Journal of Applied Biosciences 152: 15667 - 15675

ISSN 1997-5902

Prevalence of shiga toxin-producing *Escherichia coli* O157, O26 and O111 in milk, meat and faeces of cattle, sheep and pigs slaughtered in Benin

Chakirath Folakè Arikè Salifou[1], Cyrille Boko[1], Isidore Houaga[2], Raoul Agossa[1], Isabelle Ogbankotan[1], Christie Agonsè Gnonnandé Adèle Ahokpossi[1], Benoit A. Mehoba[1], Bernadette M. Konsaka[1], Serge Gbênagnon Ahounou[1], Issaka Youssao Abdou Karim[1]

[1] Department of Animal Health and Production, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi

[2] Research Unit "Vector-Borne Diseases and Biodiversity" (UMaVeB), Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), 01 BP 454 Bobo-Dioulasso 01, Burkina Faso.

Corresponding author: chakiraths@yahoo.com

Original submitted in on 16th June 2020. Published online at www.m.elewa.org/journals/ on 31st August 2020

https://doi.org/10.35759/JABs.152.6

ABSTRACT

Objectives: The study aimed to search for *E. coli* O157 and non-O157 in milk, meat and faeces of cattle, sheep and pigs slaughtered in Cotonou.

Methodology and Results: One hundred and Seventy-Five (175) samples including 25 meat, 25 faeces per species and 25 milk from cattle were analysed for *E. coli* O157; O26 and O111 and the virulence genes were identified by PCR. The SAS software (1998) and the bilateral Z test were used to calculate and compare the identification frequencies. *E. coli* O157 was identified in 4% of cattle faeces, 4% of sheep faeces, and 20% of beef and, in 20% of milk samples. *E. coli* O26 was identified in 12% of cattle faeces and, in 8% of beef samples. *E. coli* O111 was identified at frequencies of 8%, and 12% in faeces of sheep and pigs, respectively. The *eae* gene was detected in 4% of beef, ovine meat, milk, pig faeces and in sheep faeces. *stx1* was detected in 8% of milk, and in 4% of bovine and sheep faeces. The strains possessing the gene were all of *E. coli* O157 with the exception of one from pig faeces identified as O111.

Conclusions and application of findings: The presence of these serogroups of *E. coli* with virulence genes poses a real food safety problem in Benin. This study findings must be taken into account for risk assessment and management related to the consumption of food of animal origin.

Keywords: Benin, *E. coli* O157, O26, O111, faeces, meat, milk

INTRODUCTION

The urban population explosion in West African countries has fostered the development of a dynamic traditional food production sector which provides producers with a substantial income and a wide range of inexpensive food (Elwert-Kretschmer, 2001; Djevi & Outtier, 2004). However, food processing, distribution conditions and practices are sources of contamination by pathogens and expose consumers to the risk of foodborne illness. According to Salifou *et al.* (2013), enteric infections and foodborne illness are an important public health concern because of their frequency and severity. They cause more than 2 million deaths a year in the world and more

than half come from the African continent (Saba & Gonzalez-Zorn, 2012). Among the microorganisms involved, *Escherichia coli* and particularly those producing Shiga-toxins (STEC) are considered to be one of the most important groups of emerging pathogens. Food is contaminated by animals and humans who may be healthy carriers of *E. coli* and also by previously contaminated surfaces (Estrada-Garcia *et al.*, 2004). The strains of pathogenic *E. coli* are numerous and vary according to the regions of the world (AFSSA, 2010). Benin, like other developing countries, has high rates of diarrheal diseases (407200 cases in 2016 including 207 deaths) (DPP, 2016). That suggests significant underlying food safety issues. Food-borne diseases include typhoid and paratyphoid fevers, shigellosis, amoebiasis, cholera and other undiagnosed diarrheal diseases (Salifou *et al.*, 2010). Potentially pathogenic Shiga-toxin producing *E. coli* (STEC) has been identified as responsible for most childhood diarrhea in developing countries (Filopon, 2005). Serotypes O157, O26 and O111, have been listed as STECs with high prevalence in both Africa and India (Lebbie & Ramsay, 1999; Jajarmi *et al.*, 2017). Ogura et al (2009) found that serotype O157 has virulence genes very similar to those of O26 and O111. The strains of Shiga toxin-producing *E. coli* (STEC group), synthesize Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2) or both (Obrig *et al.*, 2003; Rahal *et al.*, 2012), and additional toxin subtypes have also been described (Scheutz *et al.*, 2012). At the slaughterhouse in Cotonou in Southern Benin, a heavy load of *E. coli* was counted on the surface of the carcasses produced (Salifou *et al.*, 2013b) and *E. coli* O157 was identified in sandwiches and monyo (local soup) marketed on the campus of Abomey-Calavi University (Ahoyo *et al.*, 2010). Considering the abundant consumption of meat in restaurants and in street foods in Benin, a search for potentially pathogenic Shiga-toxin-producing *E. coli* (STEC) and particularly *E. coli* O157, *E. coli* O26 and *E. coli* O111 will make it possible to access the real risk incurred by consumers, especially children. The overall objective of the study was to characterize the prevalence of *E. coli* O157 and non-O157 in cattle milk, meat and faeces of species such as cattle, sheep and pigs slaughtered in the municipality of Cotonou. Specifically, this involved: a) assessing the prevalence of *E. coli* O157, O26 and O111 in cow's milk, carcasses and faeces, of cattle, of sheep, of pigs and, b) determination of the virulence genes of the presumptive strains *E. coli O157*, O26 and O111 isolated.

