors. Furthermore, seasonal variability has been documented for EPEC in cattle and infants (Yabiki and Hamada, 1969; Amisano et al., 2011).

The prevalence of EPEC in chicken cloacal samples ranged from 6 to 28% among sampling occasions, indicating that chickens carry EPEC in their feces, in concordance with Kariuki et al. (2002), Kobayashi et al. (2002), Wani et al. (2004), Krause et al. (2005), and Farooq et al. (2009), who detected EPEC in chicken fecal samples with prevalences ranging from 2.3 to 15%. It is valid to consider that the sampling method and methodology used for sample processing may influence the variability in prevalence (Johnson et al., 1996).

High percentages (39 and 56%) of carcasses sampled before the washing step (unwashed carcasses) were contaminated with EPEC, suggesting an improper evisceration (intestinal breakage), which may increase carcass contamination with bacteria from the intestinal tract of the chicken. In the plant sampled in this study, the visceral package was removed from the carcass after the evisceration process. However, Russell and Walker (1997) reported that this system produced carcasses with much less visible contamination, total coliforms, and *E. coli* from eviscerated carcasses than another system in which the viscera remains attached to the carcass.

Jiménez et al. (2003) demonstrated that high percentages of carcasses with visible contamination after the evisceration step remained visibly contaminated after the washing step, suggesting that this step was not effective in removing the visible contamination and reducing microbiological contamination. In addition, in our study, EPEC was detected in washed carcasses after the chilling step in all samplings and in the water of one chiller during the third sampling (data not shown). This finding indicates that the chilling step could contribute to carcass contamination, although Huezo et al. (2007) demonstrated that chilling reduced the level of *E. coli* and coliforms contamination.

In washed carcasses, EPEC was found in a similar proportion at the external surface, visceral cavity, or both; however, in the second sampling a high proportion of carcasses were contaminated at the visceral cavity surface. No studies compare EPEC contamination at both surfaces of poultry carcasses, although reports exist about *E. coli*, coliforms, and other bacteria. Bodnaruk et al. (1998) found no differences of *E. coli* counts between external (breast skin area) and visceral cavity of turkey carcasses, but these areas were less contaminated than external tight or back skin areas. In another study (Smith et al., 2007), a fecal contaminant containing coliforms and *E. coli* was applied to the external and visceral cavity surface of eviscerated broiler carcasses. After the washing step, remaining fecal residue was occasionally observed in carcasses with external contamination, but no contaminant residue was observed inside carcasses with internal contamination, suggesting that contaminants adhere less readily to visceral cavity surface and that the washing step removes the internal contamination more easily than the external contamination.

To the best of our knowledge, ours is the first study to report EPEC contamination at different stages of the slaughtering process in a chicken processing plant. Enteropathogenic *E. coli* was found in cloacae, visceral cavity surface of unwashed eviscerated carcasses, and washed carcasses after chilling (at both surfaces, only at the external surface or only at the visceral cavity). Our results indicate that EPEC contamination remained or even increased during the chicken slaughtering process, suggesting that the washing step is not sufficient to reduce the contamination of eviscerated carcasses and that the chilling step could contribute to carcass contamination. Therefore, poultry processing plants should improve not only their evisceration process but also the efficiency of the washing steps and the hygienic measures throughout chicken processing in order to reduce EPEC contamination in the final products.

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