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DETECTION OF *Escherichia coli O157*:H7 IN SELECTED PIGGERIES IN SAMARU, ZARIA, KADUNA STATE OF NIGERIA

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ABSTRACT

Escherichia coli is a commensal organism that live as normal flora in warm- blooded animals and the serotype Escherichia coli O157:H7 is a major foodborne pathogen that inhabit the hindgut of some animals. The major reservoir of E. coli O157:H7 are cattle and small ruminants that contaminate the environment through their faecal material although pigs have also been known to carry it. Infection to human occur through faecal oral route of transmission. This study investigated the presence of E. coli O157:H7 in the faeces of pigs. A total of 100 samples were collected, 50 each from two different piggeries in Samaru, Zaria in Kaduna State of Nigeria. The samples were analysed to isolate E. coli O157:H7 using Rapid Latex Agglutination test Kit and Enzyme Immunoassay (RIDA$^{(R)}$ Quick Verotoxin/O157 Combi). The result of the study showed biochemically characterized isolation rate of E. coli as 74% in from Samaru new extension and 34.1% at Samaru hayin danyaro piggery. A 1% isolation rate of E. coli O157:H7 from Samaru new extension using Rapid Latex Agglutination test and 0% using Enzyme Immunoassay were also obtained. In conclusion, the study has established 1% prevalence of E. coli O157:H7 at Samaru new extension piggery. It was therefore recommended that public health education be carried out by government to sensitize people rearing pigs in their neighbourhood on the need to maintain strict hygiene to minimize environmental contamination with the pathogen.

Key words: *Escherichia coli, piggeries, faecal matter*

INTRODUCTION

Escherichia coli is the leading cause of morbidity and mortality in newborn and weaned pigs (*Flores, 2004*) with post weaning diarrhea (PWD) as the main health problem in piggeries and has caused significant losses in the swine industry worldwide (*Fairbrother et al, 2005*). The main causal agents of PWD are verotoxin (VT)-producing *E. coli* (VTEC), also known as Shiga-toxin (Stx)-producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC) (*Fairbrother et al, 2005*). Enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) have also been found associated with PWD (*Botteldoorn et al, 2003*). VTEC is characterized by the production of VTs (VT1 and VT2) which disrupt protein synthesis whereas ETEC is characterized by the production of heat labile enterotoxin (LT) and heat stable enterotoxin (ST) (*Kaper et al, 2004*). Among the pathogenic *E. coli*, VTEC O157 is often the main focus of most surveillance programs due to its association with severe human infections (*Strockbine et al, 1998)*.

Pathogenic *E. coli* with an inter-human circulation represent a leading cause of diarrhoea, often with high mortality rates, in developing countries (Van den Beld *et al*, 2012). On the other hand, STEC have gained increasing global concern as food-borne pathogens worldwide (Caprioli *et al*,

