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Original Research Article

Prevalence of *Escherichia coli* O157:H7 in Cattle Faeces and Manure from Abattoirs, Cattle Farms and Livestock Markets from Bauchi State North-Eastern Nigeria

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Abstract

A research was conducted to determine the occurrence and distribution of Escherichia coli (E. coli) O157:H7 in cattle faeces and manure, aimed at isolating and identifying shiga toxin producing E. coli (STEC) using phenotypic methods from cattle faeces and manure. Faecal samples (n=990) from apparently healthy cattle and manure samples (n=165) were collected between March to August, 2016 for this study, all samples were transported on ice to bacterial Zoonoses lab., department of Veterinary Public Health and preventive medicine Faculty of Veterinary Medicine, A.B.U. Zaria for analysis. Approximately 1 ml/1 g of faeces (homogenized when possible) were suspended into 9 ml of modified tryptone soya broth. Samples were vortexed and incubated overnight at 41°C. After selective enrichment, fifty micro liters of the product was streaked onto Eosin methylene blue (EMB), incubated at 37°C overnight. Preliminary results indicated 374/1155 (32.3%) of the samples showed greenish metallic sheen and were considered presumptive positive for E. coli and for further analysis. The 374 positive samples were tested biochemically using indole, methyl red, Voges Proskauer and citrate (IMViC), triple sugar iron and motility tests respectively, positive isolates 80/374 (4.7%) were subjected to Microbact 40E (U.K) where 48/80 (60%) were positive and were subjected to serological testing using E. coli O157:H7 agglutination test kits. The overall prevalence was determined to be 4.16% for all the samples collected in the three LGA, while a prevalence of 0.34%, 0.7% and 3.4% were found for Bauchi, Darazo and Katagum LGA respectively. Of the overall prevalence 2.51% were confirmed E. coli O157:H7 while 1.64% non O157. The prevalence based on sample locations were 0.09%, 3.37% and 0.69% for abattoir, cattle farms and livestock market respectively.

Keywords: Escherichia coli, occurrence, distribution, abattoir, cattle farms, livestock markets.

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Introduction

The *E. coli* organisms are normal inhabitants of the intestines of most animals, including humans [1] Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases, such as urinary tract infections and neonatal meningitis [2]. *E. coli* strains may exhibit characteristics that have been acquired from a wide variety of sources [2]. This research is design to determine the occurrence and distribution of *E. coli* O157:H7 in cattle faeces and manure, aimed at isolating and identifying shiga toxin producing *E. coli* (STEC) using phenotypic methods from cattle faeces and manure.

Some assays for the detection of diarrheagenic *E. coli* available include biochemical reactions, serotyping, phenotypic assays based on virulence characteristics, and molecular detection methods [3]. *E.*

coli O157:H7 in humans causes food-borne infections with symptoms that range from mild diarrhea to haemorrhagic colitis [4]. Most patients recover from the infection within the first 10 days, but in a small percentage of patients (up to 10%), particularly young children and the elderly, the infection may lead to haemolytic uraemic syndrome (HUS) [5].

Enteropathogenic *E. coli* are present in the faeces of humans, wildlife and domestic livestock, although, they may be found in faeces, water and soil, only a small proportion (< 1%) of the bacteria are harmful [6]. Most strains of *Escherichia coli* inhabiting the intestines of healthy humans, domestic livestock and wildlife are harmless, and in fact are a beneficial component of the natural intestinal flora [7].

There are several pathogenic strains of *E. coli*, with one of the best known and of zoonotic concern is *E. coli* O157:H7. This strain (*E. coli* O157:H7) and some other pathogenic ones produce toxins that can cause serious human illness. All *E. coli* are classified on the basis of the presence or absence of surface antigens (O, H, K) and a numerical system that distinguishes them based on harmless and harmful bacteria [7].

Humans are considered to be incidental hosts of *E. coli* O157:H7, although infection may also spread among patients due to the low infectious dose [8]. Cattle and sheep have been most often linked to human disease due to *E. coli* O157:H7 and cattle in particular, are considered the primary reservoir host for the organism [4, 9]. Majority of *E. coli* O157:H7 outbreaks were traced to cattle products or vegetable products that were contaminated with cattle waste [10].

