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THE OCCURRENCE OF *ESCHERICHIA COLI* O157:H7 IN MARKET AND ABATTOIR MEAT IN PLATEAU STATE, NIGERIA

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ABSTRACT

Escherichia coli O157:H7 is a newly emerging pathogen frequently associated with the consumption of foods of bovine origin. The severity of the infections caused by this food borne pathogen in the young and the elderly has had a tremendous impact on human health and food industry. The present study evaluated the occurrence of *E. coli* O157:H7 in abattoir and market meat in seven selected sites in Plateau State, Nigeria. One hundred and forty samples of each of the various meat parts including muscle, liver, heart, kidney and intestine were collected to give a total of 700 meat samples. The samples were aseptically collected at monthly intervals from December 2008 to November 2009 after which they were transported to the laboratory for bacteriological analysis. Enrichment medium Tryptone Soya Broth (TSB) and selective agar media, namely; sorbitol MacConkey agar (SMAC)and cefixime tellurite-sorbitol MacConkey agar (CT-SMAC) were used for the isolation of *E. coli* O157:H7 from the various meat samples. The results of this study indicated that the intestinal samples had the highest percentage occurrence of the organism (3.39%), while muscle tissue had the lowest (0.43%). The organ meats had comparable percentage occurrence of the organism, with 1.0% for liver, 0.86% for kidney and 0.71% for heart samples. Significant difference ($P<0.05$) existed between the occurrence of the pathogen during the wet and dry seasons, but no significant difference ($P>0.05$) existed in the occurrence of the organism with respect to the sampling sites and the sources (abattoir and market). The presence of *E. coli* O157:H7 in beef should be regarded as being highly hazardous to consumers and handlers alike because of the severity of the infections associated with the organism. Thus, hygienic and good management of meats and meat environments are very essential.

KEY WORDS: *E. coli* O157:H7, Pathogen, Abattoir, Market, and Infections

INTRODUCTION

Escherichia coli are bacteria that normally inhabit the intestines of humans and animals. Most strains are known to be harmless, but several of them can cause mild to serious diseases. One strain in particular, named *E. coli* O157:H7 can cause severe bloody diarrhoea leading to hemorrhagic colitis and in some cases leads to serious complications such as hemolytic uremic syndrome (which is a major cause of acute renal failure in children) and thrombocytopenic purpura (which causes loss of platelets, skin colouration, fever and nervous disorders) (Karmali, *et al*.,1985; Pai *et al*., 1988; Doyle and Padye, 1989; Rowe *et al*., 1993; Griffin and Tauxe, 1991; Mead and Griffin, 1998; Ahmed and Dongly, 1998).

Many studies have indicated that cattle are the major reservoir of *E. coli* O157:H7 (Armstrong *et al*., 1996; Hancock *et al*., 1998). Thus, this pathogen has been isolated from apparently healthy animals during investigation of sources of human infection (Borczyk *et al*., 1987; Ostroff *et al*., 1990; Chapman *et al*., 1997; Wells

et al., 1999). Cattle harbour this pathogen on their hides and in their intestinal contents, and carcass contamination may occur from either hides or the leakage of faecal material during intestine removal (Grau, 1987; Ayres, 1995; Elder *et al*., 2000). Since the first outbreak of *Escherichia coli* O157:H7 infection, which occurred in USA in 1982 as a result of consumption of hamburger prepared from contaminated ground beef, the organism has been detected throughout the world (Griffin, 1995), including Nigeria (Olorunshola *et al*., 2000; Mawak and Ashamu, 2006, Dahiru *et al*., 2008; Janet and Agina, 2010). Although, undercooked ground beef and beef products have been identified as the leading vehicle of *Escherichia coli* O157:H7 infections (Doyle and Schoeni, 1987; Padhye and Doyle, 1991; Kim and Doyle, 1992; Bell *et al*., 1994; Griffin *et al*., 1994), fresh fruits, vegetables, raw un-pasteurized milk and juice are becoming increasingly important vehicles of food borne transmission of this pathogen (Besser *et al*., 1993; Hillborn *et al*., 1999; Beuchat, 2002).