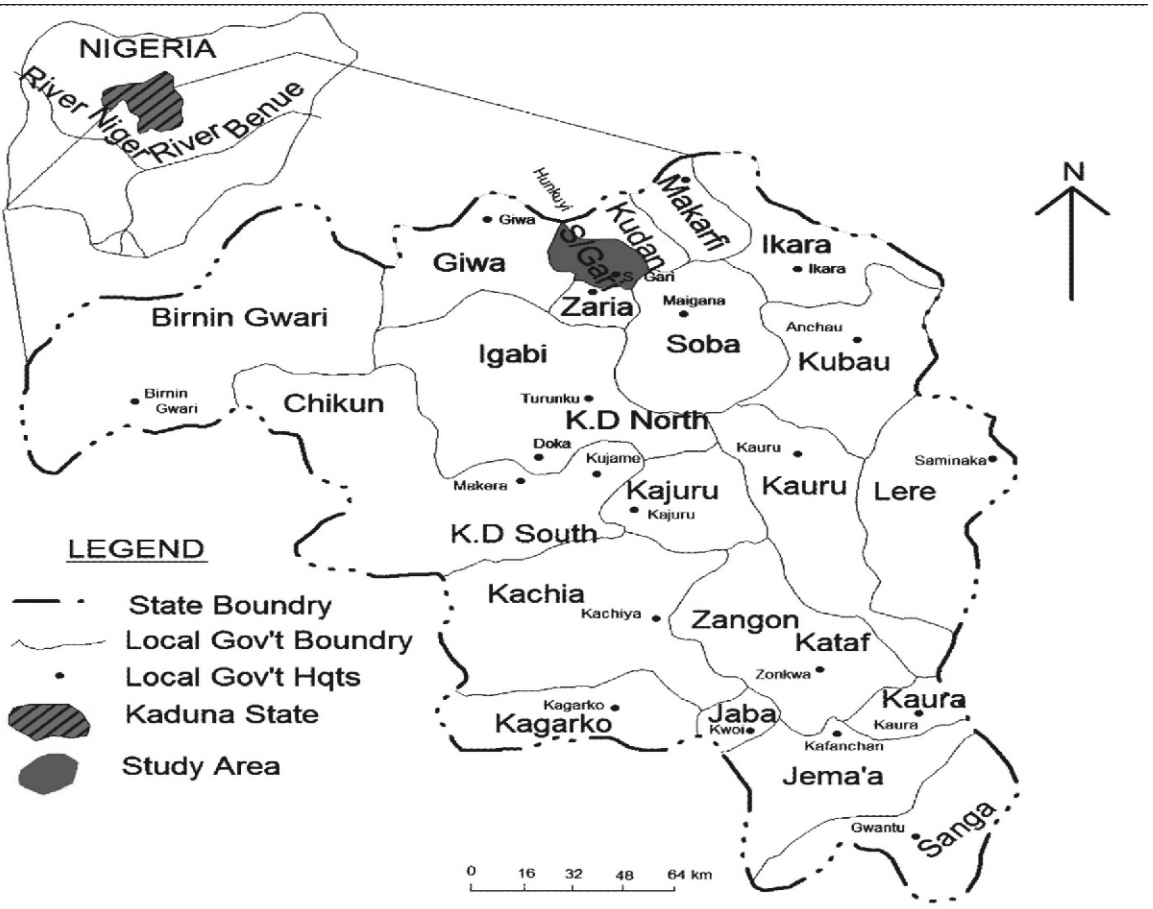
2005, Koch *et al,* 2001 and Tarr *et al,* 2005) and are the only diarrheagenic *E. coli* pathogroup with an ascertained zoonotic origin, with ruminants being regarded as the main animal reservoir (Faith *et al,* 1996 and La Ragione *et al,* 2009). *E. coli O*157:H7 is a significant food-borne pathogen that has emerged in the past two decades which colonizes the lower intestinal tract and is commonly shed through faeces (Bach *et al,* 2002).

This study investigated the presence of E. coli O157:H7 in the faeces of pigs in selected piggeries in Samaru, Zaria in Kaduna State of Nigeria as a possible source of water and environmental contamination with the pathogen due to the semi-intensive management system of pigs in the area.

MATERIALS AND METHODS

Study Area

The study was carried out in Samaru, Sabo Gari Local Government Area of Kaduna state, Nigeria situated on latitude 112 ° 12" N and longitude 07 ° 37" E, at an altitude of 550-700 metres. Zaria comprises two Local Government Areas, namely Zaria and Sabon Gari. The study area is characterized by a natural and stable ecosystem in the Northern Guinea Savannah zone, with a discontinuous layer of sparsely distributed short trees followed by relatively continuous layers of tall, medium and short grasses (Lawan *et al*, 2015). Most of the people in the study area were engaged in farming activities both crop and animal production. The two location study pigs were the commonest animal reared.



Source: Modified from Lawan *et al*., 2015.

Figure 1: Map of Kaduna state showing Sabon Gari Local Government Area.

Sampling strategy

Two piggery farms located at new extension and hayin danyaro Samaru were selected using convenience sampling based on willingness to participate. A total of 100 fecal samples: 50 from each site were collected. 10g of faecal material was collected using a clean sterile polythene bag as described by Lawan *et al* (2015). The pig fresh void was also collected at the time of sampling. The fecal material was then placed in Trypticase soy broth (TSB, Oxoid, Basingstoke Hampshire, UK) and transported to the laboratory.

