

Shiga Toxin - Producing *Escherichia Coli* (STEC) in Meat: Selected Studies, Review

M. ABD Elgadir

Department of Food Science & Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia

Abstract: Wide range of food stuffs currently are associated with toxin - producing *Escherichia Coli* (TPEC) and potentially increased the risk of human disease. Raw meats and ready-to-eat meat products obtained from some animals such as cattle, goats, sheep and pork have been identified as an important source of Shiga Toxin - Producing *Escherichia Coli* (STEC) which could cause infection to humans. Contamination of meat and meat products with shiga toxin-producing *Escherichia coli* (STEC) has become preeminent of the world concern for the last few decades due to the increasing of numerous number of illness occurring from this microorganism. The toxin of STEC is well recognized and associated with causes of diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. The human infections occur due to consumption of animal origins with STEC-contaminated meat in addition to other foodstuffs. The aim of this investigation was to summarise detection and prevalence of toxin-producing *Escherichia coli* (STEC) in different types of meat and meat products in order to give a clear picture about this microorganism.

Keywords: Meat, Shiga, Toxin, *Escherichia coli*, STEC

1. Introduction

Meat and meat products have been involved in many disease outbreaks cases caused by numerous diarrheagenic *Escherichia coli* all over the world (Karmali et al., 2010; Rhoades et al., 2009). Earlier, Nataro and Kaper (1998) clinically investigated diarrheagenic *E. coli* strains and revealed that there were five most common pathogroups namely Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and enteroinvasive *E. coli* (EIEC). These pathogens can be classified into several pathogroups based on their virulence traits. STEC could be remained as the major food safety concern which associated with meat, especially red meat products. Shiga toxins that produced by *E. coli* are responsible for Hemolytic Uremic Syndrome and hemorrhagic colitis. Potter (1992) reported that STEC is an emerging pathogen responsible for epidemic outbreaks of diseases such as diarrhoea and haemolytic-uraemic syndrome (HUS).

Karch et al. (2005) stated that domestic ruminants such as cattle are considered to be the main source of STEC. Many researchers have found that large game animals including wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) could be carriers of STEC (Díaz et al., 2011; Eggert et al., 2012; Sánchez et al., 2009, Sánchez et al., 2010, Ahn et al., 2009; Keene et al., 1997a, Keene et al., 1997b; Rabatsky-Ehr et al., 2002; Rounds et al., 2012, Díaz-Sánchez et al., 2012). Recently there are several studies dedicated with the objective of studying shiga-toxin producing *Escherichia Coli* (STEC) in different types of food stuffs ((Franz et al., 2014, Li et al., 2016, Pokharel et al., 2016, Elder et al., 2016, Khalil et al., 2016, Álvarez-Suárez et al., 2016 and Kerangart et al., 2016, Topalcengiz et al., 2017). This study aims at highlighting detection and prevalence of STEC in different types of meat and meat products and summarizing selected methods used in this regards.

Shiga-toxin Producing *Escherichia Coli* (STEC) and people health

Outbreaks of STEC normally occur after consumption of processed contaminated meat such as minced beef in forms of hamburgers, meatballs and sausages (Ammon et al., 1999 and Soler et al., 1999, Jackson et al., 2000; EFSA, ECDC, 2013). Levre` and Velentini, (2001) and Bouvet et al., (2002) reported that although strains of verocytotoxic *E. coli* (VTEC) have also been detected in pork meat but the risk appeared much lower compared to that of STEC. Paton and Paton (1998) stated that STEC could cause gastroenteritis that may be complicated by hemorrhagic colitis or the hemolytic-uremic syndrome which is the main cause of acute renal failure in children. Schmidt (2010) investigate Enteropathogenic *E. coli* (EPEC) and observed that this strain produces characteristic histopathology known as attaching and effacing on intestinal cells. However, Nataro and Kaper (1998) and Schmidt (2010) found that EPEC can further divided into two subtypes, typical atypical (aEPEC) and (tEPEC), depending on the presence or absence of the EPEC adherence factor (EAF) plasmid and bfpB gene. On the other hand, Trabulsi et al. (2002) noticed that infection by Strains of aEPEC occur most frequently in developed countries whereas infection by tEPEC is the leading cause of infantile diarrhea occur in developing countries. (Kaper et al., 2004) reported that enterotoxigenic *E. coli* (ETEC) could be able to produce heat-stable enterotoxin which consider as an important cause of diarrhea in infants. It was found that domestic ruminants, mainly goats, sheep and cattle have been recognized as the major natural reservoirs for STEC and they can play a significant role in human infections (Griffin and Tauxe, 1991). However, during processing of these mentioned ruminants carcasses, transfer of bacteria or fecal contamination from the animal's hide to the carcass can assist and facilitate transmission of pathogenic *E. coli* to the meat (Elder et al., 2000). According to Islam et al., (2010), this is because in most of the developing countries living with the domestic animals within the same premises is a common practice in both rural and urban areas and hygienic conditions are severely compromised. Thus the living style of the people in developing countries could be an effective

