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The Occurrence of *Escherichia Coli* Serotpye O157: H7 among Humans in Some Parts Plateau State, Nigeria

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ABSTRACT

The present study evaluated the occurrence of *E. coli* O157: H7 among human subjects in seven selected experimental sites in Plateau State, Nigeria. Seven hundred patients' stool specimens were examined for the presence of *E. coli* O157: H7. The specimens were aseptically collected at monthly intervals between November 2008 to October 2009, from government and private hospitals, clinics, medical laboratories, after which they were transported to the laboratory for bacteriological analysis. Tryptone Soya Broth (TSB) and selective agar medium namely; cefixime tellurite-sorbitol MacConkey (CT-SMAC) agar were used for the isolation of the pathogen from various samples. The results of this study revealed that out of the seven hundred patients' stool specimens examined, 35 (5.00%) were found with *E. coli* O157: H7. The male subjects had a higher occurrence of the organism 24 (3.43%) than the female counterparts 11 (1.57%). The infection rate of *E. coli* O157: H7 disease was recorded highest 12 (2.45%) among the age group of 0-9 years. This was followed by the age group of 50 years and above 7 (1.43%). *E. coli* O157: H7 had the highest occurrence among the patients that attended government owned hospitals 18 (2.58%), followed by patients that attended private hospitals (1.14%), while patients that attended clinics had the least 4 (0.57%). Patients with bloody diarrhoea had the highest infection rate of *E. coli* O157: H7 disease 23 (3.29%), whereas patients with non bloody diarrhoea had 12 (1.71%). None of the individuals without diarrhoea yielded the pathogen. The percentage occurrence of *E. coli* O157: H7 infection among the infected patients was significantly higher ($P<0.05$) during the wet season than during the dry season. The high occurrence of this organism among patients with diarrhoea in the experimental sites suggests that the awareness should be created among the public and within the medical practices on the dangers of the infection caused by this pathogen and that the organism should be included

among the diarrhoegenic pathogens that are routinely screened in our medical laboratories.

Keywords: *Escherichia coli*, Serotype, O157: H7, Diarrhoegenic, Humans.

INTRODUCTION

Escherichia coli is part of the healthy bowel faecal flora of both humans and animals; however, some strains of *E. coli* can cause severe and life threatening diarrhoea (Abbott and Janda, 1992). Although there are more than 50 serotypes of *E. coli* that cause gastrointestinal diseases (Farmer and Kelly, 1990; Griffin and Tauxe, 1991), *E. coli* O157: H7 has attracted most attention because of the severity of the illness it causes.

E. coli O157: H7 was first recognized as a human pathogen following an outbreak of hemorrhagic colitis associated with the consumption of undercooked ground beef in 1982 (Riley *et al.*, 1983). Since then, there have been many reports form throughout the world in including Nigeria, describing the severe disease associated with this organism (Griffin, 1995; Ogunsanya, 1994; Keilly, 1998; Olurunsho *et al*., 2000). Several studies have shown that infection with *E. coli* O157: H7 is responsible for most causes of Hemolytic uremic Syndrome (HUS) which is a major cause of acute renal failure in children (Karmali *et al*., 1985; Bitzan *et al*., 1993; Rowe *et al*., 1993 Wells *et al*., 1998) and thrombotic thrombocytopenic purpura, which involves loss of platelets, skin colorations, fever, nervous disorders and sometimes leads to death (Doyle and Padye, 1989). In addition, the infection with this organism is known to be a common cause of bloody and non bloody diarrhoea (Bell *et al*., 1994; Thomas *et al*., 1995). *E. coli* O157: H7 is usually the most common bacterial pathogen isolated from bloody stools and has been isolated from as many as 40% of all bloody stools in United States of America (Griffin and Tauxe, 1991). Other symptoms related to *E. coli* O157: H7 infection include severe abdominal cramps, vomiting, nausea and mild fever (Riley *et al*., 1983; Ostroff *et al.*, 1989). *E. coli* O157: H7 infection is generally found to affect both male and female and also people of all age groups, but has a more devastating effects on the young and elderly (Thomas *et al*., 1995).

