Escherichia coli O157:H7: local epidemiology and disease spectrum in Brazil

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ABSTRACT

Escherichia coli O157: H7 is one of the most important foodborne pathogens nowadays, since it has been responsible for severe outbreaks worldwide. Even though this food pathogen has been isolated in many countries, Brazilian foods were considered *E. coli* O157:H7-free until recently. However, the presence of *E. coli* O157:H7 has been reported in diverse foods produced in Brazil and an increasing number of isolation from cattle feces has been observed, demonstrating that this pathogen is present in different parts of Brazil, and severe foodborne outbreaks may occur in the near future if adequate control measures are not implemented.

Keywords: Escherichia coli O157:H7; Brazil; food contamination; animal contamination

Escherichia coli O157:H7 was recognized as a food pathogen for the first time in Oregon and Michigan in the United States, in 1982, causing two outbreaks of severe bloody diarrhea, involving at least 47 people, after eating sandwiches containing beef hamburgers, onion sauce, and pickles distributed by a fast food chain. *E. coli* O157:H7 was isolated from nine out of 12 victims with diarrhea from the outbreaks, and also from a hamburger sample collected in Michigan[1]. This serovar had already been isolated from a sporadic case report of hemorrhagic colitis in 1975[1]. Despite the severity of the symptoms, outbreaks were not widely publicized at that time. After more than a decade without being associated with new foodborne outbreaks, in 1993, *E. coli* O157:H7 was responsible for an outbreak involving about 700 people due to the consumption of undercooked beef hamburgers distributed by another fast food company in the U.S. Among the affected people, more than 40 cases progressed to Hemolytic Uremic Syndrome (HUS) and 4 people died[2]. From this episode, which was considered the “September 11” of food safety in the U.S., *E. coli* 0157:H7 gained wide notoriety and has been isolated from many foods involved in several foodborne illnesses in different countries[3].

Most *E. coli* strains do not cause diseases and are actually part of the normal flora of the intestinal animal tract, including that of humans[4-6]. Some types of *E. coli*, however, may be pathogenic, causing diarrhea, urinary tract infections, respiratory diseases, pneumonia, and other diseases[4-7], while others may cause serious foodborne diseases, as is the case of Shiga toxin-producing *E. coli* (STEC).

There are more than 400 STEC serotypes[8]; however, the group clinically associated with Hemorrhagic Colitis (HC) is designated EHEC (*Enterohemorrhagic E. coli*)[5-8]. EHEC is often involved in serious human diseases[9] and, among them, the *E. coli* O157:H7 is the serotype most frequently associated with severe foodborne outbreaks. The strains of this serotype are more pathogenic or more transmissible than other *E. coli* serotypes[10]. Until recently, Brazil was considered an *E. coli* O157:H7-free country because no food contamination by this microorganism had been described by official reports. However, diverse scientific publications have demonstrated the isolation of *E. coli* O157:H7 in Brazil, mainly from animal feces and more recently from food samples. Based on this, the objective of this review is to present the current situation about the epidemiology and disease spectrum of *E. coli* O157:H7 in Brazil.

DISEASES CAUSED BY *ESCHERICHIA COLI* 0157:H7

Overall, *E. coli* O157:H7 can cause three known diseases named Hemorrhagic Colitis (HC), Hemolytic Uremic Syndrome (HUS), and Thrombotic Thrombocytopenic Purpura (TTP)[11].

