ENTERPATOPHGENIC ESCHERICHIA COLI O157 IN BANGUI AND N’GOILA, CENTRAL AFRICAN REPUBLIC: A BRIEF REPORT

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Abstract. Escherichia coli O157:H7 producing Shiga like toxins is a food-borne pathogen frequently isolated in Bangui from patients with hemorrhagic colitis (HC). This survey provides comprehensive data on the high prevalence of E. coli O157:H7 infection in Bangui: carriage of E. coli O157:H7 by zebu (Bos indicus) and fish, contamination of the fields at N’Goila where the butchers kill the zebus, and contamination of the field surface water along the M’Poko River upstream of the Oubangui River where fish are caught, appear to be important contributory factors. We also describe novel strains of serogroup O157:NM isolated from zebu and from fish; a variety of assays indicate that these strains belong to the enteropathogenic pathotype, though they lack certain genetic elements thought to be diagnostic for this pathotype.

INTRODUCTION

An outbreak of bloody diarrhea was attributed to E. coli O157:H7 in 1996 in Zémio. This is a village in the eastern region of Central African Republic (CAR), 200 km from Bangui (the capital city of CAR), along the Oubangui River. Since this epidemic, an increasing number of cases of acute bloody diarrhea in infants and adults have been reported in Bangui. In several hospital patients with hemorrhagic colitis (HC), hemolytic anemia, and renal insufficiency, sorbitol non-fermenting E. coli O157:H7 that had the genes encoding Shiga toxins 1 and 2 were identified. Between 1996 and 1999, in diagnosed cases of HC or hemorrhagic uremic syndrome (HUS), serum samples were collected from hospitalized patients and tested for antibodies to the O157 lipopolysaccharide (kindly provided by P. Grimont, Institut Pasteur, Paris), by enzyme-linked immunosorbent assay. The combined use of microbiologic and serologic techniques provided evidence of Shiga toxin (Stx)-producing E. coli O157 in 438 (65.6%) of 668 cases examined. We did case control studies in 1996 (33 HC versus 140 controls) and 1998 (12 HC and 9 HUS versus 58 controls). During these studies, because of civil unrest in the city, coups, and military rebellions, patients were not paired. The questionnaires from these studies included demographic and socio-economic characteristics, environmental factors, and habitual food consumption. Food histories often pointed to uncooked (carpaccio) zebu (a domesticated bovine mammal, Bos indicus, of Africa) meat or fish, and smoked zebu meat (a meal named kanda).

In Bangui there is no slaughterhouse and the zebus are killed in a field in a suburb named N’Goila, along the M’Poko River. All the zebu herds that come from the Northern regions of CAR, Chad, and Sudan are regrouped in pasturelands 12 km North of Bangui where butchers buy them. The day before they are to be killed, zebus are held at N’Goila where they eat the grass and drink the water on the surface of the fields. Each day, early in the morning, zebus are killed. The carcasses are butchered on the ground and the entrails are left on the killing field. Transportation of the meat is carried out at ambient temperature.

Several fisheries are located on the Oubangui River downstream from M’Poko River, less than 1 km from N’Goila, because water is full of fish near the area where zebus are killed. After being captured in fishing nets, fish are kept alive by immersion in the Oubangui River until they are sold. Because E. coli is a common contaminant of food in the tropics, a survey was conducted in Bangui to determine if zebu and fish could be the potential reservoir hosts of these O157 pathogenic E. coli.

Between 1997 and 2001, zebu dung (collected with rectal swabs), entrails of fish from Oubangui River (for the study, the fish were kept alive in the river, near the beach, and sampled directly by the investigators), and surface water collected in the fields where zebus are held or killed, were studied. Zebu dung was also collected 20 km north of Bangui before they arrived in the fields of N’Goila. Zebu dung and entrails of fish were plated on purple bromocresol lactose agar (BCP) and sorbitol/MacConkey agar and were incubated overnight at 37°C. Water samples were vacuum filtered, and the filters were subcultured onto BCP lactose agar and sorbitol/MacConkey agars. All lactose-fermenting colonies and all sorbitol-nonfermenting colonies were selected and streaked on blood agar plates. All the E. coli isolates were confirmed biochemically, characterized by using API 20E strips (Biomerieux, France) and anti O157 and anti H7 antisera (Difco).

