SEROPREVALENCE OF ENTEROHAEMORRHAGIC SHIGATOXIN-PRODUCING *ESCHERICHIA COLI* 0157:H7 ISOLATES FROM ABATTOIR EFFLUENTS IN ZANGO ZARIA, NIGERIA

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SUMMARY

The occurrence of verotoxin-producing *Escherichia coli* 0157:H7 (VTEC 0157:H7) in abattoir effluent samples from Zango, Zaria was investigated. A total of 210 samples were obtained from 2002 to 2006. The samples were enriched at the point of collection using Modified Tryptone Soya Broth supplemented with Novobiocin and incubated at 37°C for 24 hours. The enriched samples were plated on Eosin-Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. Colonies showing a greenish metallic sheen on EMB agar were biochemically screened and those that were *E. coli* were stored on nutrient agar (NA) slants at 4°C. The selected colonies were later plated on Cefixime Sorbitol MacConkey (CR-SMAC) agar and incubated at 37°C for 24 hours. Colourless colonies on CR-SMAC agar were tested for verotoxin production by reverse passive latex agglutination using VTEC-RPLA *Escherichia coli* verotoxin detection kit. Isolates that produced verotoxin 1(Stx1) and/or verotoxin 2(Stx2) were finally tested serologically using remel Wellcolex *E. coli* 0157:H7 Kit. *E. coli* 0157:H7 was detected in one sample (1/210) or 0.5%. Abattoir effluents are a risk factor to man for *E. coli* 0157:H7 infection and the pathogen is present in our food animals. Vegetables and fruits from abattoirs should be washed thoroughly with fresh water and/or disinfected using vinegar before consumption since it was observed that such effluents are often used for agricultural purposes on farms near the abattoirs.

KEY WORDS: Enterohaemorrahagic, *Escherichia coli 0157:H7*, Shigatoxin

INTRODUCTION

Escherichia coli 0157:H7 is a pathogen that was first reported as a public problem by Riley et al. (1983). During the past two decades, it has becomes important worldwide as a significant human pathogen acquiring the status of an emerging infectious disease that has attracted a lot of interest (http://www.about-ecoli.com/main.html). The organism was reported as causing a diarrhea problem in Lagos the decade after it was associated with human illness (Akinyemi et al., 1998), and as a major cause of diarrhea in many other African countries, including South Africa, (Browning et al., 1990); Kenya, (Sanga et al., 1996); Cameroon, (Cunnin et al., 1999); Cote d'lvoire, (Dadie et al., 2000) and Egypt where Abdul-Raouf et al. (1996) isolated the organism from samples of raw ground beef, chicken, lamb and unpasteurized milk. E. coli 0157:H7 causes illness in humans through the colonization of the intestinal tract and elaboration of verotoxins (Shigatoxins), leading to diarrhea, haemorrhagic colitis, hemorrhagic uraemic syndrome and death (Beutin et al., 2004; Bidet et al., 2005; Sanga et al., 1996). The ruminant gastrointestinal tract is considered to be the reservoir site and cattle have been reported as the primary reservoir for E. coli 0157:H7 (Paiba et al., 2003; Paiba et al., 2002). Extensive studies have been carried out on isolates of the organism from human, animal and environmental sources in many other countries. Beutin et al., (2004) in Germany; Blanco et al. (2004) in France; Bryne et al. (2003) in the USA. Perna et al. (2001), reported the genome sequence of enterohaemorrhagic Escherichia coli 0157:H7.

Abattoir effluents are used for irrigation and as manure at vegetable and fruit farms around Zango abattoir, Zaria, so it was the primary objective of this study to find out if the effluents contain Escherichia coli 0157:H7.

MATERIALS AND METHODS

Sampling of Zango abattoir effluents was carried out from September 2002 to May 2006. Samples were collected two weeks after the first heavy rains each year, at the peak of each rainy season, the peak of the harmattan and the peak heat period during the dry seasons. The sampling sites were at the point just before the fresh effluents arrived at the first sedimentation tank (which has crumbled), at the point where the effluents were used for agricultural purposes (manure and irrigation) and at the point that the effluents drains unprocessed into a nearby stream.