The dangerous health implication associated with *Escherichia coli* O157:H7 infection and the increased reports of outbreaks linked to consumption of foods of bovine origin have heightened concern regarding the pathogen contamination of raw beef and beef products all over the world. The objective of this study was to determine the occurrence of *Escherichia coli* O157:H7 in various beef parts sold in abattoir and market environments in Plateau State, Nigeria and to determine the effect of seasons and sources where samples were obtained on the percentage occurrence of the organism.

MATERIALS AND METHODS

One hundred and forty samples each of various beef parts including muscle, liver, heart, kidney and intestine were collected to give a total of 700 samples. The samples were randomly collected during one year period (2007 – 2008) from retail markets and abattoirs in sterile polythene bags, from seven sampling sites in Plateau State, Nigeria namely: Jos , Bukuru, Vom, Miango, Bassa, Riyom and Barkin Ladi. All meat samples were fresh the day they were collected and were taken to the laboratory for of 4^0C for not more than 2 hours until analyzed to reduce multiplication of microorganisms.

Ten grams of each of the samples were weighed, blended using sterile electric blender and transferred into 90ml Tryptone Soya Broth (TSB) and incubated at 37^OC for 2 hours (Weagant *et al*., 1995). After then, 1ml each of the incubated broth was serially diluted in peptone water to 10^8. The diluents were aseptically inoculated onto selective agar media, namely; sorbitol MacConkey agar (SMAC) and cefixime tellurite-sorbitol MacConkey agar (CT – SMAC) and then incubated at 37°C for 18 – 24 hours (March and Ratnam, 1986; Chapman *et al*., 1994; Karch *et al*., 1996). From each plate, the numbers of non-sorbiol fermenting colonies (NSFC) were noted and ten colonies were randomly selected and then subjected to gram staining reaction and biochemical tests such as motility test, formation of gas from glucose, production of indole, methyl-red reaction, voges, proskaur reaction, citrate utilization, catalase test, oxidase test and ureas test (Cheesbrough, 1991). It was necessary to test up to 10 NSF colonies, to ensure a high probability of detecting any *Escherichia coli* O157:H7 strains which may be in mixed culture with other NSFC organisms (Griffin, 1995). Presumptive colonies of *Escherichia coli* O157:H7 were serologically confirmed by using *Escherichia coli* O157:H7 latex agglutination assay and *E. coli* antiserum H7 assays as described by Nataro and Kaper (1988). The colonies that agglutinated when tested were considered to be *E. coli* O157:H7.

The data were subjected to statistical analyses using analysis of variance (ANOVA). Each value presented represents a mean of five values, each consisting of 3 replicates.

RESULTS AND DISCUSSION

The results of the occurrence of *Escherichia coli* O157:H7 isolated from the various beef parts obtained from the sampling sites are shown in Table 1. Out of the 700 beef samples investigated, 44(6.29%) were contaminated with *E. coli* O157:H7. The results in Table 1 also indicate that among the beef parts examined, the intestinal samples had the highest percentage occurrence of the pathogen 23(3.29%), while the muscle samples had the

The direct comparison of the occurrence of *Escherichia coli* O157:H7 with respect to the sampling sites shows that the pathogen occurred equally in some of the sites and varied in others (Table 1). However, statistical analysis of the result showed that there was no significant difference ($P>0.05$) in the occurrence of the organism with respect to the sampling sites.

Table 2 shows the results of the percentage occurrence of *Escherichia coli* O157:H7 isolated from beef parts in relation to sources (abattoir and markets).The results show that out of the 44(6.29%) meat samples contaminated with *Escherichia coli* O157:H7, 20(2.86%) were from abattoirs, while 24(3.43%) were from retail markets. Statistical analysis of the results in Table 2 indicated that significant difference did not exist ($P>0.05$) between the percentage occurrence of *Escherichia coli* O157:H7 isolated from the two sources.