factor that supports the occurrence of pathogenic *E. coli* infections (Simpore et al., 2009, Kagambega et al., 2011).

Selected investigations on detecting Shiga-toxin Producing *Escherichia Coli* (STEC) on various types of meat

Most human infections of Shiga toxin-Producing *Escherichia coli* causes are due to consumption of STEC-contaminated animal origins or other foodstuffs. Bai et al. (2015) evaluated prevalence of STEC on retail raw meats from different animals' resources including pork, beef, mutton, chicken and duck. They revealed that 166 out of 853 samples were positive to STEC which means that high genetic diversity of STEC in retail raw meats that have potential to cause human diseases could be found in raw meats from the mentioned animals. On the other hand, Shiga Toxin-Producing *Escherichia coli* strains were investigated in retail ground meat from different types of animals including beef, chicken, deer, boar, bison, pork and rabbit (Magwedere et al., 2013). From a total number of 83 ground beef, pork, and chicken samples, 17 (20%) carried O121, 9 (10%) carried O45, 8 (9%) carried O157, 3 (3%) carried O103, and 1 (1%) carried O145. None of the samples were positive for O26, O111, or the stx gene. All deer samples (100%) were positive for O45, O103, or both, 2 (10%) and all bison samples (100%) were contaminated with either O121, O145, or O157.

Selected studies on methods used to detect shiga-toxin Producing *Escherichia Coli* (STEC) and its toxin on meat

Chapman et al. (2001) investigated 120 samples of various naturally contaminated raw meats including raw beef, lamb and mixed meat products for the presence of shiga-toxin producing *Escherichia coli* using three different methods including PCR, BioSign and Path-Stik in order to compare sensitivity of these three methods in detecting STEC on the meats. They found that 80 (67%) of the samples were positive to STEC by PCR method, 70 (58%) were positive by BioSign and 67 (56%) were positive by Path-Stik method. They concluded that it is important to use sensitive

methods such as PCR to detect STEC because some times the numbers of this pathogen in contaminated meat products may be low during investigations of outbreaks, surveillance and quality control. So, the detection method used can have a marked effect on the results of surveillance studies. Sánchez et al. (2013) investigated large game meat and meat products using PCR method for screening the presence of subtilase cytotoxin (subAB) of STEC using the primer pair RTsubABF/RTsubABR as described by Paton et al. (2004). That was because SubAB is an AB5 toxin produced by Shiga toxin (Stx)-Producing *Escherichia coli* (STEC) strains and it was recognized usually with lacking the eae gene product intimin. Two allelic variants of SubAB encoding genes have been described as subAB1 with located on a plasmid and the second one is subAB2 that located on a pathogenicity island (PAI). subAB1 has been reported to be more frequent among bovine strains while subAB2 has been mainly associated with strains from small ruminants. The authors investigated the two variants of subAB among 59 eae-negative STEC from large game animals including deer, wild boar and their meat and meat products in order to assess the role of other species in the epidemiology of subAB-positive, eae-negative STEC. They detected subAB genes in 71.2% of the strains: 84.1% of the strains from deer and 33.3% of the strains from wild boar. Most of them (97.6%) possessed subAB2. This could approved that large game animals mainly deer could be represent as an important animal reservoir of subAB2-positive, eae-negative STEC which highlight the risk of human infection posed by the consumption of large game meat and meat products. Momtaz et al. (2013) investigated the presence of Shiga Toxin-Producing *Escherichia Coli* serogroups in ruminant's meat obtained from sheep, beef, goat and camel using using culture, PCR and disk diffusion methods. A total of 820 raw meat samples were evaluated for the presence STEC. Totally, 238 (29.02%) samples were reported as positive for presence STEC. They demonstrated that mainly meat from cattle, sheep, goat and camel were contaminated Shiga Toxin-Producing *Escherichia Coli* (STEC) and its toxin.

