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Occurrence of *Escherichia coli* and *Escherichia Coli* O157:H7 in cattle, farm environment, milk and beef

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ABSTRACT

Aims: The aim of this study was to determine the occurrence of *Escherichia coli* and *E. coli* O157:H7 in the cattle, farm environment beef and milk samples.
Methodology and results: A total of 192 samples were collected from cattle (rectal faeces) (96) and their farm environment (96) including feed, floor of stall swabs, pooled flies and water samples and 30 milk samples from dairy cattle. A total of seven markets were selected to collect 60 beef samples. The presence of *E. coli* and *E. coli* O157:H7 was determined using culture method and confirmed using PCR assay. The overall occurrence rate of *E. coli* in the farm was 49%. In milk, the occurrence of *E. coli* was 33.3% and in beef was 10%. The isolated *E. coli* were then screened for *E. coli* O157 using latex agglutination test (Oxoid) and 34.2% were found positive to the test. The identified *E. coli* O157 isolates were then subjected to multiplex PCR with five primer pairs. They were all negative except for 3.6% of the *E. coli* O157 (10.5%) isolates which were positive for the presence of *fli*CH7 genes.
Conclusion, significance and impact of study: This study showed the absence of *E. coli* O157:H7 in the cattle, farm environment, milk and beef. This study may provide a base for conducting a broader spectrum study by having more risk factors included in the study of *E. coli* in cattle in Malaysia. Further detailed studies may provide a platform to control emergence and distribution of pathogenic bacteria and ensure good control and surveillance strategies and policies.

Keywords: *E. coli* O157, cattle, environment, milk, beef

INTRODUCTION

Food borne illness is an unavoidable and is of great public health concern worldwide due to consumption of contaminated raw and uncooked food (Jianghong Meng, 2007). Most pathogens cause infection while a number produce toxins inside the host after ingestion which may lead to mild to severe diarrhea and even death in severe infection. There are two types of *E. coli*, pathogenic and non-pathogenic *E. coli.* The non-pathogenic strains of *E. coli* are present in the normal intestinal microflora which are harmless, hinder the growth of harmful bacteria, produce vitamins and are described as commensal *E. coli* (Nataro and Kaper, 1998; Beauchamp and Sofos, 2010). Based on the pathogenicity of the disease and presence of virulence factors, at least seven pathotypes of pathogenic *E. coli* also known as diarrheagenic *E. coli* have been identified and studied, namely, enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC); including *Shigella*, enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) (Beauchamp and Sofos, 2010; Jafari, *et al*., 2012; Allocati *et al*., 2013) and the recently emerged, adherent invasive *E. coli* (AIEC) (Allocati *et al*., 2013; Martinez-Medina and Garcia-Gil, 2014).

Of all diarrheagenic *E. coli* identified, Shiga-toxin or Vero toxin producing (STEC/VTEC) *E. coli* is the most significant pathotype in human diseases (Wani *et al*., 2003). There are many serotypes in STEC and among them, the EHEC serotype O157:H7 are found to be highly virulent, responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) worldwide. Ruminants are found to be the natural reservoir hosts for these organisms (Nataro & Kaper, 1998). STEC was first recognized as a human pathogen in 1982 (Riley *et al*., 1983). In animals, STEC is found in the intestines of newborn animals. However the healthy adult animals act as subclinical carriers for STEC and considered as the common source of O157 serotype in humans worldwide (Wani *et al*., 2003).

For identification of different pathotypes of *E. coli*, polymerase chain reaction (PCR) method is used and is based on amplification of specific virulent genes (Nataro and Kaper, 1998). Watterworth *et al.* (2005) designed a multiplex PCR assay by using six sets of primers to identify four different pathotypes. Chang *et al*. (2013)

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designed a duplex PCR assay by using two sets of primers for the detection of *E. coli* O157:H7.

In Malaysia, limited studies were reported on the occurrence of pathogenic *E. coli* in cattle, farm environment, milk and beef. The presence of *E. coli* O157:H7 may possibly be due to a number of factors such as farm husbandry practices and environment differences. Therefore, the aim of this study was to determine the occurrence of *E. coli* O157:H7 in cattle, the farm environment, milk and beef.

MATERIALS AND METHODS

Sample collection

A total of eight cattle farms were visited. Faecal samples from twelve cattle in each farm were directly collected from the rectum using sterile gloves. Three of each type of environmental samples were collected from all farms which comprised feed, pooled flies of five flies per pooled sample, swabs of floor of stalls and water. Six milk samples each from five dairy cattle farms were directly collected from the teats of each dairy cattle. Sixty beef samples were purchased from seven wet markets. Each of the sample collected was kept in individual sterile plastic bag and put in a cool box with ice packs. The samples were transported to the Veterinary Public Health Laboratory, UPM and processed within 3 h following collection.