Within a mean of 3 days of contaminated food consumption, the patients present diarrhea without blood and severe abdominal pains. After this period, vomiting and, in some cases, low-grade fever may occur. Next, there is an increase in abdominal pain, and bloody diarrhea (hemorrhagic colitis) begins, which results in bloody evacuation, which usually lasts 1 week. Within 4 days of the onset of symptoms, positive cultures can be obtained from feces. About 85% of HC are self-limiting, however in 15% of cases it progresses to HUS, which is the most severe complication of enteric infection. It is considered the main cause of acute renal failure in children[9,10], and people affected by HUS are mainly children under 5 years old and elderly over 65[12]. HUS can be diagnosed on average 7+/-2 days after the onset of diarrhea[12]. The majority of HUS cases (70% to 95%) are associated with gastroenteritis caused by *E. coli* O157:H7[13,14]. HUS is defined by a triad consisting of hemolytic anemia, thrombocytopenia, and acute renal failure. This syndrome occurs when toxins produced by *E. coli* 0157:H7 affect the kidneys[4]. Clinically, patients with HUS have become seriously ill, or sometimes with jaundice and often with hypertension. Patients may present problems with the cardiovascular system and central nervous system with cardiac infarction, sudden attacks of apoplexy, coma, and hypertensive encephalopathy. The disease can lead to death[15]. Most patients (90%) will recover with appropriate treatment, but from 3 to 5% of children will die, and about 12 to 30% will present severe consequences, including expressive renal failure, hypertension, and/or central nervous system manifestations[9,10]. As there is no specific therapy for HUS, the majority of patients require prolonged treatment involving dialysis, blood transfusion, or kidney transplant[16-18].

The incidence of HUS will vary according to the country. For example, in 2009, the incidence of HUS in children below 5 years old was 5.8 cases per 100.000 children in the U.S. and America[13,14]. HUS was considered an endemic disease in Argentina, presenting one of the highest rates in the world, i.e., 10.4 and 12.2 cases per 100.000 children in 2000 and 2001, respectively[19].

Infection by *E. coli* O157:H7 can also trigger the TTP clinical state, characterized by micro angiopathic hemolytic anemia, thrombocytopenia, neurologic manifestations, fever, and kidney failure. TTP patients exhibit clinical and pathological characteristics similar to the HUS patients, but the involvement of the central nervous system is the primary characteristic[15]. TTP is predominant in 30-year-old adults and the female/male rate is 3:2[20]. Little data is available on the incidence of TTP caused by *E. coli* O157:H7.

RESERVOIRS AND INFECTIOUS DOSE

Bovines have been identified as the major reservoir of *E. coli* 0157:H7, and the pathogen may be excreted with feces, thus contaminating food, water, and the environment[21-23]. Although the infectious dose is unknown, there is a suspicion that it is similar to that of *Shigella* sp. (10 microorganisms)[5-10]. The ingestion of raw milk[24], lettuce[25,26], potatoes[27], radish sprouts[28], alfalfa sprouts[29], and beef hamburgers[1] has been associated with outbreaks. There are also cases where the transmission occurred while people were swimming or drinking sewage contaminated water (untreated water sources)[30], and also due to contact with other persons[18].

CLINICAL CASES OF HEMOLYTIC UREMIC SYNDROME IN BRAZIL

Human infections associated with STEC in Brazil are mainly sporadic cases of diarrhea,

bloody diarrhea, hemolytic anemia, and HUS[31]. Although most cases of diarrheal diseases were associated with non-O157 STEC serotypes, isolation of O157:H7 has been reported in some Brazilian States and it was in general related to more severe cases of bloody diarrhea and HUS. Besides the O157:H7 cases identified in São Paulo, two other O157:H7 STEC strains were isolated in the States of Minas Gerais and Espírito Santo, in 2007, and were confirmed by the Instituto Adolfo Lutz, São Paulo[31]. A likely explanation for the lack of association between *E. coli* O157:H7 and foodborne outbreaks would be the absence of routine enforcement to investigate this microorganism in foods involved in outbreaks. Furthermore, conventional microbiological techniques for *E. coli* are not capable of detecting *E. coli* O157:H7 or its Shiga toxin[32,33]; to detect both of them it would be necessary to use specific methods based on immunomagnetic separation and molecular biology techniques[33,34]. Besides requiring skilled technicians and being more expensive, these techniques have not been implemented in the majority of official Laboratories in Brazil yet. Furthermore, several difficulties have been faced for the detection and isolation of STEC from foods, as they are often present in low amounts, and it may be difficult to isolate them in the presence of high numbers of competitor organisms[31].