Table 1: Selected methods used to detect and identify virulence genes of Shiga-toxin Producing *Escherichia Coli* (STEC)

S.no.	Target gene	Product size	Concentration (µM)	References
1	eaeA	482	0.1	482 0.1 Vidal et al. (2005)
2	ent	629	0.4	629 0.4 Müller et al. (2007)
3	escV	544	0.4	Müller et al. (2007)
4	EHEC-hly	688	0.1	Antikainen et al. (2009)
5	stx1	244	0.2	Müller et al. (2007)
6	stx2	324	0.4	Müller et al. (2007)

Table 2: Selected studies from different countries on detection of Shiga-toxin producing *Escherichia Coli* (STEC) in meats

S. no.	Source of infected meat	Country	Reference
1	Minced beef, beef meatballs, beef burgers	Italy	Stampi et al., 2004
2	Chicken, turkey, pork, ground beef	United States of America	Doi ET AL., 2010
3	Ground beef, beef sausage, beef burger, kofta, and beef luncheon	Egypt	Mohammed et al., 2012
4	Cattle meat	Argentina	Brusa et al., 2012
5	Carcasses, meat and meat products	Spain	Sánchez, 2013
6	Raw sheep meat	Iran	Tahmasby, 2014
7	Retail poultry meats	Canada	Aslam et al., 2014
8	Pork, poultry, beef, venison	The Czech Republic	Skočková, 2015
9	Pork, beef, mutton, chicken, duck	China	Bai et al., 2015
10	Patties, pork cutlets	Republic of Korea	Ro et al., 2015

2. Conclusion

Escherichia coli *E. coli* and its toxin such as STEC are found in a wide variety of animal species, including cattle, sheep, goats, pigs and wild ruminants all over the world. The toxins produced by various serogroups of *E. coli* may cause some serious infections such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome. The main reasons of growth, proliferation and survival of *E. coli* which cause infection and several disorders for humans could be prevented by (1) using sanitary methods in slaughterhouses, (2) butchers should apply sanitary practices in slaughterhouses, (3) avoiding of consuming raw meat without cooking (4) perform animals' vaccination (if necessary), (5) checking slaughterhouses in order to STEC, (6) improving methods of meat preparation, (7) observing hygiene during boiling and cooking of meat, (8) keeping meats in cool and dry places away from direct sun light and (9) preventing contamination of meats with extrinsic factors such as insects and dust.