Laboratory procedure for isolation and identification of *E. Coli.*

The Tripticase soy broth containing the sample was incubated at 37°c and analysed within 24 hours. One hundred µL of enrichment broth of the samples were streaked on Eosin Methylene Blue (EMB, Oxoid) agar and incubated at 37°c for 24 hours. Colonies producing greenish metallic sheen on EMB agar were considered as having *E. coli.* These were then inoculated into nutrient agar slants and incubated at 37°c for 24hours. The slants were then stored at 4°C awaiting biochemical test and further studies. The Positive isolates were characterized biochemically as described by Barrow and Feltham (1993) and sugars fermentation test was also carried out using 5ml solutions of Mannitol, Sorbitol, Inositol, Lactose and Sucrose prepared according to manufacturer's instruction

Detection of *E. coli* O157:H7 using rapid latex agglutination test and screening for STEC using a commercial enzyme immunoassay (EIA). All the Isolates biochemically characterized to be *E. coli* were further screened using Latex agglutination test (Remel, Dartford, UK) for the presence of *E. coli* O157:H7 and commercial enzyme immunoassay (R-Biopharm, Darmstadt, Germany) to detect Shiga toxins producing *E. coli* all of which were prepared according to Manufacturer's instructions.

RESULTS

The one hundred samples were inoculated onto EMB with 46 each from both sites producing colonies producing greenish metallic sheen of *E. coli* showing an overall isolation rate of 92%. Biochemical confirmation of single colonies (one colony per sample) produced 34 *E. coli* positives from Samaru new extension and 18 *E. coli* positives from Samaru hayin danyaro indicating an isolation rate of 57%. Biochemical sugars fermentation test showed slow rate of innositol fermentation as shown in figures 1 and 2. One Out of the 100 samples collected was identified as *E. coli* O157:H7 from New Extension using the rapid Latex agglutination test and none was positive to the EIA as shown in Table 1.

DISCUSSION

The result from the Latex agglutination test suggested that pigs harbor *E. coli* O157:H7 apart from ruminants which serve as reservoirs of the pathogen (Caprioli *et al*., 2005). There was high isolation rate of 92% of *E. coli* from the two sites both on EMB agar and biochemically characterized to be *E. coli* (Table 1). This is because *E. coli* is a normal flora in the gastrointestinal tract of animals as described by Singleton (1999). However this indicate high number of *E. coli* were shed into the environment via pig faeces and if adequate hygiene is not maintained may cause environmental contamination including water and vegetables which is of public health concern. There was a higher isolation rate of *E. coli* from New Extension when compared to Hayin Danyaro as shown in (Table 1).

This variation may be due to the high exposure of the pigs in New Extension to contaminated water in the drainages and run-offs adjacent to the piggery were the pigs wallow continually and in the process of wallowing ingest the contaminated water which was observed during the study. During the biochemical sugar fermentation test, it was observed that there was a slow rate of innositol fermentation within 24 hours despite the isolates were confirmed to be *E. coli* which further accentuates the presence of a slow inositol fermenting *E. coli* as shown in Figures II and III. *E. coli* are not slow fermenter of inositol as previously described by (Siegrist, 2016).

The 1% isolation rate of *E. coli* O157:H7 using the Rapid Latex agglutination test from this study is slightly higher than that of Ji-Yeon *et al*., (2005) who obtained a prevalence of 0.65% in Korea although they had a larger sample size and their samples were collected from pig faeces at slaughter houses but less than that of Ojo *et al*., (2010) who obtained 5.6% at Ibadan using PCR. This study was unable to detect O157 and non O157 Verotoxin-producing *E. coli* using the commercial enzyme immunoassay which is similar to that of Pinna *et al*., (2004) in which no isolate of *E. coli* serogroup O157 was isolated even though he used PCR in his study. Although several work has shown that the commercial enzyme immunoassay used in this study was similar in sensitivity to the PCR in detecting STEC (Zidan *et al*., 2014). Further reason for this finding can be attributed to the small sample size used in this study when compared with Callaway *et al*., (2004) with a sample size of 240 and prevalence rate of 2.1%.