E. coli O157: H7 strains are water and food-borne and can lead to severe outbreaks (Riley *et al*., 1983; Wells *et al*., 1983; Ryan *et al*., 1986). Although most outbreaks of *E. coli* O157: H7 infection have been linked to foods of bovine origin, such as undercooked ground beef and dairy products (Griffin, 1995; Itelima and Agina, 2010b), other food items of non-bovine origin such as salad vegetables, fruits and apple cider have also been associated with the infection (Abdul-Raouf, 1993; Morgan *et al*., 1988; Besser *et al*., 1993; Islam *et al*., 2004; Janet and Agina, 2009; Itelima and Agina, 2010a). Transmission of *E. coli* O157: H7 infection through person-to-person contact, particularly in institutionally settings, like day care centres, nursing homes and hospitals have been documented (Pavia *et al*., 1990). The spread of *E. coli* O157: H7 infection from person-to-person is a result of the low infectious dose of the pathogen (Thomas *et al*., 1995; Makino *et al*., 2000).

It is interesting to note that despite the severity of the infection caused by *E. coli* O157: H7 and the fact that the infection cuts across all age groups, clinical laboratories at the various hospitals and other medical centres in Plateau State were stool specimens were collected do not include this pathogen when stool cultures are being screened for other enteric pathogens. Thus, there is still dearth of information on the occurrence of the organism in Plateau State. This study was undertaken specifically to determine the occurrence of *E. coli* O157: H7 among human subjects in some selected parts of Plateau State, Nigeria, and to determine the effects of sources (where specimens were collected) and season on the percentage occurrence of the pathogen and how the percentage occurrence of this pathogen in stool specimens compares with that of other pathogens commonly screened in our clinical laboratories.

MATERIALS AND METHODS

Sample Collection

A total of 700 human stool specimens comprising of bloody diarrhoea, non bloody diarrhoea and non diarrhoea presented for routine analysis in government hospitals, private hospitals, clinics and diagnostic laboratories located at seven different experimental sites in Plateau State, Nigeria. The experimental sites are Jos, Bukuru, Vom, Miango, Bassa, Riyom and Barkin Ladi. The stool specimens were collected within the space of one year through the months of November 2008 to October 2009 from the various sources and then transported to Microbiology Laboratory of Department of Plant Science and Technology University of Jos where they were examined for the presence of *E. coli* O157: H7.

Isolation of Organism

A loopful of patients' stool specimens were aseptically inoculated into 10ml of Typtone Soya Both (TSB) which is an enrichment medium used for the isolation of *E. coli* O157: H7 (Weagant *et al*., 1995). The mixture was homogenized for about 30 seconds, treated with 0.1 mg/ml ampicillin to reduce the load of normal flora (Islam *et al*., 2004) and then incubated at 37^{O}C for 2 hours. A 0.5ml of the enriched suspension of the samples were further subculture onto selective solid media namely Sorbitol MacConkey agar (SMAC) and Sorbitol MacConkey agar supplemented with cefixime and tellurite (CT-SMAC) by streaking and re-incubated at 37^{O}C for18-24hours (March and Ratnam, 1986). The plates with non-sorbitol fermenting colonies (NSFC), which are usually colourless colonies, were noted, after which ten colonies were randomly selected and then examined for the presence of gram-negative rods. It was necessary to test up to 10 separate NSFC to ensure a high probability of detecting any *E. coli* O157: H7 strains which may be in mixed culture with other NSFC organisms (Griffin, 1995). Presumptive colonies of *E. coli* O157: H7 were streaked on SMAC agar to ensure purity before subjecting to biochemical tests as described by Cheesbrough (1991). *E. coli* positive colonies were serologically confirmed by using *E. coli* O157 latex agglutination assay (oxoid) and *E. coli* antiserum H7 assay (Difco) test as described by Nataro and Kaper (1998). The colonies that agglutinated when tested were considered to be *E. coli* O157: H7 colonies. The Stool specimens used for

culturing *E. coli* O157: H7 were also analyzed for the presence of other common enteric pathogens using standard biochemical tests described by Cheesbrough (1991).

Statistical Analysis

The data were subjected to statistical analysis of variance (ANOVA) and chi-square (X^2) test. Least significant difference (LSD) was used to test whether there was a significant difference between the means or otherwise. Each value presented represents a means of five values, each consisting of 3 replicates.

RESULTS

The results of the frequency of occurrence of *E. coli* O157: H7 isolated from the human stool specimens in the various experimental sites in relation to sex of patients is represented in Table 1. Of the700 patients' stool specimens examined for the presence of *E. coli* O157: H7, 35 (5.0%) were found with the organism. The results in Table 1 also reveal that male subjects had a higher occurrence 24 (3.43%) of *E. coli* O157: H7 infection than their female counterparts 11 (1.57%).

