Research and characterization of *Escherichia coli* O157 strains isolated from ovine carcasses of two slaughterhouses of Algiers city

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Abstract. Objective: This study aims to identify and characterize *Escherichia coli* O157 strains isolated from ovine carcasses of two slaughterhouses of Algiers city. Material and method: One hundred and fifty-one (151) parts of sheep carcasses were swabbed by a non-destructive method based on double swabbing process (wet/dry) at two slaughterhouses of Algiers city for the identification and investigation of *E. coli* O157 strains. The *E. coli* O157: H7 isolation required a non-selective enrichment step followed by one of immuno-concentration of the bacteria throughout the immunomagnetic separation (IMS) technique and another one for bacteria isolation (on CT-SMAC agar - Cefixime Tellurite Sorbitol–MacConkey). The presence of O157 somatic antigen and H7 flagellar antigen in the isolated suspicious doubtful colonies was confirmed by the anti-O157 latex and H7 antisera agglutination assays. Confirmation of the virulence of the isolated *E. coli* O157: H7 strain was obtained performed by gene amplification (PCR – Polymerase Chain Reaction). Results: The presence of *E. coli* O157: H7 was shown in eleven ovine carcasses out of the 151 tested with a prevalence rate of 7.26%. Nine of these carcasses came from the first slaughterhouse and two of them from the second one. Amongst the eleven positive carcasses, thirteen strains of *E. coli* O157: H7 were isolated: ten strains are sorbitol and β-glucuronidase-negative, and three of them are sorbitol and β-glucuronidase-positive. Among them, seven strains (53.85%) showed an *eae* stx2 pathotype, one strain (7.69%) showed *eae* stx1 pathotype, one strain (7.69%) showed the *eae* pathotype, and three strains (23.08%) showed *stx2* pathotype. Only one strain (7.69%) had no gene encoding the virulence factors. Conclusion: The obtained results revealed that sheep are carriers of *E. coli* O157: H7 in Algeria. Appropriate hygiene and control measures at the slaughterhouse level must be implemented to provide a safe product for the consumer.

Key Words: *E. coli* O157, gene encoding the virulence factors, ovine carcasses, swabbing, Algiers City Slaughterhouses.

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Introduction

Since their first discovery, *Escherichia coli* O157: H7 strains and other enterohemorrhagic *E. coli* (EHEC) are known to be the main infectious agents responsible for hemorrhagic diarrhea, their main reservoir being represented by ruminants (Riley et al. 1983). These *E. coli* produce one or more Shiga-toxins (Stx), and that’s why they are called Shiga-toxins (Stx), and EHECs have an arsenal of virulence factors whose exact list and roles are yet to be determined. The two major proteins involved in their pathogenicity are, on the one hand, the intimin, encoded by the *eae* gene and responsible for specific lesions of the enterocytes known as “attaching-effacing lesions (A/E)” and diarrhea, and, on the other hand, the Shiga-toxins, encoded by the *stx1* and *stx2* genes, which are responsible of the death of intestinal, vascular and renal cells (Fegan & Desmarchelier 1999). The epidemics identified to date are mainly related to the consumption of food of bovine origin, but the ingestion of other foodstuffs and/or water, in direct contact with animals and fecal-oral human-to-human transmission are also implicated (Cappioli et al 2005). 5 to 10% of infected people, particularly young children and the elderly, develop severe complications, such as the hemolytic and uremic Syndrome (HUS) (Griffin & Tauxe 1991; Seibt et al 1997). Since 1982, EHEC, particularly those of O157: H7 serotype, have often been implicated in Hemorrhagic colitis epidemics (Cappioli et al 2005). 5 to 10% of infected people, particularly young children and the elderly, develop severe complications, such as the hemolytic and uremic Syndrome (HUS) (Griffin & Tauxe 1991; Seibt et al 1997). EHECs have an arsenal of virulence factors whose exact list and roles are yet to be determined. The two major proteins involved in their pathogenicity are, on the one hand, the intimin, encoded by the *eae* gene and responsible for specific lesions of the enterocytes known as “attaching-effacing lesions (A/E)” and diarrhea, and, on the other hand, the Shiga-toxins, encoded by the *stx1* and *stx2* genes, which are responsible of the death of intestinal, vascular and renal cells (Fegan & Desmarchelier 1999). The epidemics identified to date are mainly related to the consumption of food of bovine origin, but the ingestion of other foodstuffs and/or water, in direct contact with animals and fecal-oral human-to-human transmission are also implicated (Cappioli et al 2005). Since these germs are not systematically sought even in hospitals, their involvement as pathogens may go unnoticed. This study aims to assess the contamination level of ovine meat obtained in two slaughterhouses in Algiers by the *E. coli* O157. For this, we first sought the presence of these strains on the surface of the ovine carcasses and tried to confirm their virulence by the identification of the present genes.
Materials and methods