The samples were collected by aspirating 10mls of the effluent using sterile syringes. Each sample was immediately enriched in 20mls of Modified Tryptone Soya Broth (Oxoid Ltd., Hampshire, England), supplemented with Novobiocin (Oxoid Ltd., Hampshire, England) and was incubated at 37°C for 24 hours. Following enrichment, 50il of each sample was cultured on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. Colonies showing a greenish metallic sheen were picked and biochemically screened. Those identified as Escherichia coli were stored on nutrient agar (NA) slants at 4°C. The stored colonies were eventually removed and cultured on Cefixime Sorbitol MacConkey (CR-SMAC) agar and incubated at 37°C for 24 hours. Colourless colonies on CR-SMAC agar were stored at 4°C on NA slants (Paiba et al., 2003).

The stored colonies were tested for Shigatoxin (Verotoxin) production by Reverse Passive Latex Agglutination using VTEC-RPLA Escherichia coli Verotoxin Detection Kit (Oxoid Ltd., Hampshire, England) and positive colonies where identified as E. coli 0157:H7 by a Rapid latex agglutination test using Remel Wellcolex E. coli 0157:H7 Kit (Remel Europe Ltd., Kent UK). All the isolates that were E. coli 0157 were cultured on brain heart infusion (BHI) agar containing 7% human blood and incubated at 37°C for 24 hours.

For this study an isolate that was citrate ve; urease ve; produced Acid/Acid reaction with gas but was H_2S ve on TSI agar; Indole +ve, H_2S ve and motile on SIM medium; Methyl Red +ve and Voges-Proskauer ve using the MVRP medium; produced gas from glucose fermentation; was colourless on CR-SMAC agar, produced latex agglutination in the test

wells for Stx1 (VT1) and/or Stx2 (VT2); produced agglutination of the 0157 test latex accompanied by a lack of agglutination of the control latex and agglutination of the H7 test latex accompanied by a lack of agglutination of the control latex, was considered to be E. coli 0157:H7. The control strain EHEC EDC 933 was used as control in assays involving 0157 and H7.

RESULTS

Only one isolate was serologically conformed to be E. coli 0157:H7. This makes the isolation rate to be 0.5% (1/210). The isolate was also positive for the production of Shigatoxin (VT2) (Table 1) and showed haemolytic activity on BHI agar.

TABLE I: Detection of shigatoxin-production and 0157 and H7 serotypes from the isolates.

STRAIN[1]		Shigatoxin		Antigens	
	CR-SMAC	Stx1	Stx2	0157	H7
2002/3 ZA	+				
2002/5 ZA	+				
2002/7 ZA	+				
2002/16 ZA	-	-	+		
2002/20 ZA	+				
2003/1 ZA	+				
2003/4 ZA	+				
2003/5 ZA	-	-	-		
2003//11 ZA	-	-	-		
2003/17 ZA	-	-	-		
2003/28 ZA	-	-	+	+	-
2003/40 ZA	-	-	+	+	-
2003/43 ZA	+				
2003/46 ZA	+				
2004/3 ZA	-	-	-		
2004/8 ZA	+				
2004/9 ZA	+				
2004/11 ZA	+				
2004/13 ZA	-	-	-		
2004/31 ZA	+				
2004/35 ZA	+				
2004/36 ZA	-	-	-		
2004/43 ZA	+				
2004/44 ZA	-	-	+	-	-
2004/49 ZA	-	-	-		
2005/1 ZA	-	-	-		
2005/3 ZA	+				
2005/7 ZA	+				
2005/14 ZA	+				
2005/33 ZA	-	-	+	+	+
2005/37 ZA	-	-	+	-	-
2005/40 ZA	+	-	-		
2005/41 ZA	+	-	-		
2005/44 ZA	+	-	-		
2005/49 ZA	-	+	-	+	-
2005/50 ZA	-	-	+	+	-
2006/2 ZA	-	-	-		
2006/7 ZA	-	-	-		
2006/8 ZA	-	-	-		
2006/12 ZA	-	-	+	-	-
2006/14 ZA	-	-	+	-	-

