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Molecular Characterization of *E. coli* Isolated from Chicken, Cattle and Buffaloes

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Abstract: *E. coli* plays an important role in maintaining intestinal physiology. However, there are pathogenic strains that cause distinct syndromes of diarrheal disease. In this study we used a collection of 28 *E. coli* isolated from cattle, buffaloes and chicken obtained in the same geographical area to perform a detailed analysis of the molecular epidemiology of O157 and non O157 strains by using PCR for identifying general similarities and differences in the genetic composition of *E. coli* populations.The isolates showed a degree of diversity in PCR of *stx1*, *stx2* and *eae.* 12 strians had *stx1* gene, 16 strains had *stx2* gene and 10 strains had *eae* gene. 76.92% of cattle isolates had *stx2* and 38.46% possessed both *stx1* and *stx2*, also *stx1* was detected from 38.46% of the examined cattle strains. Using restriction enzyme (RE) it is clear that between 3 to 6 fragments were obtained with hindIII digested DNA of chicken and buffalo isolates and between 3 to 5 fragments of cow isolates. Among EcoRI between 5 to 9 fragments were obtained with digested genomic DNA of chicken and cattle and 7 to 9 fragments of buffalo isolates. HindIII and EcoRI ribotyping could reveal only minor differences in non O157:H7 strains belonging to the same serotypes. This indicates that ribotyping which is regarded to be a useful tool for epidemiological investigation, was not able to discriminate between STEC isolates belonging to the same serotype. It is believed that in future a better understanding of moleculer diversity of *E.coli* strains of different sources would provide and assist the design of approaches to epidemiological studies.

Key words: Escherichia coli · O157 · *stx1* · *stx2* and *eae* genes · Restriction enzyme

INTRODUCTION

Escherichia coli is considered as an important pathogen of animals, comprising the normal flora of the gastrointestinal tract and the cause of septicemic disease in foals, calves, piglets and lamb [1]. Most *E. coli* strains do not cause disease in human but certain types may cause serious illnes and death. Shiga toxigenic *E. coli* (STEC) enteritis and hemolytic uremic syndrome (HUS) are associated with significant mortality and morbidity, especially among patients with severe renal and neurological disorders [2]. Since the initial identification of *E. coli* O157:H7 as the etiological agent of HUS and hemorrhagic colitis (HC), this pathogen has risen as a major concern of food safety in many countries [3]. Non O157 serogroupes which have emerged as a significant cause of human disease include *E.coli* O26 and O111 [4], some of them linked to cattle [5]. The pathogencity of verotoxigenic *E. coli* (VTEC) is associated with a number of virulence factors such as Vero toxins (VT1and VT2), *eae* gene that encodes intimin which is responsible for the attaching and effacing the organism to the gut epithelial cells lead to hemolytic uremic syndrome [6]. Several molecular biology based techniques have been applied in order to obtain the further discrimination required for outbreak investigations [7]. The aim of this study was to to estimate the frequency of verotoxigenic *E.coli* isolated from chichen, cattle and buffaloes. It is aimed that this work may provide additional discriminatory information of *E. coli* isolates that would be useful in studying the genetically diverse as well as the possible epidemiological typing of *E.coli* in order to trace the infection and prompting measures to prevent outbreaks.

MATERIALS AND METHODS

Samples: A total of 250 fecal samples collected from chichen (n=100), cattle (n=30) and buffaloes (n=50) and 30 mastitic cow milk samples were obtained from farms on the outskirts of Cairo. Diarecheic chichen, cattle and buffaloes were selected randomly. The health status of each animal was evaluated by clinical examination. Also 50 and 30 raw meat and milk samples respectively were collected from cattle from different supermarkets in Cairo.

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Table 1: Oligonucleotide primers used for identification of virulence genes

Virulence genes	Primers	Anneling temp.	Amplification fractions
stx1	GAAGAGTCCGTGGGATTACG	at 55°C	130-bpPollard *et al*. [11]
	AGCGATGCAGCTATTAATAA		
stx2	TTAACCACACCCACGGCAGT	at 55°C	346-bpPollard *et al*. [11]
	GCTCTGGATGCATCTCTGGT		
eae	GACCCGGCACAAGCATAAGC	at 58°C	384-bpPaton and Paton [12]
	CCACCTGCAGCAACAAGAGG		

Isolation and Identification of the *E. coli* Isolates: The collected samples were directly inoculated on MacConkey's agar (Oxoid) plates. After overnight incubation at 37°C, rose pink colonies were picked up and subcultured on eosin methylene blue agar (Oxoid) plates to observe the characteristic metallic sheen. The well-separated colonies were picked up on nutrient agar (Oxoid) slants as pure culture and subjected to standard morphological, biochemical tests as described by Koneman *et al.* [8] and Quinn *et al.* [9]. Also serological identification of the isolates was carried out as described by Sojka [10] using diagnostic *E. coli* antisera (Denka Seiken) and *E. coli* H7 antiserum (Wellcome).

Detection of stx_1, stx_2 and *eaeA* genes by PCR: *E. coli* strains were cultured in brain heart infusion broth (Difco), at 37ºC for 18-24 h. DNA was extracted using Easyquick DNA extraction kit (Genomix). The presence of the Shiga toxins was assessed in all strains by PCR using the stx_1 and stx_2 primers (Table 1) as described by Pollard *et al.* [11]. PCR conditions were described by Pollard *et al.* [11]. Also the presence of the intimin determinant was assessed in all strains by PCR using the *eae* primers (Table 1) as described by Paton and Paton [12]. PCR conditions were as described by Paton and Paton [12]. The amplified products were separated on agarose gel and examined eletrophoretically after staining with ethidium bromide (Sigma). A 100 bp DNA ladder (Stratagene, USA) was used as a molecular size marker.