Treatment of samples

All of the samples except milk samples were individually placed in buffered peptone water (BPW) (Oxoid) and incubated at 37 °C aerobically for 24 h while each of the milk samples was placed in trypton soy broth (TSB) (Oxoid) and incubated as above. The culture of water samples was according to Wong *et al*. (2007). Each 100 mL of water sample was filtered using a sterile cellulose nitrate membrane filter of 47 mm diameter with 0.45 µm pore-size (Milipore, Sartorius Stedim, Biotech, Goettingen. Germany). Then the membrane filter was put in a sterile bottle containing 100 mL BPW (Oxoid) and incubated at 37 °C aerobically for 24 h. For beef samples, novobiocin (20*u*g/mL) was added to TSB (Oxoid) in sterile stomacher bags and homogenized for 1.5 min in a stomacher. The homogenate was then incubated for 6 h at 37 °C, then 1 mL of this incubated mixture was added to 10 mL TSB (Oxoid) and incubated for 2 h at 37 °C.

Immunomagnetic separation

Each of the incubated cultures was subjected to immunomagnetic separation (IMS) with Dynabeads® anti-*E. coli* O157 according to the manufacturer's instructions (Dynal, Oslo, Norway). For qualitative analysis of *E. coli* O157, direct plating is frequently practised and the use of immunomagnetic separation (IMS) technique is reported to improve their isolation because the IMS technique performed more sensitive detection of specific microorganism in comparison with direct plating (Šafařiková and Šafařik, 2001). The beads were washed according to Cooley *et al*. (2007).

Isolation and identification

The bead bacteria complex obtained from IMS of each faeces, feed, flies, floor of stall swabs, water, milk and beef samples was inoculated on Sorbitol MacConkey agar (Oxoid-Thermo) supplemented with Cefixime Tollurite (CT-SMAC) to obtain colourless non-sorbitol fermenting colonies. The same beads obtained from IMS were also inoculated on CHROMagar O157 to obtain pink/mauve colour colonies due to the presence of chromogenic substrates in the medium (CHROMagar™) and both were incubated aerobically at 37 °C for 18-24 h. These presumptive colonies were picked and sub-cultured onto nutrient agar (Oxoid) and incubated at 37 °C for 24 h (Khanjar and Alwan, 2014). A total of 5 typical colonies based on manufacturer's instruction were obtained from Sorbitol MacConkey agar (Oxoid-Thermo) supplemented with Cefixime Tollurite (CT-SMAC) and CHROMagar O157 for biochemical tests. The presumptive colonies were subjected to gram staining and biochemical tests which were indole, methyl red, Voges-Proskauer and citrate utilization tests which are common tests for the identification of *E. coli* (Quinn *et al*., 2002). Out of 5 selected colonies, even if a single colony appeared positive then the sample was considered positive. The *E. coli* isolates were kept on nutrient agar slants (Oxoid) at room temperature until used.

Detection of *E. coli* O157 isolates by latex agglutination test

Latex agglutination test (DrySpot *E. coli* O157; Oxoid) was used for identification of *E. coli* O157 serogroup among presumptive *E. coli* isolates. The blue dried particles on the test cards are representing reactive (positive) and non-reactive (negative) antibodies. The identification of *E. coli* O157 is presumed positive when agglutination appears in the reactive blue particles and no agglutination appears in the non-reactive particles on the test cards when *E. coli* isolates are mixed with the particles. If no agglutination appears on the test and control blue particles then the test for *E. coli* O157 is considered negative.

Polymerase chain reaction (PCR) assay

Conventional boiling method was used for the DNA extraction of the isolates using sterile distilled water. A loop full of colonies was taken and added to 1000 µL sterile distilled water in a 1.5 mL Eppendorf tube. The suspension was incubated at 94 °C for 10-15 min in dry water bath and allowed to cool down to room temperature. The suspension was then centrifuged for 3 min at 13,000 rpm. The supernatant from each centrifuge tube was collected in a new 1.5 mL Eppendorf tube. The

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supernatant was used as template DNA and the pellets were discarded.

The DNA was amplified in a total 50 µL reaction volume comprised 25 µL of Master Mix (BIOLINE), 5 µL (10 mM) of primer set as described by Watterworth *et al*. (2005) and Chang *et al*. (2013), using the internal control targeted genes *st*, *lt*, *Ial*, *rfb*O157 and *fli*CH7 (Table 1), 4 µL of template DNA and 16 µL of deionized distilled water. The total volume of the mixture was 50 µL which was adjusted with deionized distilled water. The final reaction mixture was then amplified in DNA thermal cycler (Eppendorf) with the following m-PCR protocols: an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, ending with a final extension at 72˚C for 10 min.

The PCR reaction mixture was visualized by gel electrophoresis through 2% (w/v) (Promega, USA) agarose (which contained Tris-Borate-EDTA (TBE) solution (89 mM Tris Base, 89 mM Boric acid, 2 mM EDTA, pH 8.3) in 0.5 × TBE buffer solution at 80 volts for 70 min. The gel was then stained with ethidium bromide for 10 min followed by de-staining with distilled water for 30 min. The gel was then viewed under UV transilluminator by the aid of Alpha Imager (Bio-Rad). A DNA-molecular ladder (100-bp ladder) (Vivantis Technologies) was included in each gel.