The results of seasonal effect on the percentage occurrence of *Escherichia coli* O157:H7 isolated from beef parts are shown in Figure 1. The results show that the organism occurred higher in wet season than in the dry season with overall percentage occurrence of 4.86% and 1.43% respectively. Statistical analysis of the results in Figure 1 revealed that there was a significant difference ($P<0.05$) in the occurrence of the organism with respect to the two seasons.

An important finding of this work is that *Escherichia coli* O157:H7 was present in all the sampling sites and in the two sources examined in Plateau State, Nigeria. This finding provides a better understanding of the ecology of *E.* coli O157:H7 in the meat processed and sold in our environment. The results of this study indicate that good management and hygienic practices are extremely important to curtail the rate of carcass contamination by *E. coli* O157:H7 and subsequently control the spread of the infection with the organism.

E. coli O157:H7 has been detected on several types of meat, some of which included raw beef and beef products, poultry and lambs (Samadpoor *et al*., 1994). The result of this study indicated that *E. coli* O157:H7 was isolated from 44 (6.29%) out of 700 beef samples tested. This result compared favourably with the reports of several workers who also isolated this organism isolated 158 (52.7%) of the organism out of 300 samples they examined. Other surveys of retail ground beef found *E. coli* O157:H7 in 3 (2.8%) of 76 samples (Kim and Doyle, 1992) and 3 (2.4%) of 165 samples (Sekla *et al*., 1990). In addition Pai *et al*., (1984) reported that 31% of the beef samples tested were found positive with the organism, while 80% of retail store beef examined by Besser *et al*., (1997) in Italy had *Escherichia coli* O157:H7. The ability to detect this pathogen from meat may be attributed to factors such as seasons, geographical location and sampling criteria. These factors can result in different rates of carcass contamination during slaughtering procedure (Conedera *et al*., 1997).

The present study, which contrasts with the results of similar studies elsewhere in U.S.A. and Canada, suggests that the use of insensitive assays for isolation of *E. coli* O157:H7 can lead to recovery of very low numbers or failure to find the pathogen in the meat product. For examples, a total of 66 retail ground beef samples from New Found Land U.S.A. (March and Ratnam, 1986), 666 samples from Canada (Read *et al*., 1996), 310 beef products from London, England (Willshaw *et al*., 1993) all tested negative for *Escherichia coli* O157:H7. Nevertheless, a large scale survey of beef product in U.S.A., using even a more sensitive method for *E. coli* O157:H7 detection found that only 3 were positive out of 5,000 beef samples (Johnson *et al*., 1995). Reduction in isolation rates was attributed to regional differences in the prevalence of the organism in cattle (Waters *et al*., 1994) and efforts on the part of the meat industry to reduce rate of contamination of *E. coli* O157:H7 in beef products (Bryant *et al*., 1989).

The finding of the present study showed that there was no significant difference in the occurrence of *E. coli* O157:H7 isolated from beef samples with regard to the various sampling sites. Similarity in the rate of meat contamination by the organism as observed in the various sites may be due to the fact that the same abattoir practices are employed when carcasses are being processed. For example, modern mechanization used for meat processing which reduce microbial contamination was not usually applied, thus dressing, evisceration, sawing and movement of carcass were done manually in all the abattoirs visited. This poor abattoir practices

abattoirs that were visited during this investigation include the unsightly and unhygienic conditions of the abattoirs, the use of contaminated tools and unclean wash water. All this confirms that sanitary standards and good management practices are extremely important to curtail the rate of carcass contamination in case cattle infected with *E. coli* O157:H7 and other pathogens are presented for slaughter in our abattoirs.

