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PREVALENCE AND INCIDENCE OF *Escherichia coli* O157 IN DOMESTIC GOAT DUNGS OF OKITIPUPA TOWNSHIP

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I.A. Adeyemo and O.A. Waleola

Department of Biological Science, Ondo State University of Science and Technology
(OSUSTECH), Okitipupa

ABSTRACT

Escherichia coli O157 is an emerging disease pathogen whose occurrences have been reported in several parts of the world including Nigeria. Factors such as contamination of goat meat by the dungs of the goat while killing the goat, consumption of undercooked goat meat which predisposes humans to infection with the organism abound in Okitipupa, a rural town in southern Ondo state of Nigeria with about 300, 000 people and it is also the local government headquarters of Okitipupa local government. This prompted the study on the incidence and prevalence of *E. coli* O157 in domestic goat dungs in Okitipupa Township. Two hundred (200) samples from three locations were taken and analysed. Goat dungs samples collected were inoculated onto Sorbitol MacConkey Agar fortified with Cefixime and Tellurite, sorbitol negative organisms were confirmed as *E. coli* O157 using biochemical tests. Results of study showed that of the 200 samples analysed, a prevalence rate of 30% was recorded for *Escherichia coli* O157. Kids of goats harbour this organism than Nanny and Buck as a 100% prevalence was recorded of the 20 Kids' faecal samples tested. Statistical analysis shows that there was a significant difference between the incidence of *E. coli* O157 in Kids compared to Nannies and Bucks ($p < 0.05$) but there was no significant difference in occurrence of the organism in Nannies and Bucks ($p > 0.05$). It is therefore recommended that a more intense and well planned public enlightenment be mounted by our sanitary health officials on the danger of eating undercooked goat meats, while cases of gastroenteritis with bloody or non-bloody diarrhea should be more intensely investigated bacteriologically.

Keyword: *E.coli* O157, Incidence, Prevalence, goat dungs, Nannies, Bucks, Kids

INTRODUCTION

The domestic goat (*Capra aegagrus hircus*) is a subspecies of goat domesticated from the wild goat of southwest Asia and Eastern Europe. The goat is a member of the family Bovidae and is closely related to the sheep as both are in the goat-antelope subfamily

Caprinae. There are over 300 distinct breeds of goat (Hirst, 2008). Goats are one of the oldest domesticated species, and have been used for their milk, meat, hair, and skins over much of the world (Coffey, 2004). In 2011, there were more than 924 million live goats around the globe, according to the UN Food and Agriculture Organization.

Corresponding Author: I.A. Adeyemo, Department of Biological Science, Ondo State University of Science and Technology, Email: adeyemiyourfriend@gmail.com

Goats are good source of meat and their meats are highly consumed all over the world. Goat meat is low in fat and has a flavour which as believed is due to the presence of 4-methyloctanoic and 4-methylnonanoic acid (Cramer, 1983). Despite the good flavour of goat meat and other nutrition it gives to its consumers, diseases can be transmitted through the meat especially when not properly cooked or when proper hygiene is not ensured during killing which could lead to contamination of the meat by the faeces or dung of the goat. One of the contaminants that can be transmitted through this means in the deadly diarrheagenic *Escherichia coli* O157 which can cause gastroenteritis in goats as well as in humans. It has been reported that Gastroenteritis causes 1.4 million deaths every year (Elliot, 2007; Lozano 2012).

E. coli O157, which is a strain of the enterohaemorrhagic *E. coli* group, is recognized as an organism whose presence in any food material can lead to serious outbreak (Anonymous, 1999). The growth of this strain in human intestine is known to produce large quantity of toxins, which can cause severe damage to the lining of the intestine and other organs of the body (Nataro and Kaper, 1998). The organism is particularly associated with the development of hemolytic uremic syndrome, known to result in a mortality rate of 2 – 10% (Law, 2000). The potentially high mortality associated with *E. coli* O157 infection, therefore makes its presence in any food material a concern and a serious public health problem.

In spite of the wide knowledge of the organism and its interaction, there seem to be no report on the prevalence of the organism in Ondo state and particularly Okitipupa. In the light of these prevailing circumstances, and an increased reported cases of *E. coli* O157 infection outbreak worldwide, it becomes clear that a thorough study be conducted in this part of the world to ascertain its presence in our surroundings. This study is aimed at

determining the incidence and prevalence of *E. coli* O157 in domestic goat dungs in Okitipupa township since contact with goat meat killed under unhygienic condition can result in serious disease outbreak and even death.