RESULTS AND DISCUSSION

Escherichia coli is said to be highly adaptable to various types of environments and colonize wide range of mammals as well as birds (Beauchamp and Sofos, 2010). Due to their invasiveness in penetrating the mucosal surfaces some strains of *E. coli* are considered to be pathogenic strains, as they can cause a variety of illnesses in humans (Nataro and Kaper, 1998; Beauchamp and Sofos, 2010; Croxen and Finlay, 2010).

In this study, a total of 192 samples which consisted of 96 faeces, 24 feeds, 24 pooled flies, 24 floor of stall swabs and 24 water samples were collected from eight dairy and beef cattle farms. Based on biochemical tests, 49% (95/192) were positive for *E. coli*. The occurrence of *E. coli* in cattle and farm environment in each cattle farm is presented in Table 2.

In the present study, *E. coli* were found in faeces (cattle) at 55% which was similar to a study by Awadallah *et al*. (2013) in Egypt at 48%. These findings were also similar to a study by Hossain *et al*. (2014) in Bangladesh in which 49% *E. coli* were observed among faecal samples of calves. The works of Fluckey *et al*., (2007) on faeces (cattle) at abattoirs in United States found *E. coli* ranged from 98 to 55%. High prevalence was also observed in a study by Mainda *et al*. (2015) among faeces (cattle) in Zambia at 98.7%.

Previous studies had reported several risk factors associated with the colonization of *E. coli* in cattle including feed, manure, flies, water, direct contact with the infected animal and animal wastes, which contributed to the occurrence and re-occurrence of infection and contamination of the animals and farm with *E. coli* (Jiang *et al*., 2002; Dodd *et al*., 2003). Contaminated water can also serve as an important mode of spread of *E. coli* within a farm (Jiang *et al*., 2002). In the present study, a total of 43.7% farm environmental samples, including 41.6% of water samples and 62.5% of the floor of stalls were contaminated with *E. coli*. Almost similar results were reported in a study by Adzitey *et al*. (2010), in which occurrence of *E. coli* in soil was 72% and in wash water was 50%. A total of 37.5% of *E. coli* were isolated from feed samples in this study while in that of Chowdhuri *et al*. (2011) in Bangladesh, 57.1% *E. coli* was found in feed samples. A total of 33.3% of the flies' samples collected at the cattle farms carried *E. coli* which can spread easily from or to the environment. Other factors that have been reported to be sources of *E. coli* included farm equipment, utensils, containers, carts and workers.

Table 1: Primers used to detect pathogenic *E. coli*.

Target/ *E. coli* Type	Primer	Sequence (5' to 3')	Target gene	Size of PCR amplicon (bp)	Reference
ETEC	*st*-F *st* -R	TCTTTCCCCTCTTTTAGTCAGTC CCAGCACAGGCAGGATTAC	*st*	170	Watterworth *et al*. (2005)
ETEC	*lt*-F *lt*-R	TCTCTATGTGCACACGGAGC CCATACTGATTGCCGCAAT	*lt*	322	Watterworth *et al*. (2005)
EIEC	*Ial*-F *Ial*-R	TTTCTGGATGGTATGGTGAGG CACGCTGGTTGTCAATAATGCT	*Ial*	390	Watterworth *et al*. (2005)
EHEC/ *E. coli* O157	*rfb*O157-F *rfb*O157-R	CGGACATCCATGTGATATGG TTGCCTATGTACAGCTAATCC	*rfb*O157	259	Chang *et al*. (2013)
EHEC/ H7	*fli*CH7-F *fli*CH7-R	GCGCTGTCGAGTTCTATCGAG CAACGGTGACTTTATCGCCATTCC	*fliCH7*	625	Chang *et al*. (2013)

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Table 2: Proportion of *E. coli* isolates in cattle and farm environment.

Farms	Types of samples				
	Faeces (cattle)	Feed	Floor of Stall	Flies	Water
Farm 1	0/12 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)
Farm 2	5/12 (41.7%)	1/3 (33.34%)	2/3 (66.67%)	2/3 (66.67%)	1/3 (33.34%)
Farm 3	11/12 (91.7%)	1/3 (33.34%)	2/3 (66.67%)	1/3 (33.34%)	3/3 (100%)
Farm 4	11/12 (91.7%)	0/3 (0%)	3/3 (100%)	0/3 (0%)	2/3 (66.67%)
Farm 5	6/12 (50%)	3/3 (100%)	2/3 (66.67%)	0/3 (0%)	1/3 (33.34%)
Farm 6	8/12 (66.7%)	2/3 (66.67%)	2/3 (66.67%)	2/3 (66.67%)	0/3 (0%)
Farm 7	6/12 (50%)	2/3 (66.67%)	1/3 (33.34%)	0/3 (0%)	2/3 (66.67%)
Farm 8	6/12 (50%)	0/3 (0%)	3/3 (100%)	3/3 (100%)	1/3 (33.34%)
Total	53/96 (55.2%)	9/24 (37.5%)	15/24 (62.5%)	8/24 (33.3%)	10/24 (41.6%)

The highest prevalence of *E. coli* was observed in Farm 3 at 75% (18/24). During my visit to the farms, in comparison to other farms, Farm 3 was comparatively less clean, faeces were not regularly removed from the floor of stall, cattle were not regularly cleaned, personal hygiene of farm workers were also compromised and garbage heaps were observed nearby the farm. The above reasons may have contributed in high prevalence of *E. coli* in Farm 3.