The infection rate of *E. coli* O157: H7 disease was recorded highest 2.45% among the age group of 0-9 years (Fig. 1). This was followed by the age group of 50 years and above 1.43%. There were fewer cases of the infection in the age groups of 20-29 years, 30-39 years and 40-49 years with only 0.61%, 0.82% and 0.82% cases respectively. The results in Table 1 and Fig. 1 also reflect how *E. coli* O157: H7 infection is distributed among the patients in the experimental sites. Analysis of variance (ANOVA) statistical analysis revealed that there was no significant difference ($p>0.05$) in the occurrence of the organism with respect to the various sampling sites.

The results of the effect of the various sources on the percentage of occurrence of *E. coli* O157: H7 in relation to patients' stool types are shown in Table 2. The report shows that *E. coli* O157: H7 had the highest occurrence among the patients that attended government owned hospitals 18 (2.58%) and was followed by private hospitals 8 (1.14%), while patients who attended clinics had the least 4 (0.57%). The results in Table 2 also show that patient with bloody diarrhoea had the highest percentage occurrence of *E. coli* O157: H7 disease 23 (3.29%), whereas patients with non bloody diarrhoea had 12 (1.71%). None of the individuals without diarrhoea yielded the pathogen, indicating absence of asymptomatic patients of *E. coli* O157: H7 infection in the study areas. The results of the effect of season on the percentage occurrence of *E. coli* O157: H7 among male and female patients are shown in Figure 2. Overall, *E. coli* O157: H7 infection occurred significantly higher ($p<0.05$) during the wet season (4.14%) than during the dry season (0.86%) among the patients.

The results of the comparison between clinical presentations of patients with *E. coli* O157: H7 infection and enteric infections caused by other pathogens which were ascertained during routine screening of stool specimens at the different sources were shown in Table 3. The results reveal that the percentage occurrences of the pathogens isolated from patients with bloody diarrhoea were *E. coli* O157: H7 (8.6%), *Shigella dysentriae* (4.5%), *Salmonella typhi* (1.1%), *Entameoba histolytica* (1.9 %) and

intestinal worms (2.6%). Among the patients with non-bloody diarrhoea the percentage occurrences were *E. coli* O157: H7 (5.4%), *Shigella dysentriae* (0%), *Salmonella typhi* (15.4%), *Entameoba histolytica* (0%) and intestinal worms (25.3%). *Campylobacter jejuni* and *Vibrio cholerae* were not isolated from any of the patient stool specimens. The results show that *E. coli* O157: H7 infection ranked first among patients with bloody diarrhoea, and third among patients with non bloody diarrhoea (Table 4).

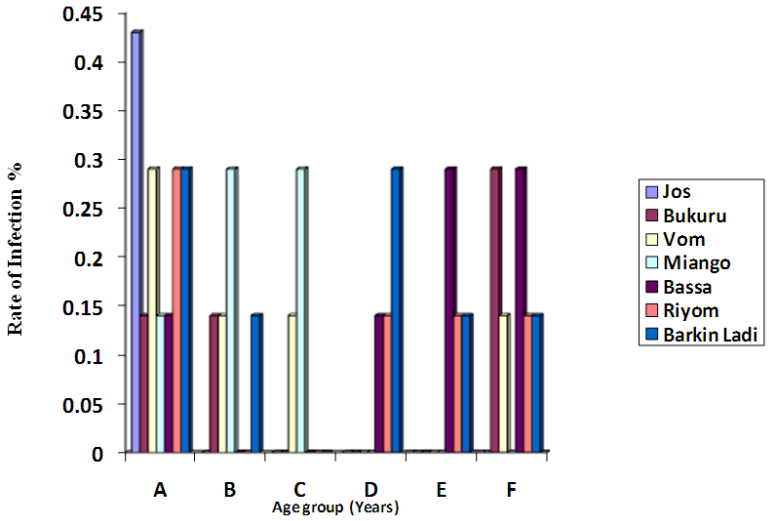

Fig.1. Rate of *E. coli* O157: H7 Infection among Human Patients in the Various Experimental Sites in Relation to Age Groups. A = 0-9, B = 10-19, C = 20-29, D=30-39, E = 40-49, F= >50