MATERIALS AND METHODS

The identification of Shiga-toxin-producing *E. coli* O157, O26 and O111 in milk, meat and faeces of cattle, sheep and pigs was carried out in the municipality of Cotonou and its surroundings in Southern Benin. It involved a total of 175 samples, of which 25 were meat and 25 faeces per species and 25 were cow's milk.

Sampling: Meat samples were taken from the carcasses by the destructive method (European Commission Regulation, 2007) and the collar, flank, shoulder and rump were the carcass sampling sites in cattle and sheep. The internal muscular surface of the ham, the posterior part of the pelvis, the sternum and the posterior aspect of the forelimb were the sites of sampling in pigs. For each sampling site, excision of the subcutaneous connective tissue (2 mm thick and 5 cm² of area) of the carcass was performed using a punch. The four samples taken per carcass were put aseptically together in a stomacher sachet, pre-identified and well labelled. For the collection of faeces, at least 100 g was recovered directly from the rectum of each animal and placed in a labeled stomacher bag. Samples were transported at 4°C in a cooler box to the Laboratory of Animal Biotechnology and Meat Technology of the Department of Animal Health and Production of the polytechnic school of Abomey-Calavi for bacteriological analyses. These analyses were performed on the same day as the sampling. Once in the laboratory, 25g of faeces were aseptically collected per bag for analysis. Twenty-Five (25) mL of milk was collected directly into labelled sterile tubes.

Microbiological analysis

Enrichment step: For each sample, different dilutions were made. A volume of 100 mL of trypticase broth (TSB) added to novobiocin for *E. coli O157*, added to cefixime, vancomycin and tellurite for *E. coli O26* and added to cefixime and vancomycin but without addition

of potassium tellurite for *E. coli O111* preheated to 41.5 ° C was introduced into each Stomacher bag containing the total test portion of 20 cm^2 of meat. After mixing in the stomacher, the supernatant was recovered in a sterile vial and formed the stock solution. For faeces, 225 mL of the same media were used for the 25 g test portion. After homogenization in Stomacher bag, the resulting solution was filtered with sterile filter and the filtrate recovered was the 10^{-1} solution. The milk taken from the farm was the stock solution and the different dilutions were made from the stock solution and with the same media used for meat and feces. The various dilutions made were homogenized and then incubated at 37 ° C for 6 h for faeces and non-*E. coli O157* and at 41.5 ° C for 24 hours for meat and milk for *E. coli* O157.

Separation/concentration step: The technique used is that of the immuno-magnetic separation which is technically simple to perform and specific for the isolation of STEC O157 and non O157 (Vernozy-Rozand, 1999). After incubation, 1 mL of the pre-enriched solution of each dilution and of each sample was taken and placed in eppendorf tubes containing 20 µl of solution of anti *E. coli O157* Dynabeads for *E. coli O157*, EPEC / VTEC O26 Dynabeads for the detection of *E. coli* O26 and Dynabeads EPEC / VTEC O111 for the detection of *E. coli* O111. The immuno-magnetic separation was made according to the recommendations of the manufacturers of Dynabeads anti *E. coli* solutions and a Dynabeads® MX1 Mixer and a washing buffer solution (PBS) were used for this purpose.

Step of isolation on selective media : The Dynabeads® bacteria complex and washing water obtained at the end of the separation phase was vortexed briefly and 50 µl were removed and inoculated on plates containing 20 mL of Cefixime Tellurite and BCIG (5-bromo-4-chloro-3-indolyl-β-D-glucuronide)-containing Sorbitol MacConkey Agar for *E. coli* O157 and plates containing 20 mL of Mac Conkey supplemented with rhamnose, cefixime tellurite (CT-RMAC) for *E. coli* O26 and on plates containing 20 mL of chromocult agar supplemented with cefixime, cefsulodine and vancomycin for *E.coli* O111. The dishes were incubated at 37°C for 18 to 24 hours.