Escherichia coli may be frequently found on raw food due to contamination by faeces, water and flies; of public health concern is the pathogenic *E. coli* in food (Costa *et al*., 2008). *E. coli* were isolated from 33.3% of 30 milk samples collected from cattle in five dairy cattle farms and ranged from 0% in two farms, 33.3%, 50% and 83.3% in three farms. Almost similar prevalence (31.6%) was observed among raw milk samples in a study by Nanu *et al*. (2007) in India while higher prevalence of *E. coli* among raw milk samples was observed in a study by Chye *et al*. (2004) in Malaysia at 65%. The factors involved in contamination of milk with *E. coli* may include the use of contaminated utensils, use of contaminated water for cleaning udder, contamination of milk with faeces, improper storage conditions, unhygienic milking equipments and dirty hands (Bagré *et al*., 2014).

Escherichia coli and other species in the *Enterobacteriaceae* group are of public health concern in the production of raw and processed meat worldwide (Adetunji & Odetokun, 2011). During slaughtering and dressing including evisceration, the carcass can be contaminated with *E. coli* through inappropriate and unhygienic practices, contaminated water, equipment, processing and storage environments at the abattoirs (Karch *et al*., 2005). In the present study, 10% of beef sampled were contaminated with *E. coli*. The rate of contamination on beef ranged from 0% in four markets, 5.5%, 25% and 33.3% in three markets. Similar results were observed in a study by Hajian *et al*. (2011) in cattle beef in Iran who reported *E. coli* at 8.9 % while Tanih *et al*. (2015) in South Africa found 67.5% of beef in abattoirs were contaminated with *E. coli*. The significant risk factors involved in contamination of beef in the market with *E. coli* included contaminated carcasses in the abattoirs, carcasses contaminated during handling and transport, contaminated clothing and hands of personnel and the physical facilities including knives, cutting boards, tables and water used for cleaning and washing at the markets (Mohammed *et al*., 2014).

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Latex agglutination test for identification of *E. coli* O157

Among the *E. coli* isolates which were subjected to latex agglutination test for identification of *E. coli* O157, 38 of *E. coli* isolated in faeces, feed, floor of stall, flies, water and milk were positive for presumptive *E. coli* O157 while none of the *E. coli* isolates from beef were positive. The occurrence of *E. coli* O157 in cattle faeces, farm environment, milk and beef was 34.2% (Table 3). Almost similar results were reported in a study by Omisakin *et al*. (2003) in faecal samples from the abattoirs in United Kingdom at 40.4% while comparatively low prevalence at 0.68% *E. coli* O157 was recorded in a study by Onwumere (2010) on floors of slaughterhouse, slabs, meat and water samples in Nigeria. Seasonal variation in the prevalence of *E. coli* O157 ranged from 4.8% during winter and 38% in spring was previously reported (Chapman *et al*., 1997; Heuvelink *et al*., 1998a; Gansheroff and O'Brien, 2000). These differences may be influenced by geographical location, season, stressful environmental condition, diet, and population density (Clarke *et al*., 1989; Kudva *et al*., 1996). A prevalence of 25% *E. coli* O157 in feed samples was observed in beef cattle in a study by Sanderson *et al*. (2006). In contrast to the current study very low level of prevalence of *E. coli* O157 was observed in feed samples from feed bunks reported by Van Donkersgoed *et al*. (2001) at 1.7%, in barley feed for cattle at 2.4% and corn feed for cattle at 1.3% in a study by Berg *et al*. (2004) in Canada. Hancock *et al*. (1998) reported 3.1% *E. coli* O157 were isolated from feedlots and in dairy farms in United States. In another study by LeJeune *et al*. (2001) 1.3% *E. coli* O157 were isolated from water troughs in dairy cattle operations. Among beef samples, Beutin (1999) in Germany, observed 0.8% *E. coli* O157 while 0.7% was observed by Tutenel *et al*. (2002) in Poland.