References

- [1] Ahn, C.K., Russo, A.J., Howell, K.R., Holt, N.J., Sellenriek, P.L., Rothbaum, R.J., Beck, A.M., Luebbering, L.J. & Tarr, P.I. (2009). Deer sausage: a newly identified vehicle of transmission of *Escherichia coli* O157:H7. *Journal of Pediatrics* 155, 587–589. <https://doi.org/10.1016/j.jpeds.2009.02.051>.
- [2] Ammon, A., Petersen, L.R. & Karch, H. (1999). A large outbreak of hemolytic uremic syndrome caused by an unusual sorbitol-fermenting strain of *Escherichia coli* 157:H. *J. Infect. Dis.* 179, 1274–1275. <https://doi.org/10.1086/314715>.
- [3] Antikainen, J., Tarkka, E., Haukka, K., Siitonen, A., Vaara, M., Kirveskari, J. (2009). New 16-plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples. *European Journal of Clinical Microbiology and Infectious Diseases* 28, 899–908. <http://doi.org/10.1007/s10096-009-0720-x>.
- [4] Aslam, M., Toufeer, M., Bravo, C., N., Lai, V., Rempel, H., Manges, A., Diarra, M., S. (2014). Characterization of Extraintestinal Pathogenic *Escherichia coli* isolated from retail poultry meats from Alberta, Canada. *International Journal of Food Microbiology*, 177 (2), 49-56. <https://doi.org/10.1016/j.ijfoodmicro.2014.02.006>.
- [5] Bouvet, J., Montet, M.P., Rossel, R., Le Roux, A., Bavai, C., Ray-Gueniot, S., Mazuy, C., Atrache, V. & Vernozy-Rozand, C. (2002). Prevalence of verotoxin-producing *Escherichia coli* (VTEC) and *E. coli* O157:H7 in French pork. *J. Appl. Microbiol.* 93, 7–14. <https://doi.org/10.1046/j.1365-2672.2002.01672.x>.
- [6] Brusa, V., Galli, L., Linares, L., H., Ortega, E., E., Lirón, J., P. & Leotta, G. (2015). Development and validation of two SYBR green PCR assays and a multiplex real-time PCR for the detection of Shiga toxin-producing *Escherichia coli* in meat. *Journal of Microbiological Methods*, 119, 10-17. <https://doi.org/10.1016/j.mimet.2015.09.013>.
- [7] Brusa, V., Aliverti, V., Aliverti, F., Ortega, E.E., de la Torre, J., H., Linares, L., H., Sanz, M., E., Etcheverría, A., I. et al. (2013). Shiga toxin-producing *Escherichia coli* in beef retail markets from Argentina. *Front Cell Infect Microbiol.* 2013 Jan 18; 2:171. <https://doi.org/10.3389/fcimb.2012.00171>.
- [8] Díaz, S., Vidal, D., Herrera-Leon, S. & Sánchez, S. (2011). Sorbitol-fermenting, betaglucuronidase-positive, shiga toxin-negative *Escherichia coli* O157:H7 in free-ranging red deer in south-central Spain. *Foodborne Pathogens and Disease* 8, 1313–1315. <https://doi.org/10.1089/fpd.2011.0923>.
- [9] Doi, Y., Paterson, D., L., Egea, P., Pascual, A., López-Cerero, L., Navarro, M., D., Adams-Haduch, J., M. et al. (2010). Extended-spectrum and CMY-type β -lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. *Clinical Microbiology and Infection*, 16 (1), 33-38. <https://doi.org/10.1111/j.1469-0691.2009.03001.x>.
- [10] EFSA, ECDC (2013). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. *EFSA J.* 11, 3129. <https://doi.org/10.2903/j.efsa.2015.3991>. <https://doi.org/10.2903/j.efsa.2013.3129>.
- [11] Eggert, M., Stüber, E., Heurich, M., Fredriksson-Ahomaa, M., Burgos, Y., Beutin, L. & Märklbauer, E. (2012). Detection and characterization of Shiga toxin-producing *Escherichia coli* in faeces and lymphatic tissue of free-ranging deer. *Epidemiology and Infection* 1–9. <https://doi.org/10.1017/S0950268812000246>.
- [12] Elder, J.R., Bugarel, M., den Bakker, H.C., Loneragan, G.H. & Nightingale, K.K. (2016). Interrogation of single nucleotide polymorphisms in *gnd* provides a novel method for molecular serogrouping of clinically important Shiga toxin producing *Escherichia coli* (STEC) targeted by regulation in the United States, including the “big six” non-O157 STEC and STEC O157. *Journal of Microbiological Methods*, 129, 85-93. <https://doi.org/10.1016/j.mimet.2016.07.005>.
- [13] Elder, R. O., Keen, J. E., Siragusa, G. R., Barkocy-Gallagher, G. A., Koohmaraie, M. & Laegreid, W. W. (2000). Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc Natl Acad Sci USA.* 2000; 97:2999–3003. <https://doi.org/10.1073/pnas.060024897>.
- [14] Franz, E., Delaquis, P., Morabito, S., Beutin, L., Gobius, K., Rasko, D. A., Bono, J., French, N., Osek, J., Lindstedt, B., Muniesa, M., Manning, S., LeJeune, J., Callaway, T., et al. (2014). Exploiting the explosion of information associated with whole genome sequencing to tackle Shiga toxin-producing *Escherichia coli* (STEC) in global food production systems. *International Journal of Food Microbiology*, 187, 57-72. <https://doi.org/10.1016/j.ijfoodmicro.2014.07.002>.
- [15] Griffin, P.M. & Tauxe, R.V. (1991). The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *Escherichia coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.*, 13, 60–98. <https://doi.org/10.1093/oxfordjournals.epirev.a036079>.
- [16] Chapman, P.A., Ellin, M., Ashton, R. & Shafique, W. (2001). Comparison of culture, PCR and immunoassays for detecting *Escherichia coli* O157 following

