International Journal of Multidisciplinary Research and Development
www.allsubjectjournal.com
Online ISSN: 2349-4182, Print ISSN: 2349-5979
Received: 03-07-2022, Accepted: 19-07-2022, Published: 04-08-2022
Volume 9, Issue 8, 2022, Page No. 8-13

Prevalence of verotoxic *E. Coli* O157: H7 in faecal samples of beef and dairy cattle at national animal production research institute Zaria

Mohammed Nura*, Abagai Rose Takai, Usman Musba Barau
Department of Biology, School of Science Education, Kaduna State College of Education, Gidan Waya, Kafanchan, Nigeria

Abstract
This study was carried out to determine the prevalence of verotoxic *E. coli* O157:H7 in beef dairy cattle at National Animal Production Research Institute (NAPRI). A total of 120 faecal samples were obtained between May – July. The samples were enriched at the point of collection using modified tryptone soya broth (MTSB) and incubated at 37ºC for 24hr on arrival to the laboratory. From the enrichment medium a loop full of the samples were obtained and streaked on EMB plates. The plates were labeled and incubated at 37º C for 24hour. The colonial morphology on each plate was then appraised and those colonies with greenish metallic sheen were considered to be *E. coli.* These were then stored on Nutrient agar (NA) slants and incubated at 37º C for 24hr. the slants were then stirred at 4ºC awaiting biochemical test. After sample collection and preservation of the positive organism on Nutrient agar (NA) slants. Gram staining was carried out to differentiate the bacterial species into two large groups based on their cell wall constituents. All colonies that were considered *E. coli* after Gram staining were further screen biochemically which involves the observation of whether or not a growth of bacterium in liquid medium will ferment a particular gas. The selected colonies were later plated on Cefixime Tellurite Sorbitol MacConkey Agar (CT-SMAC) for 24hour. *E. coli* O157H:7 was characterized as colourless colony as it does not ferment sorbitol but uses peptone as source of energy thereby liberating ammonia (NH_3) which increase the pH of the medium making the colony to appear colorless. The result of the study reveals that sex and age of cattle showed significant association with the organism. The highest prevalence was found in female weiner (16.6%), cow, bull, female calf, female weiner, male haifer, male calf and male weiner showed zero prevalence of *E. coli* 0157:H7 except female heifer with (25%) prevalence.

Keywords: *E. coli*, Vero-toxic *E. coli* O157H:7, Haemorrhagic colitis, Haemolytic Uraemic Syndrome

Introduction

Escherichia coli is a facultative anaerobic bacterium commonly found in mammalian intestinal tract. *E. coli* lives a faecal oral lifestyle and can comprise up to 1% of the gastro intestinal population of mammals and is used as an indicator of environmental faecal contamination of water supplies (Winfield and Groisman, 2003) [18]. Most *E. coli* strains are commensal; however, some *E. coli* strains can be pathogenic to human, and are harboured within food animals. Some *E. coli* can cause haemorrhagic colitis in humans; but the best known enterhaemorrhagic *E. coli* (EHEC) strain remains O157:H7. The involvement of *E. coli* in human illness has been recognized virtually since its discovery in 1885. While attempting to isolate the etiological agent of cholera in 1885 from faeces of neonate, Theodore Escherich isolated an organism Bacterium coli commune, now known as *Escherichia coli* (Jay, 1996). On the basis of their virulence and disease manifestation, described five groups of *E. coli.* These include toxins producing strains like enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) enteroaggregative (EAggEC) and nontoxin producing starins like enteropathogenic (EPEC) and enteroinvasive (EIEC) *E coli*.