MATERIALS AND METHOD

Sample Size Determination

The sample size were determined using the formula of Thrusfield, 2005,

$$(n=1.96^2 \text{ x } P_{exp}(1 - P_{exp}/d^2)$$

Cattle Faeces

The faecal sample size (n) was determined through an expected prevalence of $E.\ coli\ (P_{exp})$ of 31.2 % [11] and desired absolute precision of 5% at 95% confidence interval, sample size was arrived at 330. The same numbers were collected from three categories of cattle: abattoirs, cattle farms and in livestock markets from each of the three (3) selected LGA. The sample size was proportionately distributed across the three LGAs based on the cattle population and their distribution within the study area, thereby arriving at different sample sizes from the various sampling areas.

Manure

The Manure sample size (n) was determined through the expected prevalence of E. $coli~(P_{exp})$ of 12.2 % [12] and desired absolute precision of 5% at 95% confidence interval. The sample size was calculated at 165 which were shared among the sampling areas in the three LGAs.

Selection of sampling sites (abattoirs, cattle markets and livestock farms)

Sampling sites were selected based on estimated animal population in the local government

areas, such that three (3) farms with more than fifty (50) animals were considered where one farm in each of the three (3) districts in the LGAs will be considered. One major abattoir and cattle market were selected from each of the LGAs based on the number of animals slaughtered at the abattoirs and number of animals presented to the markets respectively.

Selection of animals in the sampling sites

Animals were selected through stratified random sampling therefore selected regardless of age, sex, health and their body condition.

Sample Collection Faecal Samples

About ten grams (10g) of faecal sample were collected from the rectum of the animals using sterile polythene bags and transported to the laboratory on ice packed cool box.

Manure Samples

Manure samples were collected from heaps of manure at the sample sites; aggregate sampling procedures were used where a heap will be thoroughly mixed using a sterile shovel. And the heap divided into four parts and about 10g collected from each part with a sterile spoon and mixed on sterile tray to form an aggregate sample. Then ten (10) grams of the aggregate sample were then transferred into a labeled sterile plastic screw capped tube, and immediately transported to the laboratory in cool or ice parked box. Sampling equipment were always clean and disinfected between sampling each separate heap.

Distribution of Samples

Sampling took place between March and August, 2016 and the period covered isolation and identification of the organisms (*E. coli*) from the samples as well as the phenotypic and genotypic identification of the isolates obtained from the samples. Faecal samples (n=990) were collected (n=330) each from the three (3) selected LGAs (Bauchi, Darazo and Katagum), sampling sites were the abattoirs, cattle farms and livestock markets. Whereas manure (n=165) samples were collected from the same LGAs and same sampling sites. The faecal and manure samples were collected from Bauchi in March and April, while sampling from Darazo was in May and June and samples from Katagum were collected between July and August (Table-1).

Table-1: Monthly distribution of faecal samples collection

		Faecal samples						Manure samples										
MONTH	Bauchi		Darazo		Katagum		Bauchi		Darazo		Katagum							
	A	CF	LM	A	CF	LM	A	CF	LM	A	CF	LM	A	CF	LM	Α	CF	LM
March	43	72	50	1	-	-	1	1	-	9	10	12	1	ı	-	ı	-	-
April	43	72	50	-	-	-	-	-	-	91	11	12	-	-	-	-	-	-
May	-	-	-	39	65	61	-	-	-	-	-	-	6	7	8	-	-	-
June	-	-	-	39	65	61	-	-	-	-	-	-	6	7	8	-	-	-
July	1	-	-	1	-	-	42	69	55	1	-	-	1	ı	-	8	10	13
August	-	-	-	-	-	-	41	69	54	-	-	-	-	-	-	7	10	12
Total	86	144	100	78	130	122	83	138	109	18	21	24	12	14	16	15	20	25

Key: A=Abattoir, CF=Cattle farms, LM=Livestock market

Distribution of cattle faeces and manure samples collected from sampling sites

A total of 990 faecal samples were collected for the study with equal number of (n=330) samples collected from each of the three Local Government Areas under the study. However, with regard to locations of sample collection, sampling was made such that 247(24.9%) samples were collected from the

abattoirs while 412(41.6%) and 331(33.5%) samples were collected from cattle farms and livestock markets respectively (Table-2). Also the samples were collected proportionately with a variation in the sample size from one sampling site to another due to estimated cattle population in the study area as confirmed via a pilot study previously carried out before the commencement of the study.