[1]Biochemically *E. Coli*

DISCUSSION

The centers for disease control in the United States of America where E. coli 0157:H7 was first identified in 1975 (Riley et al., 1983), has recommended that an initial or oral report of E. coli 0157:H7 be given to authorities when sorbitol negative colonies have been serologically tested for the 0157 antigen. This is to be followed by biochemical identification of the 0157 positive isolates. A written documentation is recommended at this stage. The final stage is the H7 antigen assay which can be carried out serologically or by molecular methods. A final report is recommended after H7 confirmation of the isolates. This is an indication of the importance of the pathogen.

The isolation rate of 0.5% is in agreement with the prevalence rates of the pathogen in cattle (Martin et al., 1986; Orskov et al., 1987; Wells et al., 1991), in the USA and (Paiba et al., 2003, 2002) in the UK and it has answered the question that the organism may be in our food animal population and can persist in the environment. The sample from which the isolate was identified came from effluent at the point for agricultural use. This was at the beginning of the rainy season two weeks after the first heavy downpour of rain in May 2005. This agrees with Effler et al. (2001) that carriage of E. coli by cattle and heavy rains with contamination of surface water and the environment are important risk factors for the emergence of E. coli 0157 in Africa.

The contamination of vegetables should be viewed as a risk factor for E. coli 0157:H7 infection in humans since it can be isolated from abattoir effluents in our environment. Vegetables and fruits produced from such agricultural practice could carry the organism since it has been reported that the organism could survive for up to 154 days in similar soil types on which carrots and onions were grown (Islam et al., 2005). However, in soil samples for carrot fields where poultry manure compost and dairy cattle manure compost were applied E. coli 0157:H7 survival was up to 196 days. At approximate dates when onions (day 140) and carrots (day 126) were harvestable high E. coli 0157:H7 counts were still on the vegetables (Islam et al. 2005). It is note worthy that Islam et al. (2005) carried out their study during winter in Tifton Georgia, USA where the average monthly temperature is low. An observation has been reported that E. coli 0157:H7 cannot grow at 5°C (Wang et al., 1997, 1996). The implication is that there could be higher survival rates and longer survival times in our environment where the temperatures in the growing season are ideal for the growth of E. Coli which occur at 20°C to 44°C (http://www.aboutecoli.com/main.html) with an optimum temperature for growth of 37°C. Moreover the infective dose for man is just 10 cells (FSA1, 1999) or less (Sekla et al. 1990).

Application of effluents as manure or as water for irrigation may result in gross contamination of the soil and agricultural produce from the fields. The danger of improper handling of the effluents is properly underestimated. It is recommended that elimination of pathogens from effluents before agricultural use is a critical intervention point in managing the pathogen on crops used as food and in managing the microbial safety of water supply to man and animals around the abattoir environment (www.fsai.ie). The impact of on-farm practices which may result in E. coli 0157:H7 becoming associated with farm produce should be explored because even a low level of contamination of E. coli 0157:H7 presents a human health hazard and constitute a public health problem.

Vegetables and fruits should be washed thoroughly using fresh water and/or soaked in vinegar before consumption to prevent E. coli 0157:H7 infection from contaminated fruits and vegetables. The production of shiga toxins, which this isolate possessed and the hemolytic activity which it demonstrated have been reported as the mechanism by which E. coli 0157:H7 is pathogenic (Fukushima et al., 1999; Griffin and Tauxe, 1991, Kaddu-Mulindwa, 2001, Liesegang et al., 2000, Martinez and Baquero, 2002, Mora et al., 2005, Wang et al., 2002 and Zhao et al., 2001).

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