Confirmation step: After incubation, five characteristic colonies were selected per plate and each subcultured according to the type of *E. coli* sought on plates containing the respective isolation media and then incubated again at 37 °C for 18 to 24 hours. One colony from each plate was subsequently confirmed with the Latex Agglutination Test (Dryspot *E. coli* O157 testkit; Prolex Latex Reagent *E. coli* O26; Prolex Latex Reagent *E. coli* O111) according to manufacturer's instructions.

Confirmation of the strains by APIE 20E strips; The biochemical characteristics of colonies that tested positive for agglutination were investigated using the API 20E strips according to the manufacturer's instructions. The same colonies were subjected to an oxidase test.

Detection of virulence genes in *E. coli* O 157: The strains identified by the APIE 20E strips as *E. coli* O157 were investigated for virulence gene using PCR. The genes sought were the *stx* genes (*stx1* and *stx2)* and the eae gene. The PCR was performed after the extraction of the DNA according to the method described by Karch et Meyer (1989). For the "master mix", the same reagents were used with the exception of the primers *eae* bact F and *eae* bact R which were used for the *eae* gene and the primers *stx1* bact F and *stx1* bact R for the *stx1* gene and *stx2* bact F and *stx2* bact R for *stx2* gene. The amplification program used included a preheating step allowing initial denaturation at 94 ° C. For 5 minutes; followed by 40 cycles consisting of a denaturation step at 94°C for 30 seconds, an annealing step at 58°C for 45 seconds and an extension step at 72° C for one minute and a final extension at 72 ° C for 10 minutes and a refrigeration step at 4 °C. The resulting PCR products were migrated on a 1.5% agarose gel. The migration was done in a tank filled with Tris Borate-EDTA run at 120 V for one hour. The PCR products were visualized under a UV light connected to a photographic device in connection with a computer to save the image.

Statistical analysis: The frequency of samples containing *E. coli* O157, O26 and O111 by type of sample (meat, milk and faeces) and by species for meat and faeces was calculated by the *Proc freq* procedure of Karch et Meyer (1989). The frequencies were compared using the pair-wise Z-test.

RESULTS

Identification of O157, O26 and O111 antigens: The agglutination latex test carried out gave a total of 21 positive strains for O157, 9 positive strains for O26 and 9 positive strains for O111. The frequencies of the identified species and serogroups are shown in Table 1. The O157 antigen was identified in meat of cattle and

pigs at frequencies of 28% and 4% respectively and in faeces of cattle and sheep at a frequency of 8%. No O157 was identified in pig meat. In milk, it has been identified at a rate of 36%. The frequencies of identification in bovine milk and meat did not differ significantly (p> 0.05). Similarly, the frequencies of O157 in faeces of sheep and bovine faeces did not differ significantly from that obtained in beef. No strain was identified in sheep meat and pig faeces unlike other meats and faeces. Some strains were positive to the agglutination latex test but also positive to the test control. Others were negative to latex test but positive to test control O26 antigen was identified in 8% and 4% respectively of beef and sheep meat samples and in 16% of cattle faeces samples. These different frequencies did not differ significantly (p> 0.05). As for the O111 antigen, it has been identified in pig meat at a rate of 8%, also in faeces of cattle, sheep and pigs at rates of 8%, 8% and, 12% respectively. No difference was also obtained between these different frequencies.

Table 1: Frequency (%) of identification of O 157, O26 and O 111 antigens

Host	Sample type	*E. coli* O 157	*E. coli* O 26	*E. coli* O111
Cattle	Faeces	8.0bc	16.0a	8.0a
	Meat	28.0ab	8.0ab	0.0a
	Milk	36.0a	0.0b	0.0a
Pigs	Faeces	0.0c	0.0b	12.0a
	Meat	4.0c	0.0b	8.0a
Sheep	Faeces	8.0bc	0.0b	0.0a
	Meat	0.0c	4.0ab	0.0

Frequencies from the same column followed by the same letter are not statistically different at 166 the threshold of 5%.

Confirmation of presumed *E. coli* O157, O26 and O111 strains by API 20^E strips: All the positive strains from agglutination test were not confirmed as *E. coli* by the API strips. From the strains positive for the O157 latex test assay, five strains isolated from bovine meat, one strain from bovine faeces and one strain from sheep faeces were identified as *E. coli*. sorbitol negative and beta-glucorinase negative. Thus, the confirmation rate of *E. coli* O157 were 20%, 4% and 4% respectively in beef, cattle faeces and sheep faeces. For milk, 5 samples were identified as *E. coli* sorbitol negative and beta-glucorinase negative resulting in an identification frequency of 20%. For the O26 test-positive strains, two strains from bovine meat and 3 from cattle faeces were identified as *E. coli*. The identification frequencies of *E. coli* O26 were 8% and 12% respectively in meat and faeces of cattle. As for O111 antigen positive strains, two from sheep faeces and 3 from pig faeces were identified as *E. coli* at the frequencies of 8% and 12% respectively. No strain from milk, cattle meat and faeces was positive for the O26 and O111 latex test. Table 2 provides frequency of positive strains from agglutination test O157, O26 and O111 confirmed *E. coli*. For four colony strains from milk that were positive for the agglutination test indicating the presence of O157 antigen, API 20^E gave *Klebsiella* for two, *Pseudomonas Aeruginosa* for one and *Serretia fonticola* for one. The four strains were sorbitol and beta-glucorinase positive.