Table-2: Distribution of cattle faecal samples from sampled Local Government Areas of Bauchi State

	Number sam			
LGA	Abattoir	Cattle farms	Livestock Market	Total
Bauchi	86 (8.7%)	144 (14.5%)	100 (10.1 %)	330 (33.3%)
Darazo	78 (7.8 %)	130 (13.1%)	122 (12.3 %)	330 (33.2 %)
Katagum	83 (8.4%)	138 (13.9%)	109 (11.2 %)	330 (33.5%)
Total	247 (24.9%)	412 (41.5 %)	331 (33.6%)	990 (100.0)

A total of 165 cattle manure samples were collected from the three selected LGAs for the study with 63(38.2%) of the samples from Bauchi LGA, 42(25.4%) from Darazo LGA and 60 (36.4%) from

Katagum LGA. Of these samples 45(27.3 %) were from abattoirs, 55(33.3%) from cattle farms and 65(39.4%) from livestock markets (Table-3).

Table-3: Distribution of cattle manure samples from sampled Local Government Areas of Bauchi State

	Sa			
LGA	Abattoir	Cattle farms	Livestock Market	Total
Bauchi	18 (10.9 %)	21 (12.7 %)	24 (14.6 %)	63 (38.2 %)
Darazo	12 (7.3 %)	14 (8.5%)	16 (9.7%)	42 (25.5%)
Katagum	15 (9.0 %)	20 (12.1 %)	25 (15.2%)	60 (36.3%)
Total	45 (27.2 %)	55 (33.3 %)	65 (39.5 %)	165 (100.00 %)

Laboratory procedure (isolation and identification of $E.\ coli$)

Faecal and Manure Samples

The procedure was in accordance with the International Standards Organization reference method (ISO 16654) for isolation of *E. coli* O157.

Primary culture – A 10g of the sample was suspended on to 90mls of 0.1% of peptone water and then homogenized. Then 10mls of the homogenate sample was inoculated on 90mls of tryptone soy broth then incubated at 37°c for 24hrs for enrichment. A loop full of the overnight culture was streaked on Eosin Methylene Blue (Oxoid, U.K) then incubated at 37°c for 24hrs for the detection of *E. coli.* A greenish metallic sheen colony was streaked onsorbitol

MacConkey Agar incorporated with cefexim tellurite selective supplement (Oxoid, U.K.) for the detection of *E. coli* O157.

Secondary culture – The well- separated colonies from above was picked up and inoculated on nutrient agar (Oxoid, U.K.) slants and incubated at 37°C then stored at 4°C for further identification.

Biochemical Test

Colonies growing on nutrient agar slants were subjected to biochemical tests; Indole, Methyl red, Voges Proskauer, Citrate (IMViC), Motility and Triple sugar iron (TSI) as described by [13,15] for further confirmation of *E. coli*. Positive isolates were also be further characterized biochemically using Microbact

12E (MB1130A+, Oxoid, U.K.) according to the manufacturer's instruction.

Serological test on Escherichia coli isolates

A serological identification of positive isolates was carried out using *E. coli* O157:H7 diagnostic kit (Wellcolex, U.K.) to identify the serogroups using test procedure as described by the kits manufacturers.

RESULTS

Isolation and phenotypic identification of *E. coli* from faecal and manure samples

Of the 990 faecal and manure samples 314(31.7%) were positive for *E. coli* with 135(42.9%) of the positive samples being from Bauchi LGA while the remaining 49(15.7%) and 130(41.4%) positive faecal samples being from Darazo and Katagum respectively (Table-4). Similarly, of the 165 manure samples, *E. coli* was isolated from only 7(4.24%) manure samples with 1(14.3%) and 6(85.7%) from Bauchi and Katagum LGAs respectively (Table-4).

Table-4: Isolation of *E. coli* from faecal and manure samples

	F	aeces	Manure		
LGA	Sample size	No. positive (%)	Sample size	No. positive (%)	
Bauchi	330	135(42.9)	63	1(14.3)	
Darazo	330	49(15.6)	42	0(0.0)	
Katagum	330	130(41.4)	60	6(85.7)	
Total	990	314(31.72)	165	7(4.24)	

Isolation and phenotypic identification of *E. coli* from faecal samples by sex

Out of the 990 faecal samples collected from the study area (abattoirs, cattle farms and livestock markets), where 656 of the samples were found to be from male cattle while 334 were from female. Of the 656 samples from male cattle 219 (33.4%) were positive for *E. coli* which comprises of 34, 60 and 125

from abattoir, cattle farms and livestock market respectively, while out of the 334 samples from female cattle, 95(28.4%) were positive (out of which 17 were from abattoirs, 47 from cattle farms and 31 from livestock markets) (Table-5). The distribution of *E. coli* between male and female cattle was highly significant (χ^2 ; p=0.008).