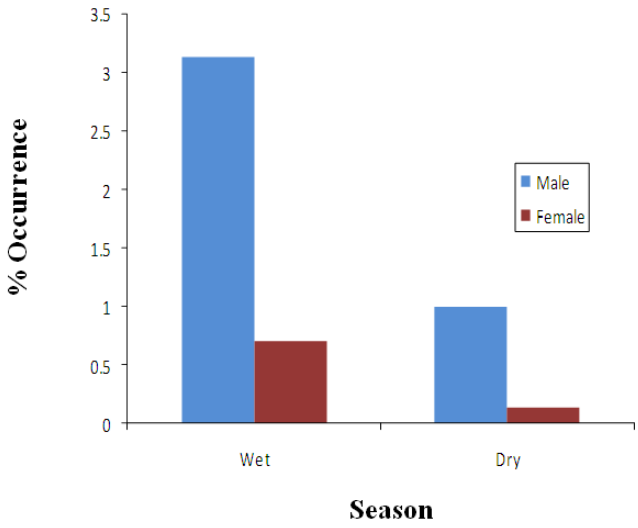

Fig. 2: Seasonal Effect on the Percentage Occurrence of *E. coli* O157: H7 Among Male and Female Patients in the Experimental Sites.

Table 1: Frequency of Occurrence of *E. coli* O157: H7 Infection among Human Patients in the Various Experimental Sites in Relation to Sex.

Experimental sites	Sample size	Sex		Total
		Male	Female	
Jos	100	2/50 (0.29)	1/50 (0.14)	3/100 (0.43)
Bukuru	100	3/50 (0.43)	1/50 (0.14)	4/100 (0.57)
Vom	100	3/50 (0.43)	2/50 (0.29)	5/100 (0.71)
Miango	100	4/50 (0.57)	1/50 (0.14)	5/100 (0.71)
Bassa	100	4/50 (0.57)	2/50 (0.29)	6/100 (0.86)
Riyom	100	3/50 (0.43)	2/50 (0.29)	5/100 (0.71)
Barkin Ladi	100	5/50 (0.71)	2/50 (0.29)	7/100 (1.00)
Total	**700**	**24/350 (3.43)**	**11/350 (1.57)**	**35/700 (5.00)**

Table 2. Effects of Various Sources on the Frequency of Occurrence of *E. coli* O157: H7 in Relation to Patients' Stool Types.

Patients Stool Types					
Source	**Sample Size**	**BD**	**NBD**	**ND**	**Total**
Government hospitals	175	15/102 (2.14)	3/45 (0.43)	0/28 (0.00)	18/175 (2.58)
Private hospitals	175	3/69 (0.43)	5/84 (0.71)	0/22 (0.00)	8/175 (1.14)
Clinics	175	2/51 (0.29)	2/52 (0.29)	0/72 (0.00)	4/175 (0.57)
Medical diagnostic laboratories	175	3/46 (0.43)	2/41 (0.29)	0/88 (0.00)	5/175 (0.71)
Total	**700**	**23/268 (3.29)**	**12/222 (1.71)**	**0/210 (0.00)**	**35/700 (5.00)**

BD=Bloody Diarrhoea, NBD=Non Bloody Diarrhoea, ND=Non Diarrhoea

Table 3: Comparison between the Percentage Occurrence of *E. coli* O157: H7 and Other Enteric Pathogens According to Patients' Stool Types.

Stool Types	Sample Size	Pathogens						
		EC	SH	SA	CB	VC	EN	IW
Bloody Diarrhoea	268	23 (8.6)	12 (4.5)	3 (1.1)	0 (0.0)	0 (0.0)	5 (1.9)	7 (2.6)
Non Bloody Diarrhoea	222	12 (5.4)	0 (0.0)	34 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	56 (25.3)
Non Diarrhoea	210	0 (0.0)	0 (0.0)	21 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	17 (8.1)
TOTAL	**700**	**35 (5.0)**	**12 (1.7)**	**58 (8.3)**	**0 (0.0)**	**0 (0.0)**	**5 (0.7)**	**85 (12.1)**

EC = *Escherichia coli* O157: H7, SH = *Shigella dysentriae,* SA = *Salmonella typhi*, CB = *Campylobacter jejuni,* VC = *Vibrio cholera,* EN = *Entamoeba histolytica,* IW = Intestinal worms