In Brazil, there is only one report in the literature describing the involvement of *E. coli* O157:H7 with a foodborne outbreak. This outbreak occurred in Campinas, in 2001, and there were two cases of diarrhea caused by the ingestion of undercooked meat[15], but no HUS.

According to the data records of authorization forms for hospital admittance of the Brazilian Unified Health System/ Ministry of Health (AIH/DATASUS/ MS) from 1998 to July 2000, there were 12 cases of HUS reported, with previous history of diarrhea and possible association with *E. coli* O157:H7, in the State of São Paulo[17]. However, there was no confirmation of the presence of the pathogen.

In 2001, an 8-month-old boy was admitted to a hospital in São Paulo city and was diagnosed with HUS. After the diagnosis, the samples of the patient's feces were analyzed. The results demonstrated the isolation of a strain of *E. coli* O26:H11. This was the first report on isolation of a STEC strain in a patient with HUS in Brazil[35].

According to data from 2001, two *E. coli* O157:H7 were isolated from patients with diarrhea, living in Campinas/SP, with history of ingestion of hamburger and other ground meat. However, a laboratory confirmation of the suspected foods was not possible so it was not possible to establish the relationship between the cases[15,36].

In the State of Minas Gerais, the analysis of epidemiological clinical and laboratorial characteristics of HUS cases reported by the Department of Pediatric Nephrology of a hospital in Uberlandia, demonstrated the possibility of the diagnosis of HUS as a cause of renal failure in children in both typical (after diarrhea) and atypical forms[37]. The samples were collected between January 1994 and January 2004.

According to data from the Center for Epidemiological Surveillance of the State of São Paulo, from 1998 to 2011, 93 cases of HUS, which could have been caused by medications, systemic or hereditary diseases, related or not with *E. coli,* were observed. In the same period, Instituto Adolfo Lutz (IAL) identified eight cases of *E. coli* O157:H7 and one (occurred in 2007) resulted in HUS[15]. However, the microorganism was not found in food. In 2002, three strains of STEC isolated from patients' feces were serotyped and molecularly characterized. The first one, in 1990, was from an HIV+ patient, aged 18; the second one was from a 4-year-old child, and the third one from an adult with bloody diarrhea. The samples were serotyped as O157:H7 and molecularly characterized as having the EHEC virulence factors; however, it was not possible to establish a relationship with food or to identify the source of infection[38].

In another study, analyzing a collection of 39 strains of STEC isolated from patients with diarrhea from 1976 to 1999, in São Paulo, Vaz et al. (2004) reported a prevalence of serogroups "O111" and "O26". The serotypes found in this study were: "O26:H11", "O55:H19", "O93:H19", "O111:NM", "O11:H11", "O118:H16", and "O157:H7"[39]. In the United States, "O26", "O45", "O103", "O111", "O121", and "O145" serogroups cause most of the cases of disease due to non-O157. These serogroups of STEC are referred to as "top six" or "Big Six"[40-43]. Other STEC serogroups, including "O113" and "O91", have also been associated to cases and outbreaks of HC and HUS in many countries[41,44-47].

Souza et al. analyzed the clinical and microbiological characteristics associated with 13 cases of post-diarrheal HUS identified in pediatric intensive care, in São Paulo city, which occurred between January 2001 and August 2005. STEC were isolated from three of the seven patients

whose fecal cultures presented bacterial growth, and the serotypes identified were O26:H11, O157:H7, and O165:HNM. The source of infection was not screened; however, the consumption of unpasteurized milk or undercooked meat was reported and they may be the major cause of infection in the majority of cases[48].