Table-5: Distribution and occurrence (No. positive) of *E. coli* in cattle faeces by sex from the selected Local Government Areas of Bauchi State, Nigeria

	Aba	ttoir	Cattle	Farm	Livestocl		
	Male	Female	Male	Female	Male	Female	
LGA	No. positive	Total					
	(%)	(%)	(%)	(%)	(%)	(%)	
Bauchi	25(7.9)	8(2.5)	16(5.0)	26(8.3)	45(14.3)	15(4.8)	135(42.9)
Darazo	9(2.9)	8(2.5)	9(2.9)	15(4.8)	6(1.9)	2(0.64)	49(15.7)
Katagum	0(0)	1(0.32)	35(11.1)	6(1.9)	74(23.6)	14(4.4)	130(41.4)
Total	34(10.8)	17(5.41)	60(19.1)	47(15.0)	125(39.8)	31(9.84)	314(100%)

Isolation and phenotypic identification of E coli from faeces samples by age

On the basis of the age of animals out of the 990 faecal samples collected from the abattoirs, cattle farms and livestock markets in the study area, 871 of the samples were from adult cattle while the remaining 119 were from young cattle. Out of the 871 samples adult cattle where 271 (31.1%) were positive for *E. coli* and that out of these 271 samples 51 were from abattoirs, 86 from cattle farms and 134 from livestock markets (Table-6) while the remaining 119 samples were young cattle where 43(36.1%) were found to be positive (and results indicate no positive sample from

the abattoir, while 21 of the positives were from the cattle farms and 22 from livestock markets). (Table 6) The distribution of *E. coli* between male and female cattle was found to be highly significant (χ^2 ; p=0.008).

Isolation and phenotypic identification of *E. coli* from faecal samples by sampling sites

Of the 314 samples positive for *E. coli* from the abattoir, cattle farms and livestock markets in the study area, abattoirs had 51(16.2%) positive samples while cattle farms had 107(34.9%) positives and the remaining 156(49.6%) of the positive samples were from livestock markets (Table-6).

Abattoir Cattle Farm Livestock Market LGA Young Adult Young Adult Young Adult Total No. positive No. positive No. positive No. positive No. positive No. positive (%) (%) (%) (%) (%) (%) 60(19.1) Bauchi 0(0)33(10.5) 9(2.8) 33(10.5) 0(0)135(42.9) 0(0) 17(5.4) 18(5.7) 1(0.31) 7(2.22) Darazo 6(1.9) 49(15.5) Katagum 0(0)1(0.31) 6(1.9) 35(11.1) 21(6.7) 67(21.3) 130(41.6) 22(7.0) 51(16.2) 21(6.7) 86(27.4) 134(42.7) Total 0(0)314(100)

Table-6: Distribution and occurrence (No. positive) of *E. coli* in cattle faeces by age from selected Local Government Areas of Bauchi State, Nigeria

Biochemical characterization of isolated *E. coli* from faeces

Out of the 990 faecal samples collected 374 showed greenish metallic sheen on EMB and were considered presumptive for *E. coli*. They were further subjected to conventional biochemical test (IMViC). The result for the biochemical test indicated that 75(20.0%) were positive for the tests, and were considered for Microbact 12E biochemical test.

Biochemical tests using the Microbact 12E test kit for faecal samples

Out of the 314 *E. coli* isolates that were subjected to the biochemical test, 75(20.0%) showed positive result and they were further tested using the Microbact 12E biochemical test kits and the result confirmed 46(61.3%) to be positive as *E. coli* while 25(33.3%) were not *E. coli* but were identified as other gram negative microorganisms such as *klebsiella*, *citrobacter*, *serratia* etc and the *E. coli* were further subjected to serum agglutination test.

Serological characterization of $E.\ coli$ isolates from faeces

Out of the 46 isolates confirmed to be *E. coli* using Microbact 12E test kits, further characterization of the isolates using the *E. coli* O157 test kits revealed 27(58.7%) to be *E. coli* O157:H7 while the remaining 19(41.3%) to be *E. coli* non O157.