Shiga like toxin *E. coli* (SLTEC) including O157:H7 are members of the enterobacteriaceae family of gamma-proteobacteria commonly found in the intestines and faeces of animals and humans (Zwadyk *et al.*, 1992) [19]. The family consists of six tribes, namely; *Escherichia*, *Salmonella*, *Klebsiella*, *Protease*, *Yersiniae* and *Edwardsiellae*. Typically, these bacteria are short, straight (coccobacillary), and Gram-negetive bacilli. Members of the genus are nonsporulating, usually motile, and exibit a relatively non fastidious facultative anaerobic metabolism (Zwadyk, *et al.*, 1992) [19]. The designation of a specific serotype as an "O" and (or) "H" type bacterium (as the case in O157:H7) is a nomenclature based on serological identification of specific antigens produced by the bacterium as structural components. The "O" type antigens (e.g., "O" 157) are somatic lipopolysacharides; whereas the "H" type antigens ((e.g., "H" 7) are components of the bacterial flagellae (Lior *et al.*, 1994) [11]. Because of the involvement of SLTEC in outbreaks of bloody diarrhea, HC, or HUS, SLTEC are now often referred to as enterohaemorrhagic *E. coli* (EHEC). Subsequent to their discovery as a cause of HC and HUS it was recognized that SLTEC produce cytotoxins that are responsible for their virulence (Riley *et al.*, 1983) [14].

Enterohaemorrhagic *Escharichia coli* O157:H7 is an important food-borne (Benjamin and Datta, 1995) [1] which was first identified in 1982 as a cause of haemorrhagic colitis during the outbreak of bloody diarrhea in Oregon

and Michigan, USA. After these cases, several outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome caused by this organism have been epidemiologically have been linked to ground beef (Schlundt 2001) [15]. The subsequent occurrence of large outbreaks and the widespread distribution of cases have led to the designation of *E. coli* O157:H7 as new, emerging pathogen. The bacterial group most commonly associated with human illness resulting from consumption of contaminated beef is Shiga-like-toxin producing *Escherichia coli* (SLTEC) such as O157:H7). It is evident that SLTEC can cause a variety of illness. These illness range from a mild diarrhea to severe haemorrhagic colitis (HC) characterized by bloody diarrhea, abdominal cramp, fever and vomiting. Approximately 10% of younger patients with SLTEC infection develop haemolytic uraemic syndrome (HUS). This is life threatening illness characterized by haemolytic anaemia, acute renal failure and various central nervous system abnormalities. Although most patients recover, some die and some may develop strokes (Griffin *et al*., 1999, Fitzpatrick *et al*., 1997, and Schmidt *et al*., 1999) [16].

Materials and Methods

Sample collection site

The study was carried out at the National Animal Production Institute (NAPRI) ABU, Zaria, Kaduna State. The average temperature of Zaria is about 27ºC and the town is located between latitude 11' 06" North and 27' 41" East of the equator. It is also located at an altitude of 700-900metres above sea level in the centre of northern Nigeria (Duze and Ojo, 1990) [5]. The National Animal Production Research Institute (NAPRI) Ahmadu Bello University, Zaria, had its primordial origin as Shika Stock Farm established in 1928 by the Department of Agriculture of the then Northern Region of Nigeria. The primary objective of the farm was to produce "by selective breeding, male stock owners". Research on sown pastures and range management was started in 1954 on the station by October 1962; the farm was upgraded to Shika Research Station under the Institute for Agricultural Research and Special Services, Samaru which became part of Ahmadu Bello University, Zaria. Staff of the Research Station became closely linked with the Faculty of Veterinary Medicine and of Agriculture with the research staff involve in teaching and supervision of students at all levels. Shika Research Institute Station became a semi-autonomous Research Institute i.e. National Animal Production Research Institute (NAPRI), as a result of the promulgation of degree No 35 of 27th August 1973 and the enactment of the research Institute Establishment order of November 1975 by the federal Government of Nigeria.The take-off of the Institute was on 1st July, 1996 with its headquarters in Shika. (www.arnigeria.or/index). The study was carried out between May-July, 2018.