The highest occurrence of *E. coli* O157:H7 recorded in the intestinal samples in the present study is as a result of high faecal contamination and improper cleaning of the intestines before they are displayed for sale. The present study also revealed that organ meat had higher percentage occurrence of *E. coli* O157:H7 than that of muscle meat. This finding is not surprising since organ meat such as liver contain large numbers of microorganisms because of the generally higher pH of the meat which favours microbial growth at a more rapid rate than in muscle meat (Nwosu, 1990). The reason for higher occurrence of *E. coli* O157:H7 in organ meats than in red muscle meat could be attributed to the fact that in the market and abattoir environments organ meats and the intestines are usually displayed on the same tables by the butchers, which may eventually lead to cross-contamination from the intestines (which are usually heavily contaminated) to the organ meats.

The results of this study showed that there was no significant difference between the percentage occurrences of *E. coli* O157:H7 in meat obtained from abattoir and market environments. The reason being that market retailers usually obtain their meat from the abattoir before conveying them to their shops where they displayed for sale. This implies that *E. coli* O157:H7 originally present in animals may contaminate carcasses during processing at abattoir before the meats are distributed to the retailers for sale (Grau, 1987; Sekla *et al*., 1990; Ayres, 1996; Elder *et al.*, 2000).

The rate of meat contamination by *E. coli* O157:H7 was higher in the wet season than in the dry season as shown in this study. Although the seasonality of *E. coli* O157:H7 in meat has not been documented, several authors have reported that carcass contamination of the pathogen depends on faecal carriage of the organism by the cattle before slaughter (Kudva *et al*., 1996; Chapman *et al*., 1997; Hancock *et al*., 1997; Hancock *et al*., 1998; Bornadi *et al*., 2001). For example, Bornadi *et al*. (2001) isolated *E. coli* O157:H7 from bovine faeces and intestinal content and also recovered the organism from 45.5% of the bovine carcass after dressing step at 3 different abattoirs, as the faecal carriage of *E. coli* O157:H7 in cattle is higher during rainy season than during dry season (Conedera *et al*., 1997; Bornadi *et al*., 1999). Consequently, meat contamination of the pathogen from infected cattle would be expected to be higher during the rainy season than during the dry season, thus affirming the findings of the present study.

Table 1: Frequency of Occurrence of *E. coli* O157:H7 Isolated from Beef Parts in the Sample Sites.

Sampling Site	Beef Parts					Total
	Muscle	Liver	Kidney	Heart	Intestine	
Jos	1/20(0.14)	1/20(0.14)	0/20(0.00)	0/20(0.00)	3/20(0.43)	5/100(0.71)
Bukuru	0/20(0.00)	0/20(0.00)	1/20(0.14)	1/20(0.14)	2/20(0.29)	4/100(0.57)
Vom	0/20(0.00)	1/20(0.14)	0/20(0.00)	0/20(0.00)	5/20(0.71)	6/100(0.86)
Miango	1/20(0.14)	1/20(0.14)	1/20(0.14)	0/20(0.00)	4/20(0.57)	7/100(1.00)
Bassa	1/20(0.14)	1/20(0.14)	3/20(0.43)	2/20(0.00)	4/20(0.57)	11/100(1.00)
Riyom	0/20(0.00)	0/20(0.00)	0/20(0.00)	1/20(0.14)	4/26(0.57)	5/100(0.71)
Barkin Ladi	0/20(0.00)	3/20(0.43)	1/20(0.14)	1/20(0.14)	1/20(0.14)	6/100(0.86)
Total	**3/140(0.43)**	**7/140(1.00)**	**6/140(0.86)**	**5/140(0.71)**	**23/140(3.29)**	**44/700(6.29)**

Table 2: Effect of Sources on the Occurrence of *E. coli* O157:H7 Isolated from Beef Parts.