Table 2: Frequency of positive strains from agglutination test O157, O26 and O111 confirmed E. coli

Host	Sample type	*E. coli* O 157	*E. coli* O 26	*E. coli* O111
Cattle	Faeces	20,00ab	8,00a	0,00a
	Meat	4,00bc	12,00a	0,00a
	Milk	20,00a	0,00a	0,00a
Pigs	Faeces	0,00c	0,00a	12,00a
	Meat	0,00c	0,00a	0,00a
Sheep	Faeces	4,00bc	0,00a	8,00a
	Meat	0,00c	0,00a	0

Frequencies from the same row followed by the same letter are not statistically different at 189 the threshold of 5%.

Detection of the virulence genes of the presumed *E. coli* O157, O26 and O111 strains: From all the strains studied by PCR, one strain isolated from a milk sample, one strain from bovine meat, one from sheep faeces and one from pig faeces have the *eae* virulence gene (Figure 1). Thus, the frequency of the *eae* gene of all 25 samples of each species was 4% in bovine meat, milk, and pig faeces and in sheep faeces. As for the *stx1* gene, it was detected in two strains from milk, one of which had the *eae* gene, in one strain from bovine meat and in one strain from the sheep faeces sample which having also the *eae* gene (Figure 2). The frequency of the *stx1* gene of all 25 samples of each species was 8% in milk and 4% in beef and sheep faeces. The *stx2* gene was not detected in any of the strains studied and the strains possessing virulence gene were *E. coli* O157 strains except for the gene identified in the feces of pig that was from *E. coli* O111 strain. The table 3 provides genes identified for strains confirmed *E. coli.*

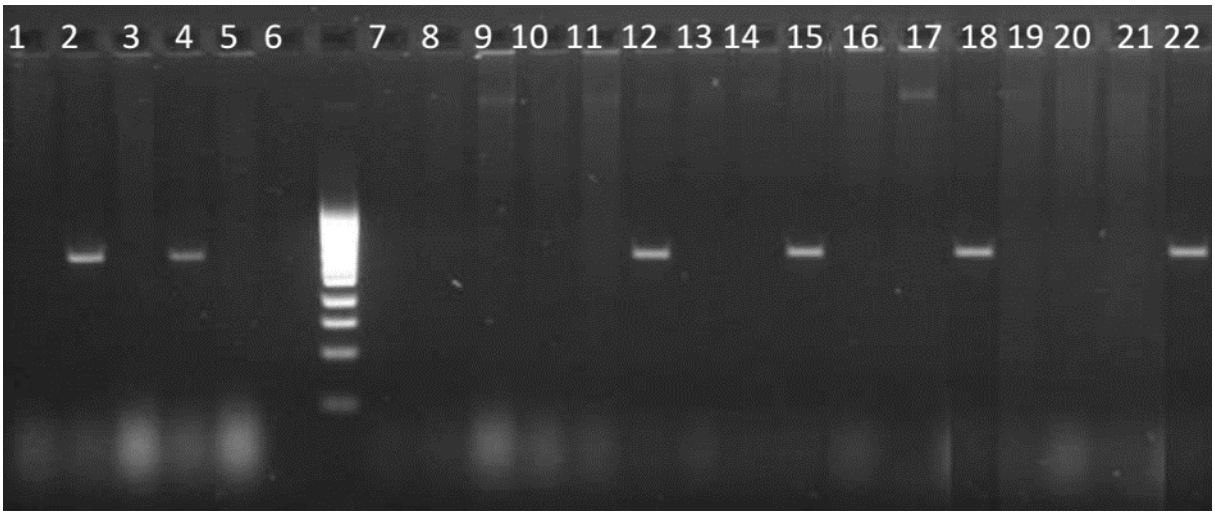

Figure 1: Detection of *eae* gene in positive strains from agglutination test

Legends:1: *E. coli* S237, 2: *E. coli* S236, 3: *E. coli* S234, 4: *E. coli* S232, 5: *E. coli* S231, 6: negative PCR, 7: strain M1, 8: strain S1, 9: strain C1, 10 : strain C2, 11: strain FC, 12: strain M2 positive, 13: strain M3, 14: strain M4, 15: strain FP1 positive, 16: strain M5, 17: strain C3, 18: strain FS1 positive, 19:strain FP2, 20: strain FP3, 21: strain C4, 22: strain C5 positive, C= cattle; S= sheep; M= Milk ; P= pig ; F= faeces.