In 2012, the Epidemiological Surveillance, in Annapolis, State of Goiás, notified the clinical suspicion of a HUS case. It was a 48-year-old housewife. This case was identified from samples sent to the Center for Epidemiological Surveillance to the Public Health Laboratory "Dr. Giovanni Cysneiros" - LACEN. The case resulted in cure without complications. The suspected food investigated was a regional cheese (Minas Frescal cheese), and a supplement for slimming[49].

Although several cases of CH and HUS have been described and the isolation of *E. coli* O57:H7 is becoming more frequent, no other cases of HUS linked with foods were reported in Brazil since 2001.

PATHOGENESIS

The mechanism by which *E. coli* O157:H7 causes HC and HUS has not been fully elucidated[7], however, much information has been raised due to the large amount of investigations related to this pathogen during the last years. Several virulence factors are involved in the capacity to colonize the human intestine, to adhere to the mucosa, damage microvilli, and release cytotoxins. The ability of excreting Shiga toxin (Stx) (encoded by *stx1* and *stx2* genes) is the primary requirement to cause HUS[30]. Stx 1 and Stx 2 differ by only one aminoacid of Shiga toxin produced by *Shigella dysenteriae* type 1[18]. Shiga toxins belong to the group of toxins known as type A/B toxins, i.e., they present one enzymatic subunit A and five subunits B, which are oligomers composed by five identic proteins[50]. The main receptor for Stx1 and Stx2 is globotriaosyl ceramide, or receptor Gb3, and its expression on the target cell surface is related to cytotoxicity[51]. In humans, these receptors are present on the epithelial cells of the intestine, on the vascular endothelium, and on the renal epithelium[52]. Shiga toxins act by inhibiting protein synthesis. The subunit A is responsible for the biological activity of the toxin, which cleaves ribosomal RNA, preventing protein synthesis in the host cell[53]. The B subunits mediate the binding of the toxin to host cell receptors[54].

The Stx toxins are produced by bacteria in the colon and reach the kidneys through the bloodstream, damaging renal cells and determining occlusion of microvascular endothelium. This is produced by a combination of toxicity and induction of local production of cytokines and chemokines, resulting in local inflammation, which can lead to HUS[53-55]. Stx also induces apoptosis of enterocites[53]. However, the precise role of Stx in mediating colonic disease, HUS, and neurological disorders has not been fully elucidated, as there is no satisfactory animal model for hemorrhagic colitis or HUS, and the severity of the disease precludes the study of experimental infections in humans[56]. Both Stx1 and Stx2 are important virulence factors of STEC, but strains producing Stx2 are more virulent and more often related to HUS[18,57].

Other important virulence genes are involved with virulence of *E. coli* O157:H7, for example the *eae (E.coli* attachment effacement) gene (encoding the intimin outer membrane protein)[58]. *E. coli* O157:H7 colonizes the large intestine and produces a characteristic histopathological feature known as the attaching and effacing lesion (A/E). This lesion is characterized by intimate attachment of the bacteria to the plasma membranes of the host epithelial cells, localized destruction of the brush border of microvilli, and assembly of highly organized pedestal-like actin structures[56]. Studies with cultured epithelial cells revealed that the A/E lesion involves conjoined action of the outer membrane protein intimin, which is encoded by the *eae* gene and several other genes[46,59]. The fliCH7 gene encodes the flagellar antigen of *E. coli* O157:H7 and it has also been investigated as a virulence factor of STEC because the identification of the H7 flagellar antigen is critical for the confirmation of *E. coli* O157:H7[60,61].

NON-HUMAN CARRIAGE OF *E. COLI* O157:H7 IN BRAZIL

In São Paulo, the first isolation of *E. coli* O157:H7 was reported by the IAL, in 1997, from a sample of well water of a small farm in Parelheiros - SP. Once this agent was detected, an interinstitutional working group was developed including professionals from the Division of Bromatology and Chemistry, the Division of Medical Biology of IAL, the Epidemiological Surveillance Center "Pr. Alexandre Vranjac" (CVE) (Division of waterborne diseases), the Health Surveillance Center, and the Epidemiological and Sanitary Surveillance of

the Regional Directorate for Health I, of the State of São Paulo. A second sample of well water, as well as samples of human feces and animals around the houses were analyzed. However, *E. coli* O157:H7 was not found[62].