Samples
Sampling was carried out in two slaughterhouses of Algiers city. The animals slaughtered there came from different livestock markets throughout the region. A total of 151 samples were collected over a period of seven months at a rate of 5 to 10 ovine carcasses per week, randomly to look for *E. coli* O157. The sampling was carried out using a non-destructive method based on the double swab of carcass surfaces, defined by ISO 17604 norm. For each ovine carcass, four zones of 100 cm², all located in the posterior-external zone of the thigh, of the flank, of the large end of the chest and the posterior side of the front limbs were harvested. The samples were conveyed to the laboratory in a cooler, and stored at + 4°C waiting for their analysis, which were done within 24 hours.

*E. coli* O157 identifying method
The swabs of each half-carcass composing of a sample have undergone the following steps: pre-enrichment of the swabs in 100 ml of buffered peptone broth (EPT, Oxoid) for 6 hours at 37°C, followed by a magnetic immuno-concentration step (Dynabeads O157, Invitrogen, Merelbeke, Belgium) using 1 ml of the enrichment broth. 100 microliters of the concentrate (magnetic beads + bacteria) were seeded on the Mac Conkey sorbitol agar supplemented with cefixime-tellurite (CT-SMAC, Oxoid) and incubated at 42°C for 18 hours. Confirmation of three characteristic colonies per box was made with anti-O157 latex (Dry-spot *E. coli* O157, Oxoid) and with antiserum H7 (Biorad). Biochemical characterization was performed on API 20E galleries (BioMérieux) and on Rapid *E. Coli* 2 agar (Biorad) for the detection of β-glucuronidase activity.

Detection of virulence genes by PCR
The pathotype of *E. coli* O157: H7 strains was determined by simplex PCR (Fig. 1).

Table 1. Primer pairs used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer and Sequence (China et al 1996)</th>
<th>Size of PCR product (pb)</th>
<th>Optimal annealing temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eae</td>
<td>B52,AGGCTTCCGTCAAGTGGA, B53, CCATCGTCACCGAGGA</td>
<td>570</td>
<td>51.9</td>
</tr>
<tr>
<td>stx₁</td>
<td>B54,AGAGCGATGTTACGTTTG, B55, TACGCCCAAGATGGAAGTG</td>
<td>388</td>
<td>52.9</td>
</tr>
<tr>
<td>stx₂</td>
<td>B56,TGGGTTTCTCTCTGACCTAC, B57, GACATTCTGGTTGACCTAC</td>
<td>807</td>
<td>53.4</td>
</tr>
</tbody>
</table>

Fig. 1 shows the different DNA bands, each one corresponding to the size of the desired gene. The numbers (7 to 13) correspond to the numbers of strains confirmed at the Pasteur Institute in Algiers.

Well A to the left: negative control strain
Well B to the left: positive control strain carrying the eae (eae + stx₂ +) gene
Well C: Size marker (100 bp)
Well A to the right: negative control strain
Well B to the right: positive control strain carrying stx₂ (eae + stx₂+) gene

Results
Prevalence
Immunomagnetic separation tests and anti-O157 latex agglutination and H7 antiserum tests conducted on the 151 analyzed samples showed that eleven ovine carcasses were contaminated with *E. coli* O157: H7 with a prevalence of 7.26%. Among these eleven positive carcasses, thirteen strains of *E. coli* O157: H7 were isolated, four strains from two carcasses at slaughterhouse No. 1 and other nine from nine carcasses at the slaughterhouse No. 2. Among the thirteen isolated strains of *E. coli* O157: H7, ten strains were sorbitol and β-glucuronidase negative while three strains were sorbitol and β-glucuronidase positive.