		Beef Parts				
Sources	**Muscle**	**Liver**	**Kidney**	**Heart**	**Intestine**	**Total**
Abattoir	1/70(0.14)	3/70(0.43)	3/70(0.43)	3/70(0.43)	10/70(1.43)	20/350(2.86)
Retail Market	2/70(0.29)	4/70(0.57)	3/70(0.43)	2/70(0.29)	13/70(1.86)	24/350(3.43)
Total	**3/140(0.43)**	**7/140(1.00)**	**6/140(0.86)**	**5/140(0.71)**	**23/140(3.29)**	**44/700(6.29)**

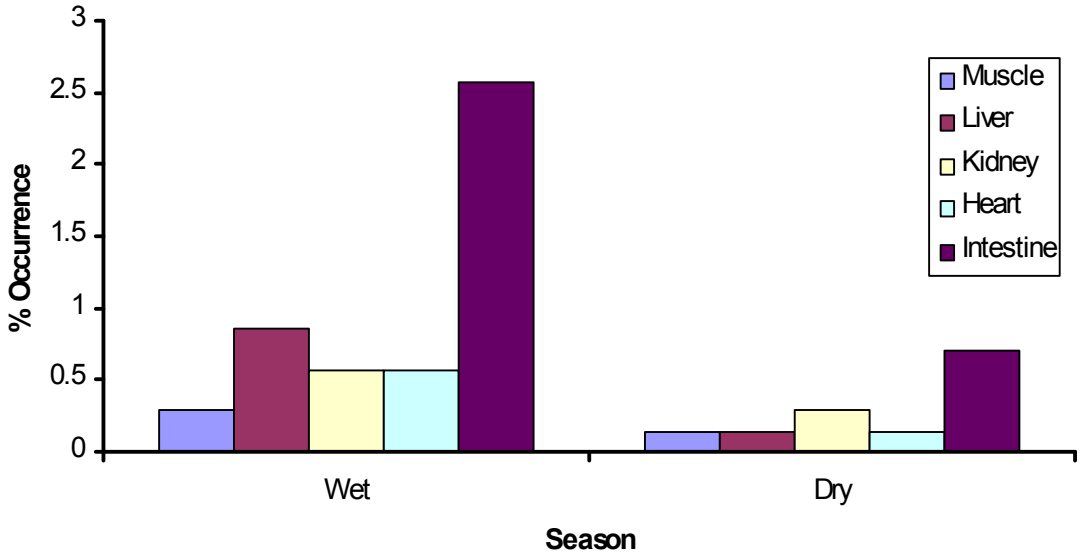

Figure 1: Seasonal Effects on the Percentage Occurrence of *E. coli* O157:H7 Isolated from Various Beef Parts.

CONCLUSION

The isolation of *E. coli* O157:H7 from meat sold in our markets and abattoirs poses some problems from the public health point of view. First, in our markets and abattoirs, meats are generally sold at atmospheric temperatures without chilling or freezing. These meats are held at warm temperatures for hours thereby encouraging the growth and activity of *E. coli* O157:H7 and other microorganisms. Secondly, organ meats such as liver and kidney are preserve their vitamin and other nutritional contents. Samples of such meat contaminated with *Escherichia coli* O157:H7 held at warm temperatures may not be heated for a long enough period to destroy all the cells of the organism. As the human infectious dose of *E. coli* O157:H7 is very low (Buchanan and Doyle; 1997; Makino *et al*., 2000); consumption of contaminated meat with even small quatity of the organism can lead to infection associated with

contamination of ready-to-eat foods and vegetables with meat may occur in the kitchen.

To prevent the transmission of *E. coli* O157:H7 from beef to man, several intervention strategies should be adopted (CDC, 2002; Wikipedia, 2006). These include the prevention of faecal contamination of beef carcass during slaughter, which is an important step to minimize contamination especially for products that will enter the food chain in the raw state. Washing hides prior to the removal from carcass has been shown to significantly reduce level of microbial contamination on the sides of the beef. Carcass decontamination using antimicrobial treatments can reduce microbial counts on meat surfaces. Thorough cooking will inactivate *E. coli* O157:H7, and is an effective strategy for cooked meat products. The prevention of cross-contamination from raw beef to cooked or ready to-eat food products is also critical. Proper personal hygiene and thorough hand washing is essential when handling beef, since low doses can cause the severe illnesses associated the organism.

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