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Research Article

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 antiserum 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 toxin *E. coli* (STEC) (Griffin & Tauxe 1991; Seibt et al 1997). Since 1982, EHEC, particularly those of O157: H7 serotype, have often been implicated in Hemorrhagic colitis epidemics (Caprioli 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 (Caprioli et al 2005). Three breeding types are distinguished in Algeria: the bovine, the ovine-caprine, and the poultry. The ovine population predominates and accounts for up to 70% of the total population with more than 12 million sheep. In North Africa and particularly in Algeria, the sheep portability of STEC is not well known yet.

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.

Table 1. Primer pairs used in this study

Gene	Primer and Sequence (China et al 1996)	Size of PCR product (pb)	Optimal anneal-ting temp (°C)
eae	B52,AGGCTTCGTCACAGTTG B53, CCATCGTCACCAGAGGA	570	51.9
stx_1	B54,AGAGCGATGTTACGGTTTG B55, TTGCCCCCAGAGTGGATG	388	52.9
stx_2	B56,TGGGTTTTTCTTCGGTATC B57, GACATTCTGGTTGACTCTCTT	807	53.4

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 cm^2, 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 confirmation of the virulence of the isolated strain was performed by gene amplification of the stx1, stx2 and eae genes. The pathotype of a part of the strains was determined by multiplex PCR according to the method of Chahed et al (2006) at the National Reference Laboratory for Food Microbiology, University of Liège. The other part of the isolated strains was confirmed by simplex PCR at the Molecular Biology Laboratory of Pasteur Institute of Algiers (IPA) for three genes *eae, stx1* and *stx2*. To detect each gene, 5 µl of DNA were transferred into a PCR tube to which were added 2 µl of MgCl2 (25 mM) (Promega), 2.5 µl of dNTP (2 mM) (Amersham Pharmacacia Biotech), 5 µl of buffer 5x Flexi Buffer (pH 8.8) (Promega), 5µl (10 µM) of the concerned primer pair (Applied Biosystems) (Table 1) and 0.5 µl of Taq polymerase (Go taq Flexi DNA polymerase) (5U/µl) (Promega), and 30 µl of sterile distilled water.

The PCR tubes were placed in the thermocycler set for the following times:

Denaturation at 94 °C for 5 minutes - 30 cycles

Denaturation at 94 °C for 30 seconds - 30 cycles

Hybridization at 50 °C for 30 seconds - 30 cycles

Elongation at 72 °C for 30 seconds

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).

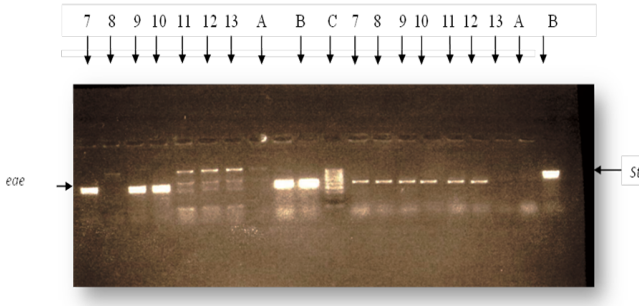

Fig. 1 shows the different DNA bands, each one corresponding to the size of the desired gene. The numbers (7 to 13) correspond to the numbers of strains confirmed at the Pasteur Institute in Algiers.

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

Table 2. Phenotypic and genotypic characteristics of *E. coli* O157: H7 strains

Slaughterhouse	N° of ovine carcasse	Strain	Serotype	Sorbitol and β-glucuronidase	Pathotype	Confirmation laboratory
Slaughterhouse n°1	1	1	O157:H7	_	*eae* stx_2	Faculty of Liège
		2	O157:H7	_	*eae* stx_1	Faculty of Liège
Slaughterhouse n°1	2	3	O157:H7	_	*eae* stx_2	Faculty of Liège
		4	O157:H7	_	*eae*	Faculty of Liège
Slaughterhouse n°2	3	5	O157:H7	_	*eae* stx_2	Faculty of Liège
Slaughterhouse n°2	4	6	O157:H7	_	*eae* stx_2	Faculty of Liège
Slaughterhouse n°2	5	7	O157:H7	_	*eae* stx_2	Pasteur Institute, Algiers
Slaughterhouse n°2	6	8	O157:H7	_	stx_2	Pasteur Institute, Algiers
Slaughterhouse n°2	7	9	O157:H7	_	*eae* stx_2	Pasteur Institute, Algiers
Slaughterhouse n°2	8	10	O157:H7	_	*eae* stx_2	Pasteur Institute, Algiers
Slaughterhouse n°2	9	11	O157:H7	+	stx_2	Pasteur Institute, Algiers
Slaughterhouse n°2	10	12	O157:H7	+	stx_2	Pasteur Institute, Algiers
Slaughterhouse n°2	11	13	O157:H7	+	-	Pasteur Institute, Algiers

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 O55: 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). 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 porting 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 this 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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References

AFSSA. The opinion of 27 May 2010 on the relevance of the definition of pathogenic STECs, specified by Afssa opinion (Opinion of 27 May 2010 on the relevance of the pathogenic definition of STECs, specified by Afssa opinion). Maisons-Alfort; 2010: 19.

Ammon A, Petersen LR, Karch H. A large outbreak of hemolytic uremic syndrome caused by unusual sorbitol-fermenting strain of *Escherichia coli* O157. J Infec Dis 1999;179:1274-1277.

Armstrong GL, Hollingsworth J, Morris JG. Emerging foodborne pathogens: *Escherichia coli* O157: H7 as model of entry of a new pathogen into the food supply of the developed world. Epidemiol Rev 1996;18:29-51.

Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J Clin Microbiol 1993;31(9):2483-2488.

Beutin L, Geier D, Zimmermann S, Karch H. Virulence markers of Shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. J Clin Microbiol 1995;33:631-5.

Blanco J, Blanco M, Blanco JE, Mora A, Gonzalez A, Bernardez MI, et al. Verotoxin-Producing *Escherichia coli* in Spain: Prevalence, serotypes, and virulence genes of O157 :H7 and non- O157 VTEC in ruminants, raw beef products, and humans. Exper Biol Med 2003;228:345-351.

Caprioli A, Morabito S, Brugere H, Oswald E. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet Res 2005;36:289-311.

Chahed A, China B, Mainil J, Daube G. Prevalence of enterohaemorragic *Escherichia coli* from serotype O157 and other attaching and effacing *Escherichia coli* on bovine carcasses in Algeria. J Appl Microbiol 2006;101:361-368.

Chapman PA, Cornell J, Green C. Infection with verocytotoxin-producing *Escherichia coli* O157 during a visit to an inner city open farm. Epidemiol Infec 2000;125:531-6.

Chapman PA, Ashton R. An evaluation of rapid methods for detecting *Escherichia coli* O157 on beef carcasses. Inter J Food Microbiol 2003;87:279-285.

China B, Prison V, Mainil J. Typing of bovine attaching and effacing *Escherichia coli* by multiplex in vitro amplification of virulence-associated genes. Appl Environ Microbiol 1996;62:3462-3465.

Espié E, Vaillant V. Toxi-infection alimentaire collective à *Escherichia coli* O148 :H8 producteur de shigatoxines (Collective food-borne infections to *Escherichia coli* O148: H8 producing shigatoxins). Rapport d'investigation 2002;44.

Fegan N, Desmarchelier P. Shiga toxin-producing *Escherichia coli* in sheep and pre-slaughter lambs in Eastern Australia. Lett Appl Microbiol 1999;28(5):335-9.

Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157: H7, other Enterohemorrhagic *Escherichia coli* and the associated hemolytic uremic syndrome. Epidemiol Rev 1991;13:60-92.

Heuvelink AE, Van Den Biggelaar FL, de Boer E, Herbes RG, Melchers WJ, Huis in 't Veld JH, Monnens LA. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. J Clin Microbiol 1998;36:878-882.

ISO 17604, Microbiology of food and animal feeding stuffs - Sampling of carcasses for microbiological analysis. 2003

Joly B, Reynaud A. Enterobacteriaceae Systematic and diagnostic methods (Enterobacteriaceae Systematic and diagnostic methods). Coll. Microbiology Monographs 2003. (2th ed.). TEC & DOC, 356p.

Karch H. Origin of EHEC. Abstracts of the second international symposium of the European study group on Enterohemorrhagic *Escherichia coli.* Acta Clin Belg 1999;54(1):48.

Khudor MH, Issa AH, Jassim FL. Detection of rfb O157 and fliC H7 genes in *Escherichia coli* isolated from humen and sheep in Basrah Province. Raf J Sci 2012;23:19-33.

Koch C, Hertwig S, Lurz R, Appel B, Beutin L. Isolation of a lysogenic bacteriophage carrying the stx OX3 gene which is closely associated with Shiga toxin-producing *Escherichia coli* strains from sheep and humans. J Clin Microbiol 2001;39:3992-3998.

Kudva IT, Hatfield PG, Hovde CJ. *Escherichia coli* O157:H7 in microbial flora of sheep. J Clin Microbiol 1996;34:431- 433.

Phillips D, Jordan D, Morris S, Jenson I, Summner J. Microbiological quality of Australian sheep meat in 2004. Meat Science 2006;74:261-266.

Riley LW, Remis RS, Helgerson SD, Mcgee HB, Wells JG, Davis BR, et al. Hemorrhagic colitis associated with a rare E*scherichia coli* serotype. N Engl J Med 1983;308:681-685.

Seibt F, Filer G, Gellermann J, Beutin L, Ehrih JHH. The heterogeneity of haemolytic uraemic syndromes in children and adults. Nephrologisches jahresgespräch 1995; Editor: Deutsche Dialysegesellschaft. IN TEXT IS 1997

Söderlund R, Hedenström I, Nilsson A, Eriksson A, Aspán A. Genetical lysimilar strains of *Escherichia coli* O157:H7 isolated from sheep, cattle andhuman patients. BMC Vet Res 2012;8:200.

Vettorato MP, Leomil L, Guth BEC, Irino K, Pestana De Castro AF. Properties of Shiga toxin-producing *Escherichia* (STEC) isolates from sheep in the State of Sao Paulo, Brazil Vet Microbiol 2003;95:103-109.

Zarei M, Basiri N, Jamnejad A, Eskandari MH. Prevalance of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* SPP. In Beef, Buffalo and lamb using multiplex PC. Jundishapur Microbiol 2013;6(8):7244.

Zweifel C, Stephan R. Microbiological monitoring of sheep carcass contamination in three Swiss abattoirs. J. Food Port 2003;66:946-952.

Zweifel C, Blanco JE, Blanco M, Blanco J, Stephan R. Serotypes and virulence genes of ovine non-O157 Shiga toxin-producing *Escherichia* (STEC) from Swiss cattle.Vet Microbiol 2005;105:37-47.

Zweifel C, Kaufman M, Blanco J, Stephan R. Significance of *Escherichia coli* O157:H7 in sheep at slaughter in Switzerland. Schwerz Arch Tierheilkd 2006;148 (6):289-95.

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