Detection of virulence genes by PCR
The pathotype of *E. coli* O157: H7 strains was determined by simplex PCR (Fig. 1).
Table 2. Phenotypic and genotypic characteristics of *E. coli* O157: H7 strains

<table>
<thead>
<tr>
<th>Slaughterhouse</th>
<th>No of ovine carcasse</th>
<th>Strain</th>
<th>Serotype</th>
<th>Sorbitol and β-glucuronidase</th>
<th>Pathotype</th>
<th>Confirmation laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughterhouse n°1</td>
<td>1</td>
<td>1</td>
<td>O157:H7</td>
<td>eae stx2</td>
<td>Faculty of Liège</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>O157:H7</td>
<td>eae stx2</td>
<td>Faculty of Liège</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°1</td>
<td>3</td>
<td>3</td>
<td>O157:H7</td>
<td>eae stx2</td>
<td>Faculty of Liège</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>O157:H7</td>
<td>eae</td>
<td>Faculty of Liège</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°2</td>
<td>5</td>
<td>5</td>
<td>O157:H7</td>
<td>eae stx2</td>
<td>Pasteur Institute, Algiers</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°2</td>
<td>6</td>
<td>6</td>
<td>O157:H7</td>
<td>stx2</td>
<td>Pasteur Institute, Algiers</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°2</td>
<td>7</td>
<td>7</td>
<td>O157:H7</td>
<td>eae stx2</td>
<td>Pasteur Institute, Algiers</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°2</td>
<td>8</td>
<td>8</td>
<td>O157:H7</td>
<td>eae stx2</td>
<td>Pasteur Institute, Algiers</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°2</td>
<td>9</td>
<td>9</td>
<td>O157:H7</td>
<td>stx2</td>
<td>Pasteur Institute, Algiers</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°2</td>
<td>10</td>
<td>10</td>
<td>O157:H7</td>
<td>stx2</td>
<td>Pasteur Institute, Algiers</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse n°2</td>
<td>11</td>
<td>11</td>
<td>O157:H7</td>
<td>stx2</td>
<td>Pasteur Institute, Algiers</td>
<td></td>
</tr>
</tbody>
</table>

Among the thirteen strains isolated from the eleven ovine carcasses collected, seven strains (53.85%) showed the pathotype *eae* stx2, three (23.08%) were of the stx2 pathotype, one strain (7.69%) had the pathotype *eae* stx1, one strain (7.69%) had the pathotype *eae*, and one strain (7.69%) had no gene encoding for a virulence factor.

The phenotypic and genotypic characteristics of *E. coli* O157: H7 isolated strains is shown in table 2.

Discussion

Eleven (7.26%) of the 151 analyzed ovine carcasses were contaminated with *E. coli* O157: H7 pathogenic bacteria. Among the thirteen isolated *E. coli* O157: H7 strains, ten contained the stx2 gene that encodes for Shiga-toxin type 2. The strains producing the stx2 toxin are 1000 times more virulent than those producing stx1 (Joly & Reynaud 2003).

We were also able to isolate two sorbitol and β-glucuronidase positive *E. coli* O157: H7 strains carrying the stx2 gene. According to Armstrong et al (1996) this new phenotype is either as a result of mutation or to membership of this uncommon phenotypic variant to a primitive clone that existed for a long time.

According to Karch (1999), this variant is originally an EPEC O157:H7 strain which has retained the ability to ferment sorbitol and to produce β-glucuronidase, but has undergone a lateral transfer of the *rfb* region due to transfection, and thereby acquired the EHEC plasmid and the stx2 gene by phage conversion. Regarding these sorbitol β-glucuronidase-positive *E. coli* O157: H7 strains isolated in this study, although they lack the intimin coding *eae* gene, they are considered potentially pathogenic.

The same pathotype was demonstrated during an epidemic in France where bacteria responsible for gastroenteritis as well as hemolytic uremic syndrome only possessed the *stx2C* gene (Espié & Vaillant 2002). The strains isolated in this study are potentially pathogenic and may cause epidemics. One of the thirteen strains isolated had only the *eae* genes. This pathotype was isolated by Zweifel et al (2006) from sheep faeces.