MATERIALS AND METHOD

Study Location

This study was carried out in Okitipupa, a town in Southern Ondo State between January and July 2015 by collecting samples from three locations (Lebi, OSUSTECH, and Ayeka) within the study area. Okitipupa is a town in southern Ondo State and the local government headquarters of Okitipupa local government with a population of about 233,565, with thousands of goats population. The average annual rainfall in Okitipupa is 1900mm and 2030mm with the rainy season lasting from April to November. The vegetation of Okitipupa area is the lowland rainforest.

Population of Study

Two hundred (200) samples were collected (all from different goats) from all three locations within Okitipupa town, twenty (20 samples) from Ayeka were analysed, fifty (50) samples from Lebi were analysed and One hundred and thirty (130) samples from OSUSTECH were analysed.

Data Analysis

Descriptive statistics such as percentage and charts were used in the discussion of the results, in order to give a clear representation of the data analyzed. Data analysis features of Microsoft Excel version 2013 was used to test for level of significance of the result obtained. The level of significance was tested using Chi-squared test at 5% ($p < 0.05$) confidence interval.

SAMPLES PROCESSING

Collection of sample

The dungs were collected using sterile polythene bags immediately they are passed out from the goats and each sample was labeled appropriately with a serial number, location mark and group mark in which the sample falls. The samples were transported immediately to the laboratory for analysis within 1 hour of sample collection. .

Isolation of Bacteria

1g of each sample was weighed and then transferred into 9ml of normal saline for partial dissolution.. The partially dissolved sample was then further diluted with the aid of a sterile pipette up to 10^{-5} after which 0.1ml was taken from three random dilutions and then transferred to a plate containing prepared Oxoid Sorbitol MacConkey Agar fortified with Cefixime and Tellurite (CT-SMAC) (Appendix) and spreading was done using a sterile glass spreader. The plates were then incubated at 37°C for 24 hours and observed.

Identification of *E. coli* O157

Cultures appearing on the plates after incubation were either pink or straw, those that appeared pink are sorbitol fermenters e.g *E. coli*, while those that appeared straw are non-sorbitol fermenters e.g *E. coli* O157. Colonies appearing straw were counted and recorded and were subjected to biochemical tests (Gram staining, Indole test, Lactose fermentation, Catalase test and Oxidase test). All biochemical tests done were typical tests for *E. coli* which is the same for *E. coli* O157 but a major difference is in the fermentation of sorbitol. *E.coli* ferments sorbitol while *E. coli* O157 does not.

RESULTS

The result of the analysed domestic goat dungs is presented in Table 1. . *E. coli* O157 was detected in 60(30%) of the total samples analysed while 140(70%) were negative.

Table 2 shows the prevalence of *E. coli* O157 as observed with respect to the groups (Buck, Nanny and Kid) of goats whose dungs were analysed. A total of 80 samples were analysed for Bucks out of which 20(25%) was positive and 60(75%) were negative for *E. coli* O157. For Kids, all 20 samples analysed was positive (100%) for the organism of interest. For Nanny, 100 samples were analysed out of which 20(20%) were positive while 80(80%) were negative. The study helps to ascertain that the prevalence of *E. coli* O157 was higher in Kids (100%) than in Nannies (20%) and Bucks (25%). Statistical analysis showed that the incidence of *E. coli* O157 differs significantly in Kids from those of Nannies and Buck ($p < 0.05$) but insignificant between Nannies and Buck ($p > 0.005$).

The prevalence of *E. coli* O157 was also observed in respect to location where samples were collected as shown in Table 3. In Ayeka where a total number of 20 samples were collected, 5(25%) were positive while 15(75%) were negative. In Lebi quarters, 50 samples were collected and analysed out of which 10(20%) were positive while 40(80%) were negative and in OSUSTECH school farm, 130 samples were collected out of which 45(34.62%) were positive and 85(65.38%) were negative. It was observed that the Prevalence of *E. coli* O157 was higher in goats of OSUSTECH farm (34.62%) compared to the other two locations. Statistical analysis shows that difference between the incidence of *E. coli* O157 in the various locations is insignificant ($p > 0.05$).