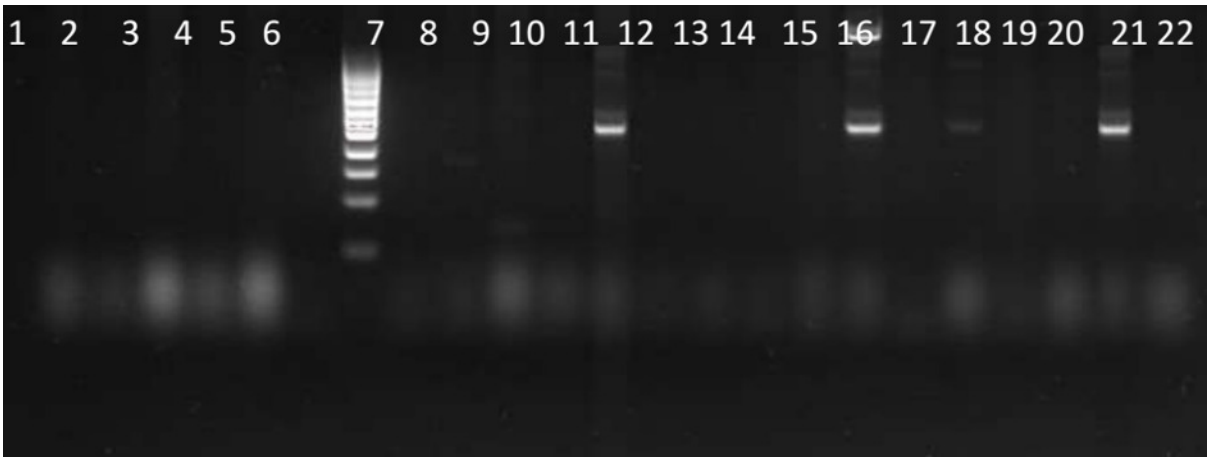

Figure 2: Detection of *Stx1* gene in positive strains from agglutination test

Legends:1 : *E. coli* S237, 2 : E. *coli* S236, 3 : *E. coli* S234, 4 : *E. coli* S232, 5 : *E. coli* S231, 6 : negative 213 PCR, 7 : strain M1, 8 : strain S1, 9 : strain C1, 10 : strain C2, 11 : strain FC, 12 : strain M2 positive, 13 : strain M3, 14 : strain M4, 15 : strain FP1, 16 : strain M5 positive, 17 : strain C3, 215 18 : strain FS1 positive, 19 : strain FP2, 20 : strain FP3, 21 : strain C4 positive, 22 : strain C5, 216 C= cattle ; S=sheep ; M=milk ; P= pig ; F= faeces.

Table 3: Identified genes for strains confirmed *E. coli*

Host	Sample type	n	O157			n	O26			n	O111		
			Stx1	Stx2	eae		Stx1	Stx2	eae		Stx1	Stx2	eae
Cattle	Cattle	5	1	-	1	2	-	-	-	0	-	-	-
	Faeces	1	-	-		3	-	-	-	0	-	-	-
	Milk	5	2	-	1	0	-	-	-	0	-	-	-
Pigs	Faeces	1	-	-	-	0	-	-	-	3	-	-	1
Sheep	Faeces	1	1	-	1	0	-	-	-	2	-	-	-