RESULTS

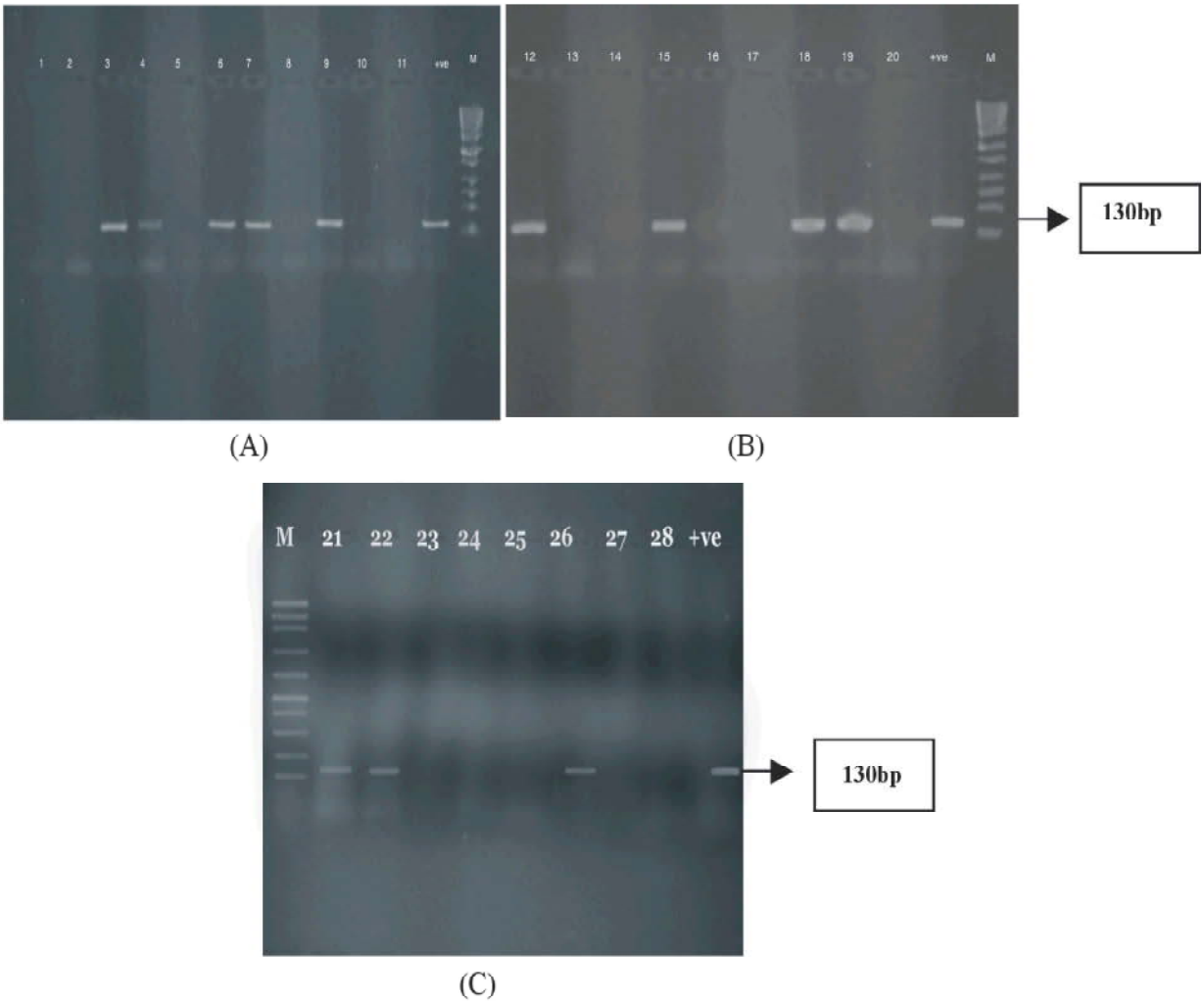
Serogrouping of *E. coli* Isolates: The identified 28 *E. coli* isolates were serogrouped. *E. coli* serogroups O2, O6, O8, O26, O27, O78, O86, O111, O128, O136 and O157 were recovered from cloacal swabs of diarrheic chicken, while serogroupes O115, O157 and O159 were recovered from milk samples of mastitic cattle and O119 were recovered from fecal swab of diarrheic cow in addition to three isolated serogrouped as O157 were recovered from fecal swab of diarrheic buffaloes. O78, O111, O112, O119 and O157 were recovered from cattle meat samples while serogroupes O111, O112, O114 and O124 were recovered from cattle raw milk samples (Table 2).

PCR Analysis: The PCR was used for detection of *stx1*, *st2* and *eae* genes among *E. coli* strains. Photos (1-3) revealed that 12 out of 28 strains of *E. coli* collected from different sources were positive to *stx1* with an incidence of 42.9% and 16 strains were positive to *stx2* with an incidence of 57.14% while, 10 strains were positive to *eae* with an incidence of 35.71%. It is clear that 4 strains from chicken cloacal swabs were positive for amplification of 130 bp specific for *stx1*, while 5 strains were positive among cattle strains. In buffaloes 3 strains were positive for *stx1* gene. Amplification of 346 bp fragment indicative to the presence of *stx2* gene was present in 5, 10 and 1strains among chicken, cattle and buffaloes strains respectively. Furthermore, the agarose gel electrophorasis