Sample collection

Random samples were collected by retrieval on rectal palpation with sterile swab stick, following adequate restraint of the animals (Chapman *et al*., 1993) [2]. The sterile swab was rolled gently to allow adequate contact between the sterile swab and the mucosa of the rectum. This was removed and inserted back into its cover and labeled approximately. The samples were kept in a cool box containing ice blocks and taken to the laboratory for bacteriological culture and isolation (Chapman *et al*., 1993) [2]. Samples were collected once a week from both sexes of cattle comprising of cow, bull, heifer, weaned and calf. Twenty four samples were collected from cow in the first week and twenty four samples were collected from bull in the second week, twenty four samples were collected from heifer likewise for weaned and calf to ensure that the animals were not sampled again.

Preparation of Sorbitol MacConkey (ASMAC) Agar (Isolation medium)

The Sorbitol MacConkey agar powder was prepared according to Manufacturer's instructions (Oxoid Ltd., Hampshire, England) by dissolving 51.1g in 1litre of distilled water supplemented with Cefixime (50ug/L) and Potassium Tellurite (2.5mg/L) at 43ºC (March *et al*., 1986) [12]. Bring to the boil to dissolve completely. It was sterilized by autoclaving at 121ºC for 15min. it was then allowed to cool to 50ºC before pouring into petri dishes. The plates were allowed to solidify and incubated at 37º C for 24hrs (Chapman *et al*., 1994) [3].

Rectal Swab Sample Processing and Enrichment

The enrichment phase was carried out on the farm soon after collection. Each of the rectal swabs was inserted into test tubes containing modified tryptone soya broth. The test tubes were shaken gently and the swab sticks were cut approximately 1cm from the cotton wool. The test tubes were labeled appropriately and incubated at 37ºC for 24hrs on arrival to the laboratory.

Culturing on Eosin Methylene Blue (EMB)

From the enrichment medium above a loopful of the samples were incubated and streaked on Eosin Methylene blue agar plates. The plates were labeled and incubated at 37ºC for 24hrs. The colonial morphology on each plate was then appraised and those colonies with characteristic greenish metallic sheen were considered to be *E. coli* (Grant *et al*., 1996). These were then stored on nutrients agar (NA) slants and incubated at 37ºC for 24hrs. The slants were then stored at 24ºC awaiting biochemical test.

Gram Staining

After sample collection, isolation and preservation of the positive organisms on nutrients agar (NA), Slants, Gram staining was carried out to differentiate the bacterial species into large groups based on their cell wall

constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by the colour of their cells i.e red or violet (Gram reaction). The organisms from (NA) slants were smeared on a glass slides, air dried and heat fixed by passing the slides over the flame three times. The slides were placed on staining rack, and crystal violet (primary dye) was added over the fixed culture and allowed to stand for 60sec (it is usually acceptable to pour stain on and off immediately). The stain was poured off and the excess stain was gently rinsed with stream of water from plastic water bottle. The objective of this step is to wash off the stain, not the fixed culture. The iodine solution was poured off and the slides were rinsed with slow-running tape water. The fixed smear was decolourized using 75% alcohol so that the solution trickles down the slide. It was then rinsed off with slow-running tap water after 5sec. further delay will caused over decolourization in the Gram positive cells, and the purpose of the staining will be defeated. The fixed smears were counter stained with safranin (Secondary dye) for 30sec after which it was rinsed off with water. The slides were placed between filter papers and press lightly to allow the excess water to be removed. It was then placed on a clean stand to air dry the smears were then examined with the oil immersion lens under magnification of 100 objectives for rod shaped organism with pinkish background indicating Gram negative *E. coli*.

Biochemical Test

All colonies that were considered *E. coli* after Gram staining above were further screened biochemically using described in details by Coghlan *et al*., (1975) [4], which involves the observation of whether or not growth of bacterium in liquid nutrient medium will ferment a particular gas. The biochemical reagents were prepared as follows according to the manufacture's instruction.

1. **Citrate utilization test:** this was prepared by dissolving 23g of citrate powder in 1000ml of distilled water; 5ml was dispensed into each test tube. It is a greenish solid medium and has not butt, it becomes dark blue if positive and retains it colour if is negative.