Isolation and phenotypic identification of E coli from manure samples

Culture and isolation and phenotypic characterization of isolates from cattle manure

Out of the 165 manure samples collected from all the three (3) LGAs, no isolation was made in samples from Darazo LGA while 1 (12%) and 6 (88%) positive samples were isolated in samples from Bauchi and Katagum LGAs respectively.

Biochemical characterization of $E\ coli$ isolates from cattle manure

Out of the 165 manure samples collected five 7(4.24%) showed greenish metallic sheen on EMB and were considered presumptive for *E. coli*, they were further subjected to conventional biochemical test (IMViC). The result for the biochemical test indicated 5(3.03%) showed positive for the tests, and were considered for Microbact 12E biochemical test

Biochemical test using the Microbact 12E test kit for manure samples

Out of the 5 \hat{E} . coli isolates that were subjected to biochemical test, 5(71.4%) showed positive result and they were further tested using the Microbact 12E biochemical test kits and the result confirmed 2(40.0%) to be positive as E. coli and were then subjected to serum agglutination test.

Serological characterization of $E.\ coli$ isolated from cattle manure

Out of the 2 isolates biochemically confirmed to be *E. coli* using the Microbact 12E test kits, further characterization of the isolates using the *E. coli* O157 test kits revealed all the 2(100%) to be *E. coli* O157:H7.

DISCUSSION

The study demonstrated that the differences in period of faecal and manure collection could have affected bacterial load in the samples. March to April is the end of dry season in the study area where there is less roughage moisture and so decreased chances for bacterial infection as cattle graze more on dry feeds. Also June to August is mid of the rainy season that could allow for greater faecal load, more moisture and greater chances for increased of load of bacterial organisms as reported in the work of [16] who stated that summer is a period of high prevalence of *E. coli*, and also as stated in the work of [17] who said animals may shed increasing loads of *E. coli* O157 from stress due to transportation to abattoirs for slaughter regardless of the season.

The research showed more faecal samples from male than female cattle animals, this is different with herd structure as reported [18] that stated no statistically significant difference (P<0.05) in the prevalence of E. coli O157:H7 between sex of animals. The result of the research indicated isolation of E. coli by sex as having more isolates from male faecal samples where feeding habits or the size of the rumen could be partly responsible to that. The result is however in conflict with that of Montenegro et al., [19] who reported 11.6% and 3.0% prevalence rates in cows and bulls using DNA hybridization technique, but with different serogroups from O157:H7. In terms of sex of animals, the result also showed no statistical significant difference (at P<0.05) in the occurrence of E. coli in

cattle faeces from Darazo LGA where male 42(86%) was found higher than female 7(14%), and that even among the male animals the occurrence was found to be slightly different between samples collected from the CF 18(37%) and samples obtained from LM 7(14%). This could probably be attributed to the large population of male than female animals presented to the sample locations as reported earlier by Biruhfesta *et al.*, [18] in a similar report with statistically significant difference ($p \le 0.05$) in occurrence of *E. coli* O157:H7 between age groups of the animals.

Similarly, the result is related to the prevalence as indicated in a study [11] in Kaduna who reported prevalence of 2.1% in male and 1% female while the relationship between sex and serogroups revealed (8.4%) male, and (3.5%) female. However, % faecal load of E. coli of 31.71% is a threat to the environment and to livestock management since livestock production in the state is largely extensive. The problem would be more severe where the management system is intensive or semi-intensive as reported [20] that suggested that environmental factors are important in E. coli O157 shedding in cattle. The fact that only 7(4.24%) of the manure samples were positive for E. coli could be due to the inadequate moisture and possibly nutrients for the organism to survive in the manure or due to inappropriate handling of the cattle manure which was found to be an important reason to control and spread of E. coli in manure as reported in a research [21].

In view of isolation of $E.\ coli$ by age the study indicated greater presence of $E.\ coli$ in adults, probably because as the adults are more matured they will therefore have more feed consumption per day and as such an opportunity to take in more of the organisms in the contaminated pasture than the young ones. The finding is similar to the findings of [22] who found an apparently cyclic pattern of $E.\ coli$ shedding where faecal prevalence was 25% from 1-7 weeks of age, decline to 0% after animals were turned out to pasture and increased to 6-14% by 2 weeks post weaning.