Detection of *E. coli* O157:H7 by PCR assay

All the *E. coli* isolates which were subjected to PCR for the detection of *rfb*O157 (EHEC) genes were negative; however, 3.6% among all *E. coli* isolates and 10.5% among presumptive *E. coli* O157 isolates showed the presence of *fli*CH7 (H7) genes at 625 bp (Figure 1). When the *E. coli* isolates were subjected to m-PCR for the detection of other pathogenic *E. coli* by observing the presence of *st* and *lt* (ETEC) and *Ial* (EIEC) genes, they were found negative. Almost similar observations were made by Apun *et al*. (2011), in which prevalence of *E. coli* was observed in rodents (43%), birds (18%) and bats (11%), however *fli*CH7 gene was detected in 23 (3.37%) while none of the isolate carry *slt-I*, *slt-II, rfbE* genes. Thus it was suggested that bats, birds and rodents do not serve as a reservoir for *E. coli* O157:H7 in Sarawak Malaysia. The cattle, farm environment, milk and beef sampled were negative for pathogenic *E. coli* and in particular *E. coli* O157. However, the four isolates positive for *fli*CH7 genes could possibly belong to other *E. coli* serotypes (such as O26, O103 and O111) which may have the potential to cause diseases. The big difference in PCR and latex agglutination test results is due to the results of latex agglutination tests being interpreted positive as per the manufacturer instructions which includes the positive results as appearance of granules only in test reagent and more granules appearance in control reagent in comparison with test reagent while if the granules appeared only in control reagent then the results would be considered uninterpreted. According to Borczyk *et al*. (1987,1990), cross reaction may occur in latex test due to the shared antigen of certain strains of *Escherichia hermanii* and *E. coli* O157.

The differences in the occurrence of *E. coli* in beef in this study to others may be due to different samples, isolation techniques, geographical locations or due to the processing and retailing conditions (Padhye and Doyle, 1992; Heuvelink *et al*., 1998b; Zhao *et al*., 2001). From this study, the occurrence of *E. coli* in the beef in wet markets were different among markets may be because the hygienic measures practiced in the wet markets were different.

The presence of birds and flies in and around the farms, the use of contaminated water and inappropriate practices of the workers in the farm may contribute in active transmission of the pathogens (Hancock *et al*., 1998; Coia *el al*., 2001; Scott *et al*., 2003; Wasteson *et al*., 2005).

The use of contaminated water for cleaning udder, contamination of milk with faeces, contaminated milking equipments and utensils and dirty milking hands may contribute in transmission of the organism in the milk (Chye *et al*., 2004).

Table 3: Proportion of *E. coli* O157 among *E. coli* isolates.

Sample Type	Total number of *E. coli* isolates	Latex agglutination positive for *E. coli* O157	Occurrence of *E. coli* O157 (%)
Faeces/Cattle	53	23	43.3%
Feed	9	2	22.2%
Floor of stall	15	6	40%
Flies	8	1	12.5%
Water	10	4	40%
Milk	10	2	20%
Beef	6	0	0%
Total	111	38	34.2%

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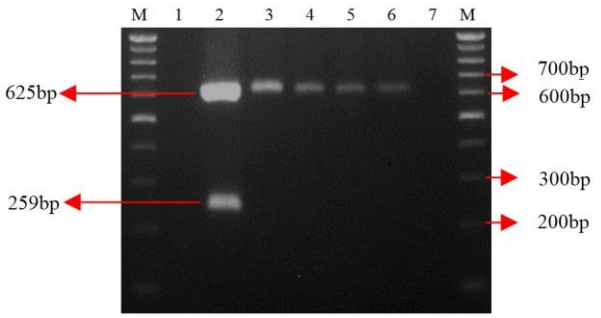

Lane M: marker 100 bp ladder, Lane 1: negative control, Lane 2: positive control (*rfb*O157 & *fli*CH7), Lane 3 to 6: H7 positive, Lane 7: negative control, Lane M: marker 100 bp ladder.

Figure 1: Representative PCR assay for detection of somatic *E. coli* O157 and H7 flagella of *E. coli* isolates.

Beef could have become contaminated with *E. coli* in the intestinal content, carcass in contact with faeces, contaminated water and equipment used during dressing (McEvoy *et al*., 2003). Beef may also become contaminated because of improper handling and cutting at the markets (Zhao *et al*., 2001). Poor or unhygienic measures in markets and equipment comprises protective equipment, workers hygiene, lack of using gloves, hygiene measures or cleanliness of stalls and display counters, contaminated storage, containers, transporting facilities and chilling facilities (Elder *et al*., 2000, Adesiji *et al*., 2011). Raw beef is highly contaminated with *E. coli* may indicate poor hygienic measures but may possibly carry other zoonotic pathogens.

CONCLUSION

There is a high occurrence of *E. coli* in cattle and farm environment. Beef in the markets and milk from cows were also highly contaminated. The high occurrence of *E. coli* in the faeces may contaminate environment as well may cross-contaminate other animals in the farms. Farm management practice, market and stall conditions, environmental factors and workers personal hygiene plays an important role in microbial contaminations. It is shown in this study that pathogenic *E. coli* were not present in the cattle and farms as well on beef in the markets that were sampled. This study may serve as a template to investigate the role of farm and environmental factors in contamination of *E. coli* and other microbes relevant to food safety.