DISCUSSION

The finding of the present study indicated that *E. coli* O157: H7 actually assisted among some of the humans in the sampling sites with percentage occurrence of 5.0%. The result of the present study is close to that of Chapman and Siddons (1996) who out of 566 human stools they examined detected 47 (8.30%). On the contrary, MacDonald *et al*. (1988) reported much lower percentage isolation of the pathogen,

25 (0.4%) out of 6485 patients stool specimens. The infection rate of the pathogen presented herein only included patients who sought medical attention and had stool cultures requested by physicians. Patients who did not seek medical attention would not be detected through this type of laboratory base surveillance; therefore, the actual rate of disease that occurred among the human population in the study areas could be higher than described. Furthermore, the recovery of *E. coli* O157: H7 has been reported to be optimal when stool specimens are obtained within 6 days after onset of diarrhoea (Well *et al*. 1983; Tarr *et al*. 1990). Thus the rate of isolation of *E. coli* O157: H7 also decreases with delay in collection of stool sample. The present report support studies performed in Canada that indicated that enteric infection with *E. coli* O157: H7 is not a rare occurrence (Grandsden *et al*. 1986) as reported by (Thomas *et al*., 1995)

Su and Brandt (1995) observed that *E. coli* O157: H7 generally affects both sexes equally. In this study however, male patients has higher percentage occurrence of *E. coli* O157: H7 infection than female patients. Thus of the 35 patients infected with *E. coli* O157: H7, 24 (69%) were males, while 11 (31%) were females. These results agree with the report of Spika *et al*. (1986) who in Atlanta, USA, found that twenty (56%) of that 36 patients infected with *E. coli* O157: H7 were males. The reason why the male subjects are more vulnerable to *E. coli* O157: H7 infection than the female subjects could most probably be attributed to the fact that the males are occupationally more exposed to the pathogen. This occupationally exposure of the males to *E. coli* O157: H7 infections may include animal husbandry practice, abattoir practice, meat butchering and rendering. The social life styles and habits of the males may also influence the vulnerability to *E. coli* O157: H7 infections; these may include their food and drinking habits, as food and drink are two very important vehicles for the transmission of the organism (Griffin and Tauxe, 1991).

The report of the present study has demonstrated that all age groups were infected by the pathogen, but the highest percentage occurrence of the infection was recorded among the age group of 0-9 years (2.45%). This was followed by the age group of 50 years and above (1.43%). The report of this study agrees with that of previous report (CDC, 2005), which revealed that the children and the elderly had been identified to be more susceptible to the infection with *E. coli* O157: H7 than other age groups. The high infection rate among the children could be due to their uncontrollable and unguarded eating as well as water drinking habits as children in this group are usually pore to indiscriminate food habits even in dirty or unhealthy environments, while the elderly may have weak immune systems and therefore lack the ability to resist most infections especially if their environments and their eating habits are hygienically substandard.

Previous study has shown that infection rate of *E. coli* O157: H7 can vary from one locality to another within the same geographical region (Chapman and Siddons, 1996). This report is not comparable with the present study in which the infection rate is not significantly different in the experimental sites. The reason for this could be that the study areas have the same climatic condition and similar human activities and agricultural practices.

The fact that *E. coli* O157: H7 infection occurred more frequently in patients who attended government-owned hospitals than other medical centres could be attributed to the fact that most patients prefer these hospitals. The reason could be economical as government hospitals enjoy some degrees of subsidy especially on drugs and so on from government; hence charge their patients considerably cheaper rate of fees than their counterparts. This could therefore attract a high patronage especially from rural dwellers that might be more exposed to the organism than those in urban areas. Furthermore, government owned hospitals seemed to be better equipped in terms of qualified medical personnel as well as facilities for service than the other medical centres hence; they enjoy greater patronage, especially from persons with severe cases of illness.

In this study, 3.29% of patients with *E. coli* O157: H7 detected in their stools had bloody diarrhoea, while 1.71% of patients had watery or non bloody diarrhoea. This finding may represent an ascertainment bias because patients with bloody diarrhoea may be more likely to seek medical attention and consequently undergo stool culturing than those without bloody diarrhoea. Investigation of outbreaks of *E. coli* O157: H7 enteric infection has demonstrated that the organism can be found in asymptomatic individuals without the evidence of bloody or watery diarrhoea (Riley *et al*., 1983; Wells *et al*., 1983; Ryan *et al*., 1986; Chapman and Siddons, 1996). However, this investigation did not incriminate *E. coli* O157: H7 infection amongst patients without diarrhoea whose stools were examined since there were no isolates from their stool samples. This suggests that there are no carriers of the pathogen among the subjects investigated. This finding is in support of the report by (Griffin, 1995) who in his study recorded the absence of *E. coli* O157: H7 strains from 500 asymptomatic individuals.