Silveira et al. investigated the occurrence of *E. coli* O157:H7 in 886 samples of hamburgers produced by eight manufacturers in Southern and Southeastern Brazil, between January and September 1997. In 17 samples (1.9%), there were *E. coli* capable of agglutinating antiserum to "O157", but further testing showed that there was no *E. coli* O157:H7 in any of the samples[63].

In 1999, *E. coli* O157:H7 was isolated from three samples collected at a slaughterhouse in the state of Rio de Janeiro. One of the three samples was beef, and the other two from dairy cattle feces. This was the first report of *E. coli* O157:H7 isolated from dairy cattle in Brazil[64]. Three (1.5%) O157:H7 *E. coli* strains were isolated from one beef and two dairy animals by the use of cefixime tellurite sorbitol MacConkey aga (CT-SMAC). To our knowledge, this was the first report of O157:H7 isolation in Brazil.

Irino et al. analyzed 153 bovine fecal samples for the presence STEC through the isolation on selective medium and production of Shiga toxin (Stx) by testing cytotoxicity on Vero cells. The samples were randomly selected from six dairy farms in São Paulo, Brazil, and the results demonstrated the presence of 202 STEC, two *E. coli* O157:H7 among them[65].

The presence of the genes for Shiga toxin (*stx*) in STEC was investigated by Leomil et al. in 344 fecal samples from both asymptomatic and diarrheic calves (n = 139, n = 205) from 12 beef cattle farms in the State of São Paulo. Among these, 44 (12.7%) animals were positive for *stx*. Diarrheic calves had a higher frequency of isolation of *stx* (28/139, 20%) compared to asymptomatic animals (16/205, 7.8%). Among strains of STEC, 16 serovars were identified, among them: O111:NM, O111:H8 (02), and O118:H16 (01). The serovar O157:H7 was not isolated[66].

In 2003, another study was carried out to investigate the presence of *E. coli* O157:H7 in raw vegetables commonly consumed in Brazil. The study analyzed 869 samples of vegetables; however, *E. coli* O157:H7 was not detected[67].

During 1999-2000, an investigation was conducted on 60 dairy farms in Pelotas, state of Rio Grande do Sul for the presence of verotoxigenic *E. coli*. In the same study, 1,127 isolates of *Escherichia coli* were found from 243 dairy cattle, water for human and animal consumption, and milk samples. The presence of verotoxigenic *E. coli* was verified in 95% (57/60) of the farms, in 49% (119/243) of the animals tested, in 5% (3/60) of the water for human consumption samples, in 8.35% (5/60) of the water for animal consumption samples, and in 5% (3/60) of the milk samples. VTEC belonging to serogroups "O157", "O91", and "O112" were isolated in animal feces (2.9%, 7/243)[68].

In the city of São Luis, State of Maranhão, the occurrence of STEC in the guts of cattle intended for slaughter was assessed through multiplex PCR for the genes *stx1, stx2,* and *eae*. Altogether, 100 stool samples were analyzed. The percentage of STEC isolated was 73%. The major virulence genotypic patterns detected were: *stx1/stx2* (68.8%), *stx1* (11.8%), and *stx2* (8.6%). Few strains of STEC (4.4%) had the *eae* gene in association with *stx* genes[69].

Stella examined the virulence factors of 473 strains of *E. coli* isolated from milk, water, and feces of dairy cattle in Ribeirão Preto, state of São Paulo, and found two stool samples positive for *E. coli* O157:H7[70].