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REFERENCES

Adetunji, V. O. and Odetokun, I. A. (2011). Bacterial hazards and critical control points in goat processing at a typical tropical abattoir in Ibadan, Nigeria. *International Journal of Animal and Veterinary Advances* **3, 349-354.**

Adesiji, Y. O., Alli, O. T., Adekanle, M. A. and Jolayemi, J. B. (2011). Prevalence of *Arcobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone Journal of Biomedical Research* **3(1), 8-12.**

Adzitey, F., Huda, N. and Rusul, G. (2010). Prevalence of *Escherichia coli* in duck intestines, faeces, soil and Wash Water samples in Penang, Malaysia. *Proceedings of the 4th International Conference Postgraduate Education: Globalisation and Liberalisation of Postgraduate Education, Nov*. **pp. 26-28.**

Allocati, N., Masulli, M., Alexeyev, M. F., and Di Ilio, C. (2013). *Escherichia coli* in Europe: An overview. *International Journal of Environmental Research and Public Health* **10(12), 6235-6254.**

Apun, K., Kho, K., Chong, Y., Hashimatul, F., Abdullah, M., Rahman, M. and Samuel, L. (2011). Detection of *Escherichia coli* O157: H7 in wildlife from disturbed habitats in Sarawak, *Malaysia. Research Journal of Microbiology* **6(2), 132.**

Awadallah, M. A., Merwad, A. M. and Mohamed, R. E. (2013). Prevalence of zoonotic *Escherichia coli* and *Salmonella* in wild birds and humans in Egypt with emphasis on RAPD-PCR fingerprinting of *E. coli*. *Global Veterinaria* **11, 781-788.**

Bagré, T. S., Kagambèga, A., Bawa, H. I., Tchamba, G. B., Dembélé, R. E., Zongo, C., Savadogo, A., Aggad, H., Traoré, A. S. and Barro, N. (2014). Antibiotic susceptibility of *Escherichia coli* and *Salmonella* strains isolated from raw and curds milk consumed in Ouagadougou and Ziniar, Burkina Faso. *African Journal of Microbiology Research* **8(10), 1012-1016.**

Beauchamp, C. S. and Sofos, J. N. (2010). Diarrheagenic *Escherichia coli*. *In:* Pathogens and Toxins in Foods. American Society of Microbiology Press. Washington DC. **pp. 71-94**.

Berg, J., McAllister, T., Bach, S., Stilborn, R., Hancock, D. and LeJeune, J. (2004). *Escherichia coli* O157: H7 excretion by commercial feedlot cattle fed either barley-or corn-based finishing diets. *Journal of Food Protection*, **67(4), 666-671.**

Beutin, L. (1999). *Escherichia coli* O157 and other types of verocytotoxigenic *E. coli* (VTEC) isolated from humans, animals and food in Germany. CABI Publishing Wallingford. United Kingdom. **pp. 121-145.**

Borczyk, A. A., N. Harnett, M. Lombos, and H. Lior. (1990). False positive identification of *Escherichia coli* 0157 by commercial latex agglutination test. *Lancet* **336, 946-947.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Borczyk, A. A., Lior, H., and Ciebin, B. (1987). False positive identifications of *Escherichia coli* 0157 in foods. *International Journal of Food Microbiology* **4(4), 347-349.**

Chang, W. S., Afsah-Hejri, L., Rukayadi, Y., Khatib, A., Lye, Y. L., Loo, Y. Y., Shahril, N. M., Puspanadan, S., Kuan, C. H., Goh, S. G. and John, Y. H. T. (2013). Quantification of *Escherichia coli* O157: H7 in organic vegetables and chickens. *International Food Research Journal* **20(2), 1023-1029.**

Chapman, P., Siddons, C., Malo, A. C. and Harkin, M. (1997). A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiology and Infection* **119(02), 245-250.**

Chowdhuri, A., Iqbal, A., Giasuddin, M. and Bhuiyan, A. (2011). Study on isolation and identification of *Salmonella* and *Escherichia coli* from different poultry feeds of Savar region of Dhaka, Bangladesh. *Journal of Scientific Research* **3(2), 403-411.**

Chye, F. Y., Abdullah, A., and Ayob, M. K. (2004). Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiology* **21(5), 535-541.**

Clarke, R., McEwen, S., Gannon, V., Lior, H., and Gyles, C. (1989). Isolation of verocytotoxin-producing *Escherichia coli* from milk filters in south-western Ontario. *Epidemiology and Infection* **102(02), 253-260.**

Coia, J. E., Johnston, Y., Steers, N. J., and Hanson, M. F. (2001). A survey of the prevalence of *Escherichia coli* O157 in raw meats, raw cow's milk and raw-milk cheeses in south-east Scotland. *International Journal of Food Microbiology* **66(1), 63-69.**

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M. T., Myers, C., Rose, C., Keys, C., Farrar, J. and Mandrell, R. E. (2007). Incidence and tracking of *Escherichia coli* O157: H7 in a major produce production region in California. *PloS One* **2(11), e1159.**