- enrichment culture and immunomagnetic separation performed on naturally contaminated raw meat products. *International Journal of Food Microbiology* 68, 11–20. [https://doi.org/10.1016/S0168-1605\(01\)00464-0](https://doi.org/10.1016/S0168-1605(01)00464-0).
- [17] Islam, M. A., Mondol, A.S., Azmi, I.J., de Boer, E., Beumer, R.R., Zwietering, M.H., Heuvelink, A.E. & Talukder, K.A. (2010). Occurrence and characterization of Shiga toxin-producing *Escherichia coli* in raw meat, raw milk, and street vended juices in Bangladesh. *Foodborne Pathogens and Disease*, 7 (11), 1381–1385. <https://doi.org/10.1089/fpd.2010.0569>.
- [18] Jackson, L.A., Keene, W.E., Anulty, J.M., Alexander, E.R., Diermayer, M., Davis, M.A., Hedberg, K., Boase, J., Barrett, T.J., Samadpour, M. & Fleming, D.W. (2000). Where's the beef? The role of cross-contamination in 4 chain restaurant-associated outbreaks of *Escherichia coli* O157:H7 in the Pacific Northwest. *Arch. Intern. Med.* 160, 2380–2385. <https://doi.org/doi:10.1001/archinte.160.15.2380>.
- [19] Jajarmi, M., Fooladi, A., A., I., Badouei, M., A. & 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*, 31, 0882–4010. <https://doi.org/10.1016/j.micpath.2017.05.041>.
- [20] Kagambega, A., Haukka, K., Siitonen, A., Traore, A. & Barro, N. (2011). Prevalence of *Salmonella enterica* and the hygienic indicator *Escherichia coli* in raw meat at markets in Ouagadougou, Burkina Faso. *Journal of Food Protection* 74, 1547–1551. <https://doi.org/10.4315/0362-028X.JFP-11-124>.
- [21] Kaper, J. B., Nataro, J.P. & Mobley, H.L. (2004). Pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.*, 2 (2), 123–140. <https://doi.org/10.3201/eid0805.010385>.
- [22] Karmali, M.A., Gannon, V & Sargeant, J.M. (2010). Verocytotoxin-producing *Escherichia coli*. (VTEC). *Vet. Microbiol.* 140, 360–370. <http://dx.doi.org/10.1016/j.vetmic.2009.04.011>.
- [23] Keene, W.E., Sazie, E., Kok, J., Rice, D., Hancock, D., Balan, V., Zhao, T. & Doyle, P. (1997). An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *Journal of the American Medical Association* 277, 1229–1231. [doi:10.1001/jama.1997.03540390059036](https://doi.org/10.1001/jama.1997.03540390059036).
- [24] Kerangart, S., Douëllou, T., Delannoy, S., Fach, P., Beutin, L., Sergentet-Thévenot, D., Cournoyer, B. & Loukiadis, E. (2016). Variable tellurite resistance profiles of clinically-relevant Shiga toxin-producing *Escherichia coli* (STEC) influence their recovery from foodstuffs. *Food Microbiology*, 59, 32–42. <https://doi.org/10.1016/j.fm.2016.05.005>.
- [25] Levre, E. & Valentini, P. (2001). Insaccati di carne suina e rischio di contaminazione di *Escherichia coli* O157. *Ig. Mod.* 116, 11 – 20.
- [26] Li, H., Garcia-Hernandez, R., Driedger, D., McMullen, L., M. & Gänzle, M. (2016). Effect of the food matrix on pressure resistance of Shiga-toxin producing *Escherichia coli*. *Food Microbiology*, 57, 96–102. <https://doi.org/10.1016/j.fm.2016.02.002>.