E. coli O157:H7 strains were isolated from 11 (5.4%) of the 205 zebu dung samples and from 6 (2.3%) of the 260 water samples collected at N’Goila, and in 3 entrails (4.7%) of the 64 fish captured in the Oubangui River. Non-motile O157 E. coli strains were isolated from two zebu dung samples collected at N’Goila and from the entrails of one fish. One non-motile O157 E. coli strain and two motile O157 E. coli lacking the H7 flagellar antigen were isolated from dung collected in 160 zebus in route to Bangui, 20 km before they arrived at N’Goila.

Polymerase chain reaction was used to detect enterohemorrhagic Shiga like toxins 1 and 2 genes, the attaching and
effacing gene eaeA, and the bundle-forming pili gene bfpA. 
Toxigenic strains of \textit{E. coli} O157:H7 were identified in 9 zebus (22 strains) from N’Goila, from 6 water samples (8 strains), and from the 3 fish (10 strains). Only two strains (isolated from the same zebu) had only \textit{sts}2 gene; the other 38 strains had the genes encoding both Shiga toxins 1 and 2. The Vero cell assay confirmed the cytotoxicity of these isolates, with most of them being seroneutralized by rabbit antiserum against Shiga toxin. 
We used pulsed-field gel electrophoresis (PFGE) to establish clonal relation and diversity among the strains. 
Restriction enzyme digestion (\textit{XbaI}) was performed as described. Twenty-one of these 40 isolates had indistinguishable PFGE patterns. This predominant clone was isolated from various clinical specimens collected in 1996 during the epidemic at Zémo, hospitalized patients at Bangui with HC between 1998 and 2001, zebu dungs, kanda, and entrails of fish. Four other PFGE patterns were identified among the 19 remaining isolates.

One of the two O157:NM strains isolated from zebus had genes encoding Shiga toxins 1 and 2. This serotype of EHEC had never been identified from clinical specimens in CAR. These two isolates were characterized by restriction digestion of the PCR-amplified \textit{fliC} gene showed a pattern characteristic of the H7 allele found in \textit{E. coli} O157:H7 isolates. They both contained the \textit{uidA} allele. These two strains had different PFGE patterns.

Two O157:NM strains, one isolated from a fish and one from a zebu, were \textit{Stx} negative. These strains are capable of autoaggregation and localized adherence but do not carry the \textit{bfpA} gene, even though these two phenotypes are usually associated with expression of bundle-forming pili. These strains were positive for attaching and effacing and localized adherence phenotype and are therefore EPEC, although they are negative for the adherence factor probe. Both strains carried an \textit{eae} gene. These two strains have different PFGE patterns. These findings emphasize the fact that \textit{E. coli} with O157 O antigen are not always EHEC and may belong to other pathotypes. The study of their phylogenetic relationships to other pathogenic \textit{E. coli} and an epidemiologic survey to determine their potential role in infant and adult diarrhea are in progress. None of the \textit{E. coli} strains (one O157:NM strain and two motile strains lacking the H7 flagellar antigen) isolated from dung collected in zebus before they arrived at N’Goila had the features of EHEC or EPEC.

Carriage of \textit{E. coli} O157:H7 by zebu and fish, and contaminations of the fields at N’Goila and of field surface water are important contributing factors to the high prevalence of HC and HUS in Bangui. Transportation of meat at ambient temperature also likely represents a risk factor. How enterohaemorrhagic \textit{E. coli} O157:H7 and O157:NM originally found their ways to zebu and fish in CAR is not known, but once there we can speculate how they spread. Recovery of toxigenic \textit{E. coli} O157:H7 and O157:NM only at N’Goila from the water on the surface of the fields, and from zebu held there, plausibly explains how these pathogens may contaminate the zebus during the 24 hours that they stay at N’Goila before they are killed. With the daily rains, water contaminated with zebu feces at N’Goila is dispersed in the Ouangui River and becomes available to livestock and fish downstream M’Poko River. PFGE has shown a clonal diversity among strains isolated from clinical and environmental specimens but results indicate that one clone could be responsible for the outbreak at Zémo and several infections at Bangui; this clone was also isolated in fish and surface water samples suggesting that \textit{E. coli} O157 was able to spread over large distances, perhaps via waterways or fish.

These findings raise concerns about a major zoonotic risk for humans, given that zebus and fish from Ouangui River are commonly used as a source of meat in Bangui. Health authorities have been informed of the urgency to build a slaughterhouse and to improve conditions of meat transportation. In the current sanitary conditions consumption of undercooked or crude zebu meat or fish must be avoided.

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ZEBU AND FISH ARE RESERVOIR HOSTS OF O157 E. COLI