Costa, D., Poeta, P., Sáenz, Y., Coelho, A.C., Matos, M., Vinué, L., Rodrigues, J. and Torres, C. (2008). Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. *Veterinary Microbiology* **127(1), 97-105.**

Croxen, M. A., and Finlay, B. B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews Microbiology* **8(1), 26-38.**

Dodd, C. C., Sanderson, M. W., Sargeant, J. M., Nagaraja, T., Oberst, R. D., Smith, R. A. and Griffin, D. D. (2003). Prevalence of *Escherichia coli* O157 in cattle feeds in Midwestern feedlots. *Applied and Environmental Microbiology* **69(9), 5243-5247.**

Elder, R. O., Keen, J. E., Siragusa, G. R., Barkocy-Gallagher, G. A., Koohmaraie, M., and Laegreid, W. W. (2000). Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proceedings of the National Academy of Sciences* **97(7), 2999-3003.**

Fluckey, W. M., Loneragan, G. H., Warner, R. and Brashears, M. M. (2007). Antimicrobial drug resistance of *Salmonella* and *Escherichia coli* isolates from cattle feces, hides, and carcasses. *Journal of Food Protection* **70(3), 551-556.**

Gansheroff, L. J., and O'Brien, A. D. (2000). *Escherichia coli* O157: H7 in beef cattle presented for slaughter in the US: higher prevalence rates than previously estimated. Proceedings of the National Academy of Sciences. **pp. 2959-2961.**

Hajian, S., Rahimi, E., and Mommtaz, H. (2011). A 3-year study of *Escherichia coli* O157: H7 in cattle, camel, sheep, goat, chicken and beef minced meat. *Proceedings of the International Conference on Food Engineering and Biotechnology*. **(IPCBEE'11).**

Hancock, D. D., Besser, T. E., Rice, D. H., Ebel, E. D., Herriott, D. E., and Carpenter, L. V. (1998). Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. *Preventive Veterinary Medicine* **35(1), 11-19.**

Heuvelink, A.E., Van Den Biggelaar, F.L.A.M., Zwartkruis-Nahuis, J.T.M., Herbes, R.G., Huyben, R., Nagelkerke, N., Melchers, W.J.G., Monnens, L.A.H. and De Boer, E. (1998a). Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. *Journal of Clinical Microbiology* **36(12), 3480-3487.**

Heuvelink, A. E., Van Den Biggelaar, F. L. A. M., De Boer, E., Herbes, R. G., Melchers, W. J. G., Huis, J. H. J., and Monnens, L. A. H. (1998b). Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. *Journal of Clinical Microbiology* **36(4), 878-882.**

Hossain, M., Rahman, M., Nahar, A., Khair, A., and Alam, M. (2014). Isolation and identification of diarrheagenic *Escherichia coli* causing colibacillosis in calf in selective areas of Bangladesh. *Bangladesh Journal of Veterinary Medicine* **11(2), 145-149.**

Jafari, A., Aslani, M. and Bouzari, S. (2012). *Escherichia coli*: A brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. *Iranian Journal of Microbiology* **4(3), 102-117.**

Jiang, X., Morgan, J. and Doyle, M. P. (2002). Fate of *Escherichia coli* O157: H7 in manure-amended soil. *Applied and Environmental Microbiology* **68(5), 2605-2609.**

Jianghong Meng, C. M. S. (2007). *Escherichia coli*. *In:* Foodborne diseases. Humana Press. **pp. 1-25).**

Karch, H., Tarr, P. I., and Bielaszewska, M. (2005). Enterohaemorrhagic *Escherichia coli* in human medicine. *International Journal of Medical Microbiology* **295(6), 405-418.**

Khanjar, A. and Alwan, M. (2014). Genotypic study of *Escherichia coli* O157: H7 isolated from stool samples of humans and cattle. *International Journal of Advanced Research* **2(6), 204-212.**

Kudva, I. T., Hatfield, P. G. and Hovde, C. J. (1996). *Escherichia coli* O157: H7 in microbial flora of sheep. *Journal of Clinical Microbiology* **34(2), 431-433.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

LeJeune, J., Besser, T., Merrill, N., Rice, D. and Hancock, D. (2001). Livestock drinking water microbiology and the factors influencing the quality of drinking water offered to cattle. *Journal of Dairy Science* **84(8), 1856-1862.**

Mainda, G., Bessell, P. R., Muma, J. B., McAteer, S. P., Chase-Topping, M. E., Gibbons, J., Stevens, M. P., Gally, D. L. and Barend, M. (2015). Prevalence and patterns of antimicrobial resistance among *Escherichia coli* isolated from Zambian dairy cattle across different production systems. *Scientific Reports* **5, 12439.**

Martinez-Medina, M. and Garcia-Gil, L. J. (2014). *Escherichia coli* in chronic inflammatory bowel diseases: An update on adherent invasive *Escherichia coli* pathogenicity. *World Journal of Gastrointestinal Pathophysiology* **5(3), 213.**