- [27] Magwedere, K., Dang, H., A., Mills, E., W., Cutter, C., N., Roberts, E., L. & DebRoy, C. (2013). Incidence of Shiga toxin-producing *Escherichia coli* strains in beef, pork, chicken, deer, boar, bison, and rabbit retail meat. *Journal of Veterinary Diagnostic Investigation*, 25 (2), 254 – 258. <https://doi.org/10.1177/1040638713477407>.
- [28] Mahmoud Ahmed Mahmoud Mohammed, Molecular characterization of diarrheagenic *Escherichia coli* isolated from meat products sold at Mansoura city, Egypt. *Food Control*, 25 (1), 159–164. <https://doi.org/10.1016/j.foodcont.2011.10.026>.
- [29] Momtaz, H., Dehkordi, F., S., Rahimi, E., Ezadi, H. & Arab, R. (2013). Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminant's meat, *Meat Science*, Volume 95 (2), 381–388. <https://doi.org/10.1016/j.meatsci.2013.04.051>.
- [30] Müller, D., Greune, L., Heussipp, G., Karch, H., Fruth, A., Tschäpe, H., & Schmidt, M. A. (2007). Identification of Unconventional Intestinal Pathogenic *Escherichia coli* Isolates Expressing Intermediate Virulence Factor Profiles by Using a Novel Single-Step Multiplex PCR. *Applied and Environmental Microbiology*, 73(10), 3380–3390. <http://doi.org/10.1128/AEM.02855-06>.
- [31] Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, 11 (1), 142–201.
- [32] Paton, J. C. & Paton, A. W. (1998). Pathogenesis and Diagnosis of Shiga Toxin-Producing *Escherichia coli* Infections. *Clinical Microbiology Reviews*, 11 (3), 450 – 479.
- [33] Pokharel, S., Brooks, J., C., Martin, J., N., Echeverry, A., Parks, A., R., Corliss, B. & Brashears, M., M. (2016). Internalization and thermal susceptibility of Shiga toxin-producing *Escherichia coli* (STEC) in marinated beef products. *Meat Science*, 116, 213–220. <https://doi.org/10.1016/j.meatsci.2016.02.016>.
- [34] Potter, M.E. (1992). The changing face of foodborne disease. *J. Am. Vet. Med. Assoc.* 201, 250–253.
- [35] Rabatsky-Ehr, T., Dingman, D., Marcus, R., Howard, R., Kinney, A., & Mshar, P. (2002). Deer Meat as the Source for a Sporadic Case of *Escherichia coli* O157:H7 Infection, Connecticut. *Emerging Infectious Diseases*, 8 (5), 525–527. <http://doi.org/10.3201/eid0805.010373>.
- [36] Rhoades, J.R., Duffy, G. & Koutsoumanis, K. (2009). Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: a review. *Food Microbiol.* 26, 357–376. <https://doi.org/10.1016/j.fm.2008.10.012>.
- [37] Ro, E., Y., Ko, Y., M., Ki Sun Yoon, K., S. (2015). Survival of pathogenic enterohemorrhagic *Escherichia coli* (EHEC) and control with calcium oxide in frozen meat products. *Food Microbiology*, 49, 203–210. <https://doi.org/10.1016/j.fm.2015.02.010>.
- [38] Rounds, J. M., Rigdon, C. E., Muhl, L. J., Forstner, M., Danzeisen, G. T., Koziol, B. S., Taylor, C., Shaw, B.T., Short, G.L. & Smith, K. E. (2012). Non-O157 Shiga Toxin-producing *Escherichia coli* Associated with Venison. *Emerging Infectious Diseases*, 18 (2), 279–282. <http://doi.org/10.3201/eid1802.110855>.
- [39] Sánchez, S., Díaz-Sánchez, S., Martínez, R., Llorente, M., T., Herrera-León, S. & Vidal, D. (2013). The new allelic variant of the subtilase cytotoxin (subAB2) is common among Shiga toxin-producing *Escherichia coli*