2. **Sulphate, Indole and Motility test:** these are prepared by dissolving 30g of SIM powder in 100ml Indole is colourless liquid which after 24hrs inoculation and incubation few drops of Kova's reagent are added. A pink ring is formed at the top if its positive and a groundnut oil ring are formed at the top if it's negative. Motility is a semi medium which after 24hr inoculation and incubation a defined line is observed if it is non-motile but if no line observed it is motile.

3. **Methyl-Red Voges-Proskauer (MRVP):** these are prepared by dissolving 17g of MRVP powder in 1000mls of distilled water; 5ml was dispensed into each test tube. These are brownish liquid media, after inoculation and incubation methyl red and Voges reagent are added it gives a pinkish colour if positive but no colour change will be observed if negative.

4. **Triple Sugar Iron (TSI):** this was prepared by dissolving 65g of TSI dehydrated powder in 1000ml of distilled water; 5ml was dispensed into each test tubes. This is a medium consisting of three types of sugar; glucose, lactose and sucrose. It consists of butt and slant. It becomes golden yellow in colour, after 24hr inoculation and incubation; a pinkish colour shows alkaline change. A black colour indicates the presence hydrogen sulphide gas (H_2S) being produced crack or empty vacuum indicates the presence of gas.

5. **Urease test:** this was prepared by dissolving 2.1g of urea dehydrated powder in 95ml of distilled water. After which 120ml of distilled water and 5ml was dispensed into each tubes. This is a brownish medium that has no butt, it becomes pink when positive and retains its original colour if negative.
   The prepared biochemical media were autoclaved at 121ºC for 15min and allowed to cool to 60ºC, they were then packed into the incubator. After 24hr, they were brought out and examined for growth of any contaminant, which there was none. The biochemical reagents were then incubated at 70º C for 24hr.
   Each isolate was tested for citrate utilization, urease production, H_2S production in triple sugar iron (TSI) agar and sulphide indole motility (SIM) medium fermentation of glucose, sucrose and/or lactose in TSI medium, methyl red (MR) and Voges proskauer (VP) reactions using MRVP medium, ability to produce an acid reaction from fermentation of lactose, arabinose, maltose, sorbitol and manitol, and gas from glucose.

Subculturing on Sorbitol MacConkey Agar (SMAC)

Colonies that showed greenish metallic sheen on Eosin Methyl Blue (EMB) and stored on the (NS) slants were selected and screened biochemically (Coghlan *et al*., 1975) [4] following Gram-staining. Isolates that were biochemically presumed to be *E. coli* were then plated on CT-SMAC agar and incubated at 37ºC for 24hr. Colourless colonies on the medium indicate *E. coli* O157:H7. This is because *E. coli* O157:H7 does not ferment sorbitol as such it utilizes peptone as source of energy thereby liberating ammonia (NH_3) which increases the pH of the medium making the colonies to appear colourless (March *et al*., 1986) [12].