McEvoy, J. M., Doherty, A. M., Sheridan, J. J., Thomson-Carter, F. M., Garvey, P., McGuire, L., Blair, I. S. and McDowell, D. A. (2003). The prevalence and spread of *Escherichia coli* O157: H7 at a commercial beef abattoir. *Journal of Applied Microbiology* **95(2), 256-266.**

Mohammed, O., Shimelis, D., Admasu, P. and Feyera, T. (2014). Prevalence and antimicrobial susceptibility pattern of *E. coli* isolates from raw meat samples obtained from abattoirs in Dire Dawa City, *International Journal of Microbiological Research* **5(1), 35-39.**

Nanu, E., Latha, C., Sunil, B., Prejit, M. T. and Menon, K. V. (2007). Quality assurance and public health safety of raw milk at the production point. *American Journal of Food Technology* **2(3), 145-152.**

Nataro, J. P. and Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews* **11(1), 142-201.**

Omisakin, F., MacRae, M., Ogden, I. and Strachan, N. (2003). Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Applied and Environmental Microbiology* **69(5), 2444-2447.**

Onwumere, O. S. (2010). Isolation and phenotyptic characterisation of *E. coli* O157 and *Salmonella* spp in a slaughter house and meat sale tables at Nsukka market, Enugu State. MSc. Thesis. University of Nigeria, Nsukka.

Padhye, N. V. and Doyle, M. P. (1992). *Escherichia coli* O157: H7: epidemiology, pathogenesis, and methods for detection in food. *Journal of Food Protection* **55(7), 555-565.**

Quinn, P., Markey, B., Carter, M., Donnelly, W. and Leonard, F. (2002). Veterinary Microbiology and Microbial Diseases. Lowa State University Press Blackwell Science. Oxford London. **pp. 1-648.**

Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., Hebert, R. J., Olcott, E. S., Johnson, L. M., Hargrett, N. T. and Blake, P. A. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine* **308(12), 681-685.**

Šafařiková, M. and Šafařik, I. (2001). Immunomagnetic separation of *Escherichia coli* O26, O111 and O157 from vegetables. *Letters in Applied Microbiology* **33(1), 36-39.**

Sanderson, M. W., Sargeant, J. M., Shi, X., Nagaraja, T., Zurek, L. and Alam, M. (2006). Longitudinal emergence and distribution of *Escherichia coli* O157 genotypes in a beef feedlot. *Applied and Environmental Microbiology* **72(12), 7614-7619.**

Scott, T. M., Parveen, S., Portier, K. M., Rose, J. B., Tamplin, M. L., Farrah, S. R., Koo, A. and Lukasik, J. (2003). Geographical variation in ribotype profiles of *Escherichia coli* isolates from humans, swine, poultry, beef, and dairy cattle in Florida. *Applied and Environmental Microbiology* **69(2), 1089-1092.**

Tanih, N. F., Sekwadi, E., Ndip, R. N., and Bessong, P. O. (2015). Detection of pathogenic *Escherichia coli* and *Staphylococcus aureus* from cattle and pigs slaughtered in Abattoirs in Vhembe District, South Africa. *The Scientific World Journal* **2015(195972), 8.**

Tutenel, A.V., Pierard, D., Uradzinski, J., Jozwik, E., Pastuszczak, M., Van Hende, J., Uyttendaele, M., Debevere, J., Cheasty, T., Van Hoof, J. and De Zutter, L. (2002). Isolation and characterization of enterohaemorrhagic *Escherichia coli* O157: H7 from cattle in Belgium and Poland. *Epidemiology and Infection* **129(01), 41-47.**

Van Donkersgoed, J., Berg, J., Potter, A., Hancock, D., Besser, T., Rice, D., LeJeune, J. and Klashinsky, S. (2001). Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *The Canadian Veterinary Journal* **42(9), 714.**

Wani, S., Bhat, M., Samanta, I., Nishikawa, Y. and Buchh, A. (2003). Isolation and characterization of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *Escherichia coli* (EPEC) from calves and lambs with diarrhoea in India. *Letters in Applied Microbiology* **37(2), 121-126.**

Watterworth, L., Topp, E., Schraft, H. and Leung, K. T. (2005). Multiplex PCR-DNA probe assay for the detection of pathogenic *Escherichia coli*. *Journal of Microbiological Methods* **60(1), 93-105.**

Wasteson, Y., Johannessen, G. S., Bruheim, T., Urdahl, A. M., O'sullivan, K. and Rørvik, L. M. (2005). Fluctuations in the occurrence of *Escherichia coli* O157: H7 on a Norwegian farm. *Letters in Applied Microbiology* **40(5), 373-377.**

Wong, T. L., Hollis, L., Cornelius, A., Nicol, C., Cook, R. and Hudson, J. A. (2007). Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. *Journal of Food Protection* **70(3), 566-573.**

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D. and Meng, J. (2001). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. *Applied and Environmental Microbiology* **67(12), 5431-5436.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538