Present study has demonstrated that there was a variation in occurrence of *E. coli* O157: H7 isolated from patients during the rainy season and dry season, with *E. coli* O157: H7 occurring higher in rainy seasons than dry season. The higher isolation rate of *E. coli* O157: H7 recorded among the infected patients during the rainy season could be due to the fact that since infection is usually through the oral route, most of the vehicles of infection such as food and water might possible have been contaminated by waste materials from humans and animals. For instance, these waste materials could be washed by rains into water bodies, judged by the terrain of the environment and this could serve as sources of infection. In like manner, fresh pasture abounds during the wet season which calls for high animals' activities in relation to grazing with subsequent soil pollution. Vegetable materials on such polluted soil when consumed could serve as a source of infection.

The result of the comparison between the percentage occurrence of *E. coli* O157: H7 and that of other enteric pathogen revealed that *E. coli* O157: H7 was the first and third most commonly isolated enteric pathogen from stool samples of patients with bloody and non bloody diarrhoea respectively when compared with other pathogens. The present report is in consonance with the report of Griffin and Tauxe (1991) which revealed that *E. coli* O157: H7 is usually the most common bacterial pathogen isolated from bloody stools in the United States of America. These findings presented herein suggest that since bloody diarrhoea occurs more frequently in patients with *E. coli* O157: H7 infection than in patients with other common enteric pathogens,

therefore it has become very necessary that the culture for *E. coli* O157: H7 should also be performed in patients who have bloody diarrhea in our hospitals, clinics and medical laboratories. Ackers *et al*. (1996) suggested that it is important to also look for *E. coli* O157: H7 in stools of patients with non bloody diarrhoea when the patients are associated with others who have bloody diarrhoea. The presence of cysts of *Entamoeba histolytica* and ova of *intestinal worms* in the stool samples agrees with the work of Ogunsanya *et al*. (1994) on their study of aetiological agents of childhood diarrhoea in which these pathogens were found in the stool samples analyzed in addition to *E. coli* O157: H7 and other microorganisms. This implies that the entire stool submitted for routine laboratory diagnosis should be subjected to both bacteriological and parasitological investigations to rule out possible omission of either infection in the stools.

CONCLUSION

Until now, the presence and the pathogenic potential of *E. coli* O157: H7 serotype in this part of the world seems to be unnoticed, but the findings of this study have not only unraveled the existence of this pathogen, but its possible impact on the public health of people of Plateau State in particular and the nation at large. Therefore, this organism should no longer be disregarded, but should be given adequate recognition just like other enteric pathogens routinely screened for when stool specimens are submitted for culture to the various medical centres in our country. The present study demonstrates several important findings. First, this study has helped to determine the human population-based occurrence of laboratory detected *E. coli* O157: H7 infection which was also compared with other enteric pathogens. Second, this study has emphasized the importance of including *E. coli* O157: H7 infection in differential diagnosis of bloody and non bloody diarrhoea along side other enteric infections. Finally, this study indicates that risk of acquiring illness was associated with the consumption of beef products and exposure to cattle and or cattle manure, raw beef, raw milk and salad vegetables. And because of the low infectious dose of *E. coli* O157: H7, coupled with the fact that some of the above mentioned foods are ready-to-eat food products; all producers of such foods should recognize the importance of hygiene and good manufacturing practice. This can be achieved most effectively through the application of food safety assurance programmes, based on the principles of the Hazard Analysis and Critical Control Point (HACCP) system. Such a system should be applied by primary producers, manufacturers, retailers and food service establishment.

Since it is evidently clear that *E. coli* O157: H7 infection does exist in experimental sites, physicians should consider culturing for this pathogen and if identified should report results to local or state health departments. Such reporting may lead to early detection of product-associated outbreaks. In addition, National Agency for Food and Drug Administration and Control (NAFDAC) and Federal Environmental Protection Agency (FEPA) should get informed of the existence of this organism in Nigeria, so that they can legislate and take appropriate measures to control outbreaks when they occur.

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