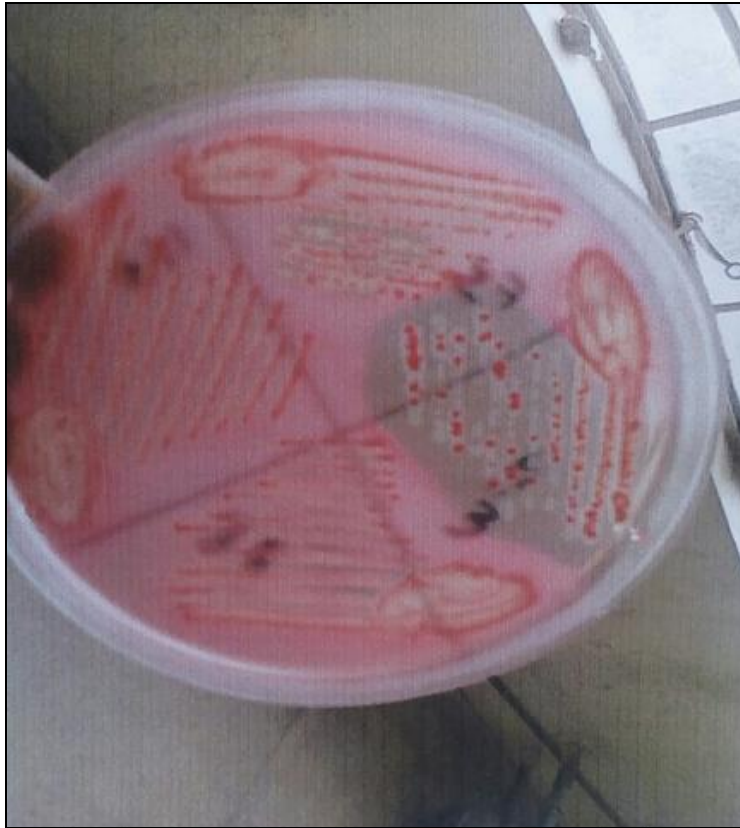

Plate 1: *E. coli* O157:H7 on SMAC with characteristics colourless colonies on the medium

Data Analysis

The prevalence was calculated by dividing the number of animals harbouring *E. coli* and *E. coli* O157:H7 by the total number of animals examined by 100 (percentage to measure the prevalence).

Table 1: Overall prevalence of *E. coli* in both sexes of cattle (matured and young)

Sr. No	Animal type	No of sample	No of sample positive for *E. coli*	Prevalence of positive *E. coli*
1	B	24	8	33.3
2	C	24	15	62.5
3	H	24	17	70.8
4	W	24	3	12.5
5	C*	24	10	41.6
Total		120	53	44.1

Table 2: Overall prevalence of *E. coli* O157:H7 in both sexes of cattle (matured and young)

Sr. No	Animal type	No of sample	No of non-sorbitol fermenting *E. coli*	Prevalence of non-sorbitol fermenting *E. coli* (%)
1	B	24	0	0
2	C	24	0	0
3	H	24	3	12.5
4	W	24	0	0
5	C*	24	0	0
		120	53	2.5

Key: B=Bull, C=Cow, H=Heifer, W=Weaner, C*=Calf

Table 3: Prevalence of *E. coli* in relation to male sex

Sr. No	Animal type	No of sample	No of sample positive for *E. coli*	Prevalence of positive *E. coli* (%)
1	B	24	6	25
2	MH	12	6	50
3	MW	12	0	0
4	MC*	12	0	0
Total		60	12	40

Table 4: Prevalence of *E. coli* O157:H7 in relation to male sex

Sr. No	Animal type	No of sample	No of non-sorbitol fermenting *E. coli*	Prevalence of non-sorbitol fermenting *E. coli* (%)
1	B	24	0	0
2	MH	12	0	0
3	MW	12	0	0
4	MC*	12	0	0
Total		60	0	0

Key: B=Bull, MH=Male Heifer, MW=Male Weaner, MC*=Male Calf

Table 5: Prevalence of *E. coli* in relation to female sex

Sr. No	Animal type	No of sample	No of sample positive for *E. coli*	Prevalence of positive *E. coli* (%)
1	C	24	17	70.8
2	FH	12	11	91.6
3	FW	12	3	16.6
4	FC	12	10	83.3
Total		60	41	68.3

Table 6: Prevalence of *E. coli* O157:H7 in relation to female sex

Sr. No	Animal type	No of sample	No of Non sorbitol fermenting *E. coli*	Prevalence of non-sorbitol fermenting *E. coli* (%)
1	C	24	0	0
2	FH	12	3	25
3	FW	12	0	0
4	FC	12	0	0
Total	60		3	5

Key: C=Cow, FH= Female Heifer, FW=Female Weaner, FC= Female Calf

Results and Discussion

A total of 120 feacal samples were collected from National Animal Production Research Institute (NAPRI) comprising (24 Cow, 24 Bull, 24 Heifer, 24 Weaner and 24 Calf), out of which 53 samples were suspected to be *E. coli*, 48 were confirmed to be *E. coli*, 5 were *Citrobacter* and 3 were identified as *E. coli* 0157:H7. Both cases include Male and female (12 males and 12 females) and were isolated over a three months period between May and July, 2012.