HUS cases have also been associated with sorbitol β-glucuronidase-positive STEC O157: H- strains in Germany. This phenotype is responsible for majority of HUS cases in pediatrics in this country (Ammon et al 1999).

Studies investigating the contamination of ovine carcasses by serotype O157: H7 and other STEC are shown in the following results:

In Australia, 1,117 sheep carcasses and 560 samples of boneless and frozen sheep meat were analyzed for *E. coli* O157: H7. Six sheep carcasses (0.54%) and one sample of boneless frozen sheep meat (0.18%) were positive for serotype O157: H7 (Phillips et al 2006). The prevalence of this pathogen in Algeria (7.26%) exceeds thirteen times what was reported in Australia. In Switzerland, the study conducted on 580 sheep carcasses by Zweifel & Stephan (2003) as part of the control of sheep slaughter at three slaughterhouses revealed that 36.6% of the samples were positive for the stx genes by PCR, without specifying the serotype. In Germany was reported an isolation rate of 2.56% of *E. coli* producing Shiga-toxins in lamb meat and 72.4% in ovine carcasses (Beutin et al 1995). Chapman & Ashton (2003) reported that 0.7% of ovine carcasses were contaminated with *E. coli* O157: H7. Similarly, *E. coli* O157: H7 was isolated in 1.8% of the samples coming from Swedish slaughterhouses (Söderlund et al. 2012). However, no contamination was detected in lamb samples, in the study conducted by Zarei et al (2013).

All of these studies show and confirm that carrying Shiga toxin-producing *E. coli* (O157: H7 and other STEC serotypes) by ovine is important and presents a significant risk that must be prevented upstream and during slaughter operations. Both ovine and bovine are important reservoirs of STEC (Kudva et al 1996). Fecal carriage in sheep breeding may vary from 4.1% to 66.6% according to some studies (Heuvelink et al 1998; Beutin et al 1993). Fecal carriage in sheep breeding may vary from 4.1% to 66.6% according to some studies (Heuvelink et al 1998; Beutin et al 1993). The most frequently found serotypes are O91: H2 (Kudva et al 1996; Blanco et al 2003, Vettorato et al 2003), O128: H2 (Kudva et al 1996; Koch et al 2001; Blanco et al 2003), O146: H21 (Koch et al 2001; Vettorato et al 2003).
According to a study conducted in Switzerland, 30% of the analyzed excrements of slaughterhouse sheep were positive for STEC (Zweifel et al 2005). In Iran, 7.3% of ovine feces and 11.1% of sheep milk samples were contaminated with E. coli O157: H7 (Khudor et al 2012). The consequences of STEC poring by sheep are hazardous, as epidemics of E. coli O157: H7 infections related to contact with sheep or with sheep meat have been described (Chapman et al 2000). In July 2002, 11 cases of gastroenteritis, including two complicated cases of HUS, were identified in France among people who ate sheep meat in a sheep barbecue during a wedding (Espié & Vaillant, 2002). The STEC strain isolated from the stool of the HUS case belonged to serotype O148: H8. In 2006, 17 cases of gastroenteritis were reported in Norway due to the consumption of sausage made from ovine meat; the incriminated serotype is the O103: H2 carrying the stx2 gene (AFSSA, 2010).

The results of the present study show that STEC, and more precisely E. coli O157: H7, are found in sheep in Algeria which is also considered a potential reservoir of these pathogens. Appropriate preventive and control measures should be implemented, as with other ruminant species. The risk of foodborne illness due to STEC, and more precisely serotype O157: H7 must be taken into account in Algeria. In order to prevent the emergence of epidemic outbreaks, of which the main actor would be this highly virulent pathogen, as was the case in the 1980s in Anglo-Saxon countries, preventive measures should be considered to avoid the occurrence of an emergency and a serious crisis that would have children and the elderly as the first victims.

Conclusions
The results of the present study showed that STEC, and more specifically E. coli O157: H7, are carried by sheep in Algeria, which are potential reservoirs of these pathogens. Appropriate hygiene and control measures at the slaughterhouse level must be implemented in order to provide a safe product to the consumer.

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