- : not found; n=Number of strains tested

DISCUSSION

The study revealed an effective presence of Shiga toxin *E. coli* O157, and O111 in meat of cattle, in faeces of sheep and pigs slaughtered in slaughterhouses in Cotonou and in raw milk from cows. The observed prevalence ranging from 4% to 20% of *E. coli* with the O157, O26 and O111 antigen indicate a poor hygienic condition of preparation of foodstuffs of animal origin and the presence of *stx1* genes in meat and milk of cattle and *eae* genes in cattle meat, indicates that a real risk is incurred by consumers. Ahoyo *et al*. (2010) have already reported the presence of E. coli O157 in sandwiches and monyos (local soup) sold on the Abomey-Calavi University Campus in Benin at frequencies of 8% and 11% respectively. The high frequencies observed in cattle, sheep and milk confirm the statement of Dadie *et al.* (2013) stating that beef, sheep, milk and dairy products are cited as the main sources of STEC transmission in Africa. Serotypes O157, O26 and O111 have also been studied in cattle by several authors (Pearce *et al.*, 2006; Ekiri *et al.*, 2014). Shridhar *et al.* (2017) reported isolated *E. coli* O157 from cattle faeces. Bonardi *et al.* (2015) obtained frequencies of 3.1%, 1.9% and 0.6% respectively of O157, O26 and O111 with the *stx1* gene in cattle faeces and frequencies of 8.8%, 1.3% and 1.3%, respectively of O157, O26 and O111 on the skin of cattle. In the present study, 4% of bovine meats and 4% of sheep faeces analysed revealed the presence of *E. coli* O157 with only the *stx1* gene. Andral *et al.* (1999) also did not obtain the *stx2* gene for O 26 and O111 serotypes. These authors identified *E. coli* O157 in meat samples from cattle carcasses and the slaughter line in France in 7 of the 50 samples analysed, representing a prevalence of 8%. A study of 100 fresh meat samples in about 15 Moroccan butcheries resulted in the isolation of *E. coli* O157 in 9% of products Beneduce *et al.* (2008). In Algeria, 18 carcasses of beef from 230 carcasses sampled at a slaughterhouse were positive for STEC O157, a prevalence of 7.8% (Chahed, 2007). Hiko *et al.* (2008) found out of 250 samples of beef tested in Ethiopia, a prevalence of 8%. In the case of small ruminants, serovars were also isolated in Ethiopia from lamb, sheep (2.5%) and goat (2%) meat (Hiko *et al.*, 2008). Chahed (2007) obtained a prevalence of 2.5% of STEC on carcass and 25.4% in faeces in sheep in Algeria. Djordjevic *et al.* (2001) identified six serotypes of STEC in 95% of the sheep tested and of these six serotypes, *E. coli* O157: H7 was identified in 2.2% of the isolates. In pigs, several authors (Heuvelink *et al.*, 1998; Parma *et al.*, 2000; Haeghebaert *et al.*, 2002) obtained low to zero (0%, 2%, 1.4%) prevalences of *E. coli* O157: H7. Similarly, in our study, no pork samples revealed the presence of *E. coli* O157 and O26. On the other hand, O111 with the virulence *eae* gene was identified at a frequency of 4%. Generally, less STEC is in milk and meat than in faeces if hygiene rules are observed during the process. STEC may therefore enter milk and meat via faecal contamination during milking and slaughter (Jaakkonen *et al.*, 2019). In the present study, there were less STEC in cattle and pig faeces for O 157 and less in sheep faeces for O 26. That may be due to the overwhelming STEC by IMS beads, Hallewell *et al.* (2017) proved the limitation of immunomagnetic separation method for detection of *E. coli* O157 and non-O 157. The lack of *eae* gene identification in *E. coli O26* strains does not mean that his gene is absent. A study on a larger sample size may reveal their possible presence. The fact that not all agglutination-positive strains were E*. coli* indicates the presence of false positives (Vernozy-Rozand, 1997). The high rate of *E. coli* O157 associated with the presence of *Klebsiella* in this study indicates that *E. coli* may be associated with presence of *Klebsiella* in raw milk due to a high *Klebsiella* prevalence noted in other studies in raw milk. Badri *et al. (*2017), found a highest prevalence of Klebsiella pneumoniae (62%) in raw milk. The self-agglutination obtained for certain colony

strains could reveal the presence of nonserotyping *E. coli*. Since the study took into account only somatic O antigens, it would be interesting to look for flagellar H antigens for complete serotyping.

CONCLUSION AND APPLICATION OF RESULTS

The study carried out on the prevalence and characterization of O157 and non-O157 Shigatoxin-producing strains of *Escherichia coli* isolated from meat, faeces of cattle, sheep, pig and milk in Southern Benin revealed that faeces and meat of those species and cow's milk contain *E. coli* O157, O26 and O111 with virulence genes *stx1* for some, *eae* for the others and both genes for some isolates. This shows that a real risk is incurred by consumers and that it is necessary to train professionals in the meat and milk sector on good hygiene practices and to put in place preventive measures in reference to HACCP throughout the food production chain of animal origin. Also, during distribution and consumption, transport conditions must be controlled by the application of good hygiene practices and the respect of temperatures. The public must also be made aware of the importance of sufficiently cooking minced meat and pasteurizing milk before consumption, especially when it is intended for children or the elderly.

Acknowledgment

The authors are grateful to the International Foundation for Science (IFS) for funding this study.

REFERENCES

AFSSA, 2010. Opinion of May 27, 2010 on the relevance of a revision of the definition of pathogenic STECs, specified by the Afssa opinion of July 15, 2008. 2010. French Food Safety Agency, Maisons-Alfort19.

Ahoyo TA, Ahissou H, Kounon F, Aminou T, Dramane K, 2010 Etude de la qualité bactériologique des aliments vendus sur le campus de l'Université d'Abomey Calavi au Bénin. International Journal of Biological and Chemical Sciences 4(4):1083–92.