Table 1 and 2 showed the age distributions of *E. coli* and *E. coli* 0157:H7 in both sexes of cattle (matured and young) respectively. The prevalence rate of *E. coli* was in the following; Bull (33.3%), Cow (62.5%), Heifer (70.8%), Weaner (12.5%) and Calf (41.6%). The highest prevalence rate was found in Heifer (70.8%) and the least was weaner (12.5%). The overall prevalence was (44.1%). The high prevalence incidence of *E. coli* may be explained by the fact that free range animal rearing is the most common animal husbandry practice where same grazing grounds are used by farmers within a community. The high prevalence rate of the pathogen in young beef and dairy cattle may be attributed to the greater susceptibility of this age group to colonization. The distribution according to age group of the animals revealed that young animals were more susceptible to *E. coli* and VTEC O157:H7 than adult cattle which agree with the finding of (Hancock *et al*., 1997, Mechie *et al*., 1997, Van Donkersgoed *et al*., 1999) [8, 13, 17]. The higher frequency in younger animals could also be due to differences in the composition of the gastrointestinal flora resulting from differences in diet (Heuvelink *et al*., 1998) [9].

Table 2 showed the prevalence of non sorbital fermenting *E. coli* 0157:H7 in both sexes of cattle (matured and young). Bull, Cow, Weaner, and Calf showed zero prevalence while Heifer has (12.5%) prevalence. The overall prevalence was (2.5%).

Table 3, 4, 5 and 6 showed the relationships between the *E. coli* and *E. coli* O157:H7 in both sexes of cattle respectively. In table 3, the highest prevalence was found in Male Heifer (50%) and the least was found in Bull. While Male Weaner and Male Calf showed zero prevalence.

Table 4 showed the prevalence of non-sorbitol fermenting *E.* coli O157:H7 in relation to sex where all the cattle showed zero prevalence. Data analysis revealed a significant difference between prevalence of the organism and sex of the animals when ($P \leq 0.05$) was set for level of significance.

Table 5 showed the prevalence of *E. coli* in relation to cow (70.8%), Female Heifer (91.6%), Female Weaner (16.6%) and Female Calf (83.3%). The highest prevalence was found in Female Heifer (91.6%) and the least Female Weaner (16.6%).

Table 6 showed the prevalence of non-sorbitol fermenting *E. coli* O157:H7 in relation to sex where Female Heifer showed (25%) prevalence while Cow, Female Heifer and Female Calf showed zero prevalence. The study revealed that the sex of animals showed significant association with the prevalence of the organism with female having a higher prevalence rate than the male. Similarly, a higher prevalence was recorded in young animals than the matured.

However, low isolation rate of *E. coli* O157:H7 in this study could be attributed to several factors such as geographic distribution, age, breed and housing condition. Other studies have shown similar rates of isolation (Chapman *et al*., 1994; Lahti *et al*., 2001) [3]. Another reason for low prevalence rate observed in our study could be sampling during the cold months, which is in agreement with the findings of other studies on seasonal variation of the infection with more cattle being found positive for VTEC O157 in late summer and early autumn (Chapman *et al*., 1997; Aslantas *et al*., 2006) [13].

Conclusion

Evidently, *E. coli* O157:H7 has been isolated in from the faeces of or gastrointestinal tract of cattle. Therefore this study identified that the organism is prevalent in the study area and is a potential risk to animal production and public health could serve as a point of outbreak or sporadic case globally as the world today is a global village.