CONCLUSION

This study has established a 57% isolation rate of *E. coli* using biochemical test which indicates poor hygienic condition of both Piggeries and a high level of environmental contamination. The study also established a 1% isolation rate of *E. coli* O157:H7 using Rapid Latex Agglutination test.

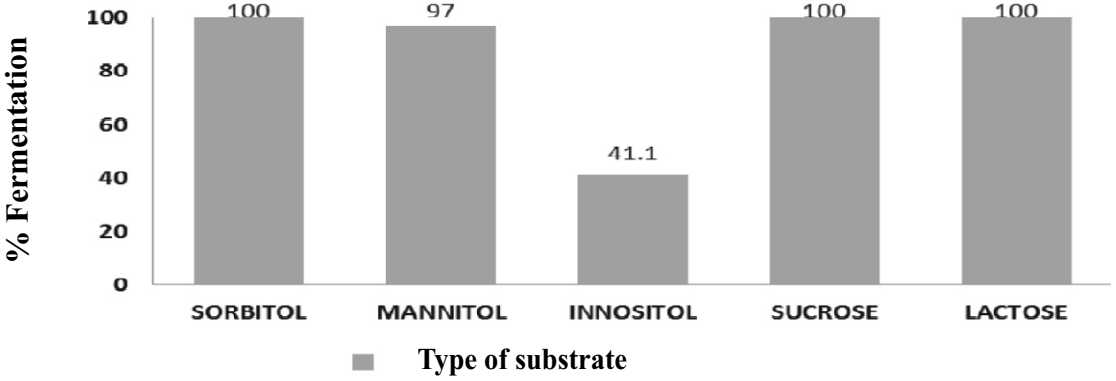


Figure 2: Percentage of sugars fermenting *E. coli* from new extension

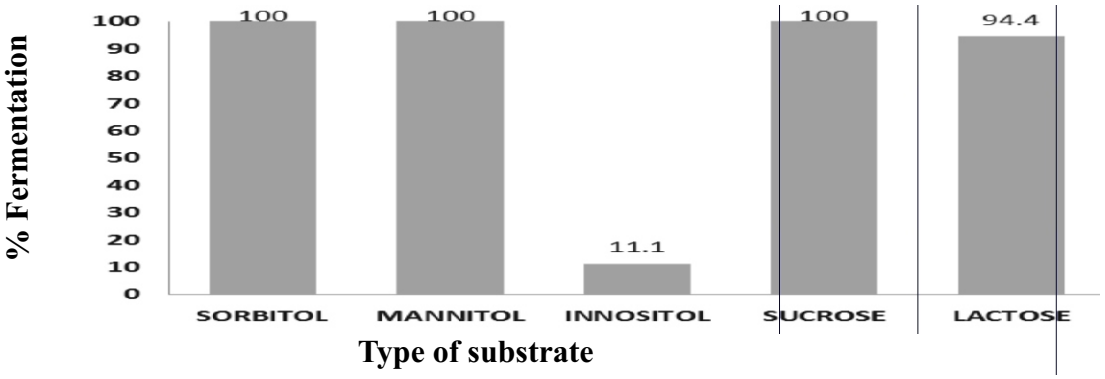


Figure 3: Percentage of sugars fermenting *E. coli* from hayin danyaro

Table 1: Isolation rate of *E. coli* on EMB and biochemical characterization of from new extension and hayin danyaro Samaru.

S/N	Location	No. of Samples		No. of colonies Biochemically Characterised as *E. coli* (%)
		Collected	Producing typical colonies on EMB (%)	
1.	New Extension	50	46(92)	34(74)
2.	Hayin Danyaro	50	46(92)	18(39.1)
TOTAL		100	92(92)	52(57)

Table 2: Isolation Rate of *E. coli* O157:H7 using Rapid Latex Agglutination Test and Ridascreen Verotoxin EIA screening from new extension and hayin danyaro Samaru.

S/N	Location	No. of Samples Collected	Positive on Latex Agglutination test for O157:H7 (%)	Positive to EIA (%)
1.	New Extension	50	1(1)	0(0)
2.	Hayin Danyaro	50	0(0)	0(0)
TOTAL		100	1(1)	0(0)

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