Andral B., Decomble C., Ducotterd B., 1999. Contamination des filières bovines par E. coli O157: H7: résultats d'une pré-étude en abattoir. Le Point Vétérinaire 30:565–67.

Badri AM, Ibrahim IT, Mohamed SG, Garbi MI, Kabbashi AS, Arbab MH, 2017. Prevalence of extended spectrum beta lactamase (ESBL) producing Escherichia coli, and Klebsiella pneumoniae isolated from raw milk samples in Al Jazirah state, Sudan. Molecular Biology, 7 (1) DOI:10.4172/2168-9547.1000201.

Beneduce L, Spano G, Nabi AQ, Lamacchia F, Massa S, Aouni R, Hamama A, 2008. Occurrence and characterization of Escherichia coli O157 and other serotypes in raw meat products in Morocco. Journal of Food Protection 71(10):2082–86.

Bonardi S, Alpigiani I, Tozzoli R, Vismarra A, Zecca V, Greppi C, Bacci C, Bruini I, Brindani F, 2015. Shiga toxin-producing Escherichia coli O157, O26 and O111 in cattle faeces and hides in Italy. Veterinary Record Open 2 e000061. doi: 10.1136/vetreco-2014-000061.

Chahed A, 2007. Prévalence et caractérisation de souches d'Escherichia coli O157 producteurs de shigatoxines isolées de denrées alimentaires d'origine animale en Belgique et en Algérie. Doctorat Sciences vétérinaires, Université de Liège. 187 p.

Dadie KA, Koua AT, Koffi RA, Dje MK, 2013. Prévalence et antibiorésistance de bactéries entériques à potentialité pathogène dans l'alimentation de rue à Abidjan; 1ère conférence de la recherche Africaine sur l'agriculture, l'alimentation et la nutrition; Côte d'Ivoire,"653–674.

Djevi H, Outtier A, 2004. Accès au marché des produits agricoles, résultats du groupe thématique "Produits Séchés ". Rapport d'étude. Cotonou, Bénin 22p.

Djordjevic SP, Hornitzky MA, Bailey G, Gill P, Vanselow B, Walker K, Bettelheim KA, 2001. Virulence properties and serotypes of Shiga toxin-producing Escherichia coli from healthy Australian slaughter-age sheep. Journal of Clinical Microbiology 39(5):2017–21.

DPP, 2016. Annuaire des statistiques sanitaires. Direction de la Programmation et de la Prospective. Ministère de la Santé, Cotonou, Bénin167.

Ekiri AB, Landblom D, Doetkott D, Olet S, Shelver WL, Khaitsa ML, 2014. Isolation and characterization of Shiga toxin–producing

Escherichia coli serogroups O26, O45, O103, O111, O113, O121, O145, and O157 shed from range and feedlot cattle from postweaning to slaughter. Journal of Food Protection 77(7):1052–61.

Elwert-Kretschmer K, 2001. L'aspiration au changement alimentaire à Cotonou, Bénin. CIRAD, 71p.

Estrada-Garcia T., Lopez-Saucedo C., Zamarripa-Ayala B., Thompson M.R., Gutierrez-Cogco L., Mancera-Martinez A., Escobar-Gutierrez A., 2004. Prevalence of Escherichia coli and Salmonella spp. in street-vended food of open markets (tianguis) and general hygienic and trading practices in Mexico City. Epidemiology & Infection 132(6):1181–84.

European Commission Regulation, 2007. EC Regulation No 1441/2007 of 5 December 2007 amending EC Regulation No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union 332, 12–29.

Filopon D, 2005. Mécanismes de régulation impliques dans la pathogénicité de Pseudomonas aeruginosa : système de sécrétion de type III, épigenèse et quorum sensing. Thèse de doctorat, Université Joseph-Fourier-Grenoble 1. 188 p.

Haeghebaert S, Gallay A, Bouvet P, Gomez M, Vaillant V, 2002. Collective food poisoning in France in 1999 and 2000. Weekly Epidemiological Bulletin 23:105–9.

Hallewell J, Alexander T, Reuter T, Stanford K, 2017. Limitations of immunomagnetic separation for detection of the top seven serogroups of Shiga toxin–producing Escherichia coli. Journal of Food Protection 80(4):598–603.

Heuvelink AE, Bleumink B, Van den Biggelaar F, Te Giffel MC, Beumer RR, De Boer E, 1998. Occurrence and survival of verocytotoxin-producing Escherichia coli O157 in raw cow's milk in The Netherlands. Journal of Food Protection 61(12):1597–1601.

Hiko A, Asrat D, Zewde G, 2008. Occurrence of Escherichia coli O157: H7 in retail raw meat products in Ethiopia. The Journal of Infection in Developing Countries 2(05):389–93.