The result further indicated that out of the total faecal samples collected from the three locations E. coli isolation was highest in adults 103(79%) cattle than in younger 27(21%) animals, and it also indicated that even within the adults, more positive samples were obtained from animals presented to the LM 67(51%) than the least number obtained from an abattoir 1(0.76%) no statistical significant difference (P<0.05). However, with regard to sampling sites and age of animals Lawal et al., [23] reported a result contrary to the findings of this research work who stated that 1.37% of STEC in effluent from the slaughter house spills in Nigeria and that young animals were the largest shedders of E. coli, because in Africa cattle encountered at the cattle markets and abattoirs are mostly adults even though he highlighted that differences could be

due to sampling method used for isolation of the organism.

For isolation and occurrence of E. coli from sample locations indicated highest isolation in cattle faeces from LM 88(67.6%) and the least being isolation from the abattoir 1(0.76%) although no statistical significant difference (P<0.05). With regard to the distribution of E. coli in faeces of animals based on sex and according to samples location, more positive isolates were found from the LM 156(49.6%), even though confirmed that the abattoir and livestock markets did not have any positive result for faecal samples of young animals. However, the result is in conflict with [24] in Gyeonggi province in Korea with low prevalence of 12.7% of STEC isolated from cattle farm samples, including faeces, ground soil and water. Also a report by [25] indicates no difference between the prevalence of E. coli in samples from organic (abattoirs) and conventional cattle farms.

The results of the biochemical test of the research indicated 75(23.9%) of the 314 isolates characterized as *E. coli* by Biochemical tests, indicates that the risk of pasture and environment is still high. Also the result of the biochemical test (Microbact 12E) of the research indicated 46(61.3%) of the 75 isolates positive for *E. coli* still indicated a risk potential for the animals and even anybody who had to be associated with faeces like the herdsmen, butchers, livestock traders and veterinary professionals as they have to interact with faeces in one way or the other.

The finding of some of the isolates to be E. coli O157 strain further increases the risk the more as there are cases enteric (typhoid) fever once in a while along with diarrhea that is poorly diagnosed in our hospitals, this can be attributed to the fact that E. coli O157:H7 cells survived for up to 77, more than 226 and 231 days in manure-amended autoclaved soil held at 5, 15 and 21°C respectively as reported by [26]. The problem is more glaring in remote villages of Nigeria where there are no medical facilities for handling cases. The same could be said for livestock as laboratory facilities for confirming diseases are not available. And the above may be as a result of animals faeces carried by rain and moves to rivers and streams which serve as common sources of drinking water in semi-urban and rural areas thereby increasing the risk of human infections as stated in a research by [27] that E. coli, have been recovered both from children with diarrhea and from children without diarrhea, though to a lower extent from the healthy children where all diarrheagenic E. coli strains were associated with diarrhea 0.02).

The result of the research indicated that isolates from manure that were confirmed to be *E. coli* (both O157:H7 and non O157) have risk to contact individuals as pointed out earlier for faeces. They have

increased risk as crop farmers are further exposed, also there is a tendency that rain water could take the organism from the crop farms to the streams which could be used as a source of drinking water as stated in [28] that the manure storage facilities must be located well outside of any stream floodplain, and should have a slight slope for drainage, and should not slope so much that runoff can cause problems.

CONCLUSION AND RECOMMENDATION

The study concluded that differences in period of sampling could have effect on bacterial load observed from the samples, and that *E. coli* were isolated in 314(31.72%) out of 990 faecal samples and in 7(4.24%) out of 165 manure samples and that 75(20.0%) of the 314 isolates from faecal samples were demonstrated to be *E. coli* by biochemical tests and the use of Microbact 12E biochemical test kits confirmed 46(61.3%) of the 75 isolates to be *E. coli* while remaining 25(33.3%) were not confirmed as *E. coli*. The *E. coli* were seen to be more in male cattle 219(69.74%) than in female cattle 95(30.25%) and that the organism was isolated more in adult cattle 271(86.31%) than in young ones 43(13.70%).

This kind of researches should be fully funded by government or non-governmental organizations for better results in order to utilize the findings from the research with a possibility of preventing risk of disease outbreaks. Results or findings obtained from this kind of research should be taken into considerations in order to safeguard the health of the public

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