Silveira analyzed 95 samples of ground beef collected in different municipalities of the State of Rio Grande do Sul (RS), near the border with Uruguay and Argentina, in order to investigate the presence of *E. coli* O157:H7. Among these samples, three isolates were identified as *E. coli* O157:H7 using the methods recommended by the USDA/FSIS. However, multiplex-PCR (*rfbO157, stx1* and *stx2*) performed at the Reference Laboratory for Regional Surveillance of HUS and bloody diarrheas in the Argentine Ministry of Health (INEI-ANLIS) revealed that the three isolates were not classified as *E. coli* 0157:H7[71].

Loiko analyzed 108 samples of bovine carcasses at an exporter slaughterhouse in the state of Rio Grande do Sul. The presence of 22 strains of *E. coli* O157:H7 was detected, and 6 PFGE profiles were identified among them. Resistance to antimicrobials was also analyzed, revealing multidrug resistance against various antibiotics tested. Among the strains identified, one of them showed the same genotypic and phenotypic profile of a strain of *E. coli* O157:H7 responsible for food poisoning in Argentina, isolated in 2005 in that country[72].

In 2013, in the state of São Paulo, a study examined 100 bovine carcasses in an exporter slaughterhouse. The presence of *E. coli* O157 was not detected in any sample[73].

In 2013, another study in the state of São Paulo analyzed the occurrence of *E. coli* O157:H7 in the same pieces of meat and carcasses of cattle finished on pasture or feedlot collected between November 2008 and October 2009. Rectal swab samples for the detection of *E. coli* O157:H7 were also taken from the same animals. A total of 100 rectal swabs, 100 samples collected before the carcasses cooled down, and 323 meat trimming samples were analyzed. According to the results, one scrap of meat sample was positive for *E. coli* O157:H7[74].

In a recent study, Rodrigues et al. isolated two strains of *E. coli* O157:H7 from irrigation water and a washing water sample in a small organic lettuce farm in Southern Brazil[75].

Although there are many registers of the presence of *E. coli* O157:H7 in different Brazilian states, the investigation of this microorganism is not, as yet, compulsory in this country. This very important information about the presence of *E. coli* O157:H7 before the occurrence of foodborne outbreaks in Brazil is a very good opportunity for the implementation of preventive measures promoted by the Brazilian government. The adoption of measures such as pasteurizing the milk, water chlorination, proper washing and disinfection of leafy vegetables, and proper cooking of meat products are measures of great importance in the prevention of foodborne outbreaks caused by *E. coli* O157:H7. Furthermore, since there are reports of the presence of this microorganism in industrial environments, hand washing and good manufacturing practices to prevent cross-contamination are also essential.

CONCLUSION

Since there is no official surveillance on *E. coli* O157:H7 and its related diseases in Brazil, and there is an increasingly frequency of isolation of this microorganism from food and animal samples, the possibility of occurrence of severe foodborne outbreaks caused by *E. coli* O157: H7 should be considered in Brazil.

This pathogen is highly virulent, and when an infection is established, there is currently little that can be done to prevent the progress of the disease to HUS, its most severe manifestation. Given that the transmission can occur by several routes, the adoption of different barriers is necessary to prevent its transmission. In addition, rapid diagnostic strategies need to be adopted and there is a need for active surveillance protocols and the use of appropriate detection methods in both clinical and food laboratories. Furthermore, rapid microbiological diagnosis of individual patients enables the prompt notification of outbreaks and the implementation of control measures to avoid more cases.

In addition, the instrumentation of official laboratories and the standardization of detection techniques are necessary in order to make possible the routine investigation of this pathogen in Brazil. We can also conclude that, given the large number of reports of clinical cases in other countries, case investigations of *E. coli* O157:H7 should be made mandatory for some foods, in the event of foodborne diseases, as it already happens in countries such as the United States and Argentina.

Finally, epidemiological studies for risk assessment, as well as studies for the characterization of isolates of *E. coli* O157:H7 found in Brazil are important because through these data it is possible to determine the incidence and distribution of the pathogen and possible reservoirs, as well as to set benchmarks for the implementation of an adequate system of monitoring and prevention.

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