Table 1: Prevalence of *E. coli* O157 in goat dungs

No of Samples	No with <i>E. coli</i> O157	% with <i>E. coli</i> O157
200	60	30%

Table 2: Prevalence of *E. coli* O157 in different groups of domestic goats whose dungs were analysed

Category	No of Samples			No of positive samples	% of positive samples
	Ayeka	OSUSTECH School Farm	Lebi Quarters	TOTAL	
Buck	8	50	22	80	20
Kid	5	8	7	20	100
Nanny	7	72	21	100	20
TOTAL				200	60

Table 3: Prevalence of *E. coli* O157 in various locations of study

LOCATION	NO. OF SAMPLES	NO. OF POSITIVE ISOLATES	% NUMBER OF POSITIVE ISOLATES
	20	5	25%
	50	10	20%
OSUSTECH FARM	130	45	34.62%

Microbial load of *E. coli* O157 was also observed and recorded in each positive sample at each dilution factor. A total of 60 samples were positive out of all 200 samples tested, 20 samples were positive for each study group (i.e 20 samples each for Buck, Nanny and Kid). Table 4 reveals microbial load vis-a-sis various dilution factors. It was observed that Kids have the

highest prevalence and incidence more than the other groups because they harbour the organism more than the other groups. For example, for positive sample 1, diluting factor 10^{-4} , there was no *E. coli* O157 colony seen for Bucks, 1cfu/ml was recorded for Nanny while 32cfu/ml was seen for Kid.

Table 4: Colony counts for *E. coli* O157

Positive Samples GROUP		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		DILUTION FACTOR/NO OF COLONY (cfu/ml)																			
BUCK	10⁻¹	44	63	20	76	54	45	43	65	30	43	22	32	40	43	44	45	65	76	65	47
	10⁻²	11	27	8	42	20	15	20	27	12	20	15	13	21	19	25	27	28	30	34	22
	10⁻³	2	16	2	26	5	4	10	14	3	9	4	7	9	10	11	15	12	15	16	10
	10⁻⁴	-	4	-	12	1	-	3	7	-	2	1	2	4	4	6	4	4	6	6	3
	10⁻⁵	-	-	-	4	-	-	-	1	-	-	-	-	-	-	2	-	1	1	2	-
NANNY	10⁻¹	45	96	76	80	86	75	75	74	86	80	96	78	82	81	79	77	69	78	79	87
	10⁻²	19	46	31	35	36	34	29	26	39	34	34	29	39	40	31	30	28	34	33	37
	10⁻³	3	11	14	14	14	16	11	12	17	12	10	13	10	16	12	11	12	14	11	10
	10⁻⁴	1	5	9	8	6	7	4	5	8	6	3	5	3	5	4	3	4	3	4	4
	10⁻⁵	-	1	3	2	2	1	-	-	2	1	-	-	-	1	-	-	-	-	1	-
KID	10⁻¹	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC
	10⁻²	TNC	TNC	150	TNC	170	TNC	TNC	TNC	TNC	130	TNC									
	10⁻³	76	60	67	86	86	80	96	88	76	82	90	92	87	67	65	87	89	84	76	73
	10⁻⁴	54	45	40	64	58	57	60	59	55	57	61	63	50	47	37	42	45	49	39	36
	10⁻⁵	32	22	19	32	30	31	36	29	30	27	28	31	23	22	19	26	25	27	15	12

Key: TNC = Too numerous to count

DISCUSSION

Escherichia coli O157 is an enterohemorrhagic serotype of the bacterium *Escherichia coli* and a cause of illness, typically through consumption of contaminated food (Karch *et al.*, 2005). Infection of *E. coli* O157 may lead to hemorrhagic diarrhea, kidney failure in humans. Transmission is via fecal-oral route, and most illness has been through distribution of contaminated undercooked meat and raw leafy green vegetables. *E. coli* O157 which are normal flora of the human and animal intestine have been identified as a leading cause of food borne illness all over the world (Agbeyegbe and Uraih, 1982). *E. coli* O157:H7 strain has been previously isolated from meat samples and has also been implicated in the contamination of vegetables such as lettuce (Anonymous, 1993; Hilborn *et al.*, 1999; Hussein, 2007).