- strains from large game animals and their meat and meat products, *Veterinary Microbiology*, 166 (3–4), 645–649. <https://doi.org/10.1016/j.vetmic.2013.06.031>.
- [40] Sánchez, S., García-Sánchez, A., Martínez, R., Blanco, J., Blanco, J.E., Blanco, M., Dahbi, G., Mora, A., Hermoso deMendoza, J., Alonso, J.M. & Rey, J. (2009). Detection and characterization of Shiga toxin-producing *Escherichia coli* other than *Escherichia coli* O157:H7 in wild ruminants. *Veterinary Journal*, 180, 384–388. <https://doi.org/10.1016/j.tvjl.2008.01.011>.
- [41] Sánchez, S., Martínez, R., García, A., Vidal, D., Blanco, J., Blanco, M., Blanco, J.E., Mora, A., Herrera-León, S., Echeita, A., Alonso, J.M. & Rey, J. (2010). Detection and characterization of O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in wild boars. *Veterinary Microbiology*, 143, 420–423. <https://doi.org/10.1016/j.vetmic.2009.11.016>.
- [42] Schmidt, M. A. (2010). LEEways: tales of EPEC, ATEC and EHEC. *Cellular Microbiology*, 12 (11), 1544 – 1552. <https://doi.org/10.3201/eid0805.010385>. 10.1111/j.1462-5822.2010.01518.x.
- [43] Simporé, J., Ouermi, D., Ilboudo, D., Kabre, A., Zeba, B., Pietra, V., Pignatelli, S., Nikiema, J. B., Kabre, G.B., Caligaris, S., Schumacher, F. & Castelli, F. (2009). Aetiology of acute gastro-enteritis in children at Saint Camille Medical Centre, Ouagadougou, Burkina Faso. *Pakistan Journal of Biological Sciences* 12, 258–263. <https://doi.org/10.3923/pjbs.2009.258.263>.
- [44] Skočková, A., Koláčková, I., Bogdanovičová, K. & Karpíšková, R. (2015). Characteristic and antimicrobial resistance in *Escherichia coli* from retail meats purchased in the Czech Republic. *Food Control*, 47, 401–406. <https://doi.org/10.1016/j.foodcont.2014.07.034>.
- [45] Soler, P., Hernandez, G. & Mateo, S. (1999). Vigilancia de *Escherichia coli* O157 en Espana. *Bol. Epidemiol. Sem.* 7, 105– 106.
- [46] Stampi, S., Caprioli, A., De Luca, G., Quaglio, P., Sacchetti, R. & Zanetti, F. (2004). Detection of *Escherichia coli* O157 in bovine meat products in northern Italy, *International Journal of Food Microbiology*, Volume 90, Issue 3, 1 February 2004, Pages 257–262. [https://doi.org/10.1016/S0168-1605\(03\)00308-8](https://doi.org/10.1016/S0168-1605(03)00308-8).
- [47] Tahmasby, H., Mehrabiyan, S., Tajbakhsh, E., Farahmandi, S., Monji, H. & Farahmandi, K. (2014). Molecular detection of nine clinically important non-O157 *Escherichia coli* serogroups from raw sheep meat in Chaharmahal-va-Bakhtiari province, Iran. *Meat Science*, Volume 97, Issue 4, August 2014, Pages 428–432, ISSN 0309-1740, <https://doi.org/10.1016/j.meatsci.2014.02.007>.
- [48] Trabulsi, L.R., Keller, R. & Tardelli Gomes, T.A. (2002). Typical and atypical enteropathogenic *Escherichia coli*. *Emerg. Infect. Dis.*, 8 (5), 508–513. <https://doi.org/10.3201/eid0805.010385>.
- [49] Vidal, M., Kruger, E., Durán, C., Lagos, R., Levine, M., Prado, V. et al. (2005). Single Multiplex PCR Assay To Identify Simultaneously the Six Categories of Diarrheagenic *Escherichia coli* Associated with Enteric Infections. *Journal of Clinical Microbiology*, 43(10), 5362–5365. <http://doi.org/10.1128/JCM.43.10.5362-5365.2005>.
- [50] Wang, H., Xin, Y., Wei, R., Tang, X., Zhao, A., Sun, H., Zhang, W. et al. (2015). Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* isolated from retail raw meats in China. *International Journal of Food Microbiology*, 200, 31–38. <https://doi.org/10.1016/j.ijfoodmicro.2015.01.018>.