Acknowledgements

My sincere appreciation and gratitude is first and foremost to Almighty God for bestowing upon me the true light of knowledge and reasoning and for granting me the opportunity, good health and courage to write this research amidst the overwhelming academic challenges.

I respectfully acknowledge the authors for their timely and unreserved help during the laboratory experiment

References

1. Benjamin MM, Datta AR. Acid tolerance of enterohaemorrhagic:61:1669-1672. Escherichia coli. Appl. Env. Microbiol*,*1995
2. Chapman PA, Wright DJ, Norman P, Crick E. Cattle as possible source of verotoxin-producing *Escherichia coli* O157:H7 infection in man. Epidemiol. Inf,1993:102:439-447.
3. Chapman PA, Wright DJ, Siddons CA. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. J.Med. Microbiol,1994:40:424-427.
4. Coghlan JA, Collee JG, Cruickshank R, Duguid JP, Gilles RR, Gould JC *et al*. Medical Microbiology: the Paractice of Microbiology. 12th Ed. Cruickshank, JP, Duguid, BP, Marnion and RHA Swain, Ed. Churchill Livingstone, Edinburgh,1975:587.
5. Duze M, Ojo A. Macmillan Senior School Atlas, revised edition, Nigeria,1990:113.
6. Fitzpatrick MM, Shah V, Trompeter RS, Dillon MH, Barrett TM. Long term renal outcome of childhood haemolytic uraemic syndrome. Med. J,1991:303:489.
7. Griffin PM, Vauxe RV. The epidemiology of infections caused by Escherichia coli O157H:7, other enterohaemorrhagic *Escherichia coli* and the associated haemolytic uraemic syndrome. Epidemiology Review,1991:13:60.
8. Hancock DD, Besser TE, Rice DH, Herriott DE, Tarr PI. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. Epidemiol.Inf,1997:188:193-195. doi: 10.1017/S0950268896007212.
9. Heuvelink AE, Van DB, De Boer E, Herbes RG, Melchers WJG, Huis Intveld JHJ, *et al*., Isolation and characterization of verocytotoxin producing Escherichia coli O157 strain from Dutch cattle and sheep. J. Clin. Microbiol,1998:36:878-882.
10. Jay J. Antimicrobial properties of diacetyl. Appl. Env. Microbiol*,*1992:44:525.
11. Lior H. Classification of Escherichia coli in Domestic Animals and Humans. C.L. Gyles (Ed.),1994:31-72.
12. March SB, Ratma S. Sorbitol MacCokey medium for detection of Escherichia coli O157:H7 associated with haemorragic colitis. J. clin. Microbiol,1986:23:869-872.
13. Mechie SC, Chapman PA, Siddons CA. A fifteen month study of Escherichia coli O157:H7 in a dairy herd. Epidemiol. Inf,1997:118:17-25.
14. Riley LW, Remis RS, Helgerson SD, Hargarett PA, Cohen ML, McGee HB, *et al*., Haemorragic colitis associated with a rare Escherichia coli serotypte*.* J. Med,1983:308:681.
15. Schlundt J. Emerging foodborne pathogens. Biomed. J. env. Sci,2001:14(2):44-52.
16. Schmidt H, Geit C, Tarr PI, Frosch M, Karch H. Non-O157:H7 pathogenic Shiga toxin-producing Escherichia coli: Phenotypic and genetic profiling f virulence traits and evidence for clonality. J. Inf. Dis,1999:179:115.
17. Van Donkersgoed J, Graham T, Gannon V. The prevalence of verotoxins, Escherichia coli 0157:H7, and Salmonella in the feces and rumen of cattle at processing. Canad. Vet. J,1999:40:332-338.
18. Winfield M, Groisman E. Role of nonhost Environments in the Lifestyles of Salmonella and Escherichia coli. Appl. and Env. Microbial,2003:3687-3694.
19. Zwadyk P. Enterobacteriaceae: General characteristics. In Zinsser Microbiology. Willet HP, Amos DB, Wilfert CM. (Eds.),1992:538-543.