Jaakkonen A, Castro H, Hallanvuo S, Ranta J, Rossi M, Isidro J, Lindström M, Hakkinen M, 2019. Longitudinal study of Shiga toxin-producing Escherichia coli and Campylobacter jejuni on Finnish dairy farms and in raw milk. Applied and Environmental Microbiology 85 (7): e02910-18. https://doi.org/10.1128/AEM.02910-18.

Jajarmi M, Fooladi AAI, Badouei MA, Ahmadi A, 2017. Virulence genes, Shiga toxin subtypes, major O-serogroups, and phylogenetic background of Shiga toxin-producing Escherichia coli strains isolated from cattle in Iran. Microbial Pathogenesis 109:274–79.

Karch H, Meyer T, 1989. Single primer pair for amplifying segments of distinct Shiga-like-toxin genes by polymerase chain reaction. Journal of Clinical Microbiology 27(12):2751–57.

Lebbie SHB, Ramsay K, 1999. A perspective on conservation and management of small ruminant genetic resources in the sub-Saharan Africa. Small Ruminant Research 34(3):231–*47.*

Obrig TG, Seaner RM, Bentz M, Lingwood CA, Boyd B, Smith A, Narrow W, 2003. Induction by sphingomyelinase of Shiga toxin receptor and Shiga toxin 2 sensitivity in human microvascular endothelial cells. Infection and Immunity 71(2):845–49.

Ogura Y, Ooka T, Iguchi A, Toh H, Asadulghani M, Oshima K, Kodama T, Abe H, Nakayama K, Kurokawa K, 2009. Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. Proceedings of the National Academy of Sciences 106(42):17939–44.

Parma AE, Sanz ME, Blanco JE, Blanco J, Viñas MR, Blanco M, Padola NL, Etcheverría AI, 2000. Virulence genotypes and serotypes of verotoxigenic Escherichia coli isolated from cattle and foods in Argentina. European Journal of Epidemiology 16(8):757–62.

Pearce MC, Evans J, McKendrick IJ, Smith AW, Knight HI, Mellor DJ, Woolhouse MEJ, Gunn GJ, Low JC, 2006. Prevalence and virulence factors of Escherichia coli serogroups O26, O103, O111, and O145 shed by cattle in Scotland. Appl. Environ. Microbiol. 72(1):653–59.

Rahal EA, Kazzi N, Nassar FJ, Matar GM, 2012. Escherichia coli O157: H7—Clinical aspects and novel treatment approaches. Frontiers in Cellular and Infection Microbiology 2 https://doi.org/10.3389/fcimb.2012.00138.

Saba CKS, Gonzalez-Zorn B, 2012. Microbial food safety in Ghana: a meta-analysis. The Journal of Infection in Developing Countries

6(12):828–35.

Salifou CFA, Boko KC, Ahounou GS, Tougan PU, Kassa SK, Houaga I, Farougou S, Mensah GA, Clinquart A, Youssao AKI, 2013a. Diversité de la microflore initiale de la viande et sécurité sanitaire des consommateurs. International Journal of Biological and Chemical Sciences 7(3):1351–69.

Salifou CFA, Boko KC, Attakpa YE, Agossa R, Ogbankotan I, Farougou S, Mensah GA, Salifou S, Clinquart A, Youssao AKI, 2013b. Evaluation de la qualité bactériologique de viande fraîche de bovins abattus aux abattoirs de Cotonou-Porto-Novo au cours de la chaîne de distribution. Journal of Animal & Plant Sciences 17(2):2567–79.

Salifou CFA, Tougan PU, Ahounou GS, Youssao AKI, 2010. Evaluation of the hygiene of the slaughtering process at the slaughterhouses of Cotonou-PortoNovo using bacteriological examination of the surface. 13th Days of Muscle Science and Meat Technology, 19th and 20th October 2010 in Clermont Ferrand, France. 175-176.

Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, 2012. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. Journal of Clinical Microbiology 50(9):2951–*63.*

Shridhar PB, Noll LW, Cull CA, Shi X, Cernicchiaro N, Renter DG, Bai J, Nagaraja TG, 2017. Spiral plating method to quantify the six major non-O157 Escherichia coli serogroups in cattle feces. Journal of Food Protection 80(5):848–56.

Vernozy-Rozand C, 1999. Les Escherichia coli vérotoxiques (VTEC) et Escherichia coli O157: H7 en clinique et en agro-alimentaire. In: Annales de Biologie Clinique. 507-516.

Vernozy-Rozand C., 1997. Detection of Escherichia coli O157: H7 and other verocytotoxin-producing E. coli (VTEC) in food. Journal of applied microbiology 82 (5) :537–551.