In the course of this study, it was discovered that few materials are available on prevalence, incidence or occurrence of *E. coli* O157 among goats in Nigeria .

The method of isolation used was direct plating on Sorbitol MacConkey Agar fortified with Cefixime and Tellurite (CT-SMAC). More techniques can be used to identify the organism up to molecular level especially polymerase chain reaction (PCR). CT-SMAC which has been used by various researchers to isolate *E. coli* O157 from various samples is in fact a selective media for *E. coli* O157. Cefixime and Tellurite was added to the media so as to eliminate interfering organisms as Cefixime is inhibitory to *Proteus spp.* Tellurite selects serogroup O157 from other *E. coli* serogroups and inhibits *Providencia* and *Aeromonas* species (Zadik *et al.*, 1993).

This study revealed the prevalence of *E. coli* O157 in goat dungs of Okitipupa to be 30% as 60 out of 200 samples analysed were positive. This result is consistent with the work of Mersha *et al.*, 2008 who reported the incidence of *E. coli* in goats of Ethiopia. It also agrees with the work of Jacob *et al.* (2013) who also reported incidence and prevalence of *E. coli* O157 in feces, hides and carcasses of goats in the United States.

In the area of correlation of sex and age of study animal to the prevalence and incidence of *E. coli* O157, from this study, *E. coli* O157 was more prevalent in Kids than in Nannies and Bucks as all samples analysed for Kids were positive (100%). This could be as a result of the immune system of Kid just developing compared to that of Bucks' and Nannies'. Kids might have also picked *E. coli* O157 up from the Nannies in the process of suckling milk from mother's breasts.

It was observed that Kids have the highest prevalence and incidence more than the other groups because they harbour the organism more than the other groups. For example, for positive sample 1, diluting factor 10^{-4} , there was no *E. coli* O157 colony observed for Bucks, 1cfu/ml was recorded for Nanny while 32cfu/ml was seen for Kid. This result is similar with the work of Willian and Harley in 1995 who both reported that the magnitude of shedding of *E. coli* O157 was greater in calves than in other groups. They ascribed the reason to the fact that adults have a fully developed forestomach compartment, where the combination of a high volatile fatty acid concentration and low pH inhibits the growth of *E. coli* O157.

This study has revealed that goats are important carriers of *E. coli* O157, hence this should prompt researchers to focus more on goats as outbreaks of *E. coli* O157 can also arise from ingesting foods contaminated with goat dungs especially in babies who crawl around and put whatever they find on the ground into their mouth. In a study conducted by Esumeh *et al.* (2011) on 50 patients who reportedly had symptoms of diarrhea in Benin, a prevalence rate of 20% was recorded for *E. coli* O157 which may suggest that even many cases of diarrhea in hospitals of Okitipupa might have been caused by *E. coli* O157. High level of unsanitary practices while killing goats and other ruminants in abattoirs and at various homes can contribute to the outbreak of *E. coli* O157 infections in Okitipupa as the contamination of the hides, skins and carcasses of the goats from the dungs is possible.

Conclusively, it is to be noted that the low level of sanitary practices observed in slaughter slabs and homes could spread *E. coli* O157 easily and possibly cause infection in human without early detection, hence it is important to engage in best hygiene practices to prevent transfer of *E. coli* O157 to man. The results of the present study also

suggest the need for further detailed epidemiological and molecular studies on *E. coli* O157:H7 involving different abattoirs and species of food animals in the study areas. Consequent upon this, it is recommended that due to the public health risks arising from *E. coli* O157:H7 and other foodborne pathogens from food animals such as goats, high level of hygiene is to be practiced at different levels, including abattoirs, in reducing skin and faecal sources and carcass contaminations at different stages of any slaughter operations. Hence public awareness should be created by the public health workers to warn the populace on the dangers of consuming undercooked meat. Also, physicians should investigate for *E. coli* O157 infection whenever patients present at their clinics with bloody or non-bloody diarrhea, so as to curb possible outbreaks early enough. It is also recommended that consumers cook their meat properly before they are consumed and also avoid indiscriminate eating of meat sold in the open that is not properly reheated. Fruits and vegetables should be washed properly as outbreaks from fruits and vegetables has been reported especially in developed countries and the presence of *E. coli* O157 in fruits and vegetables has also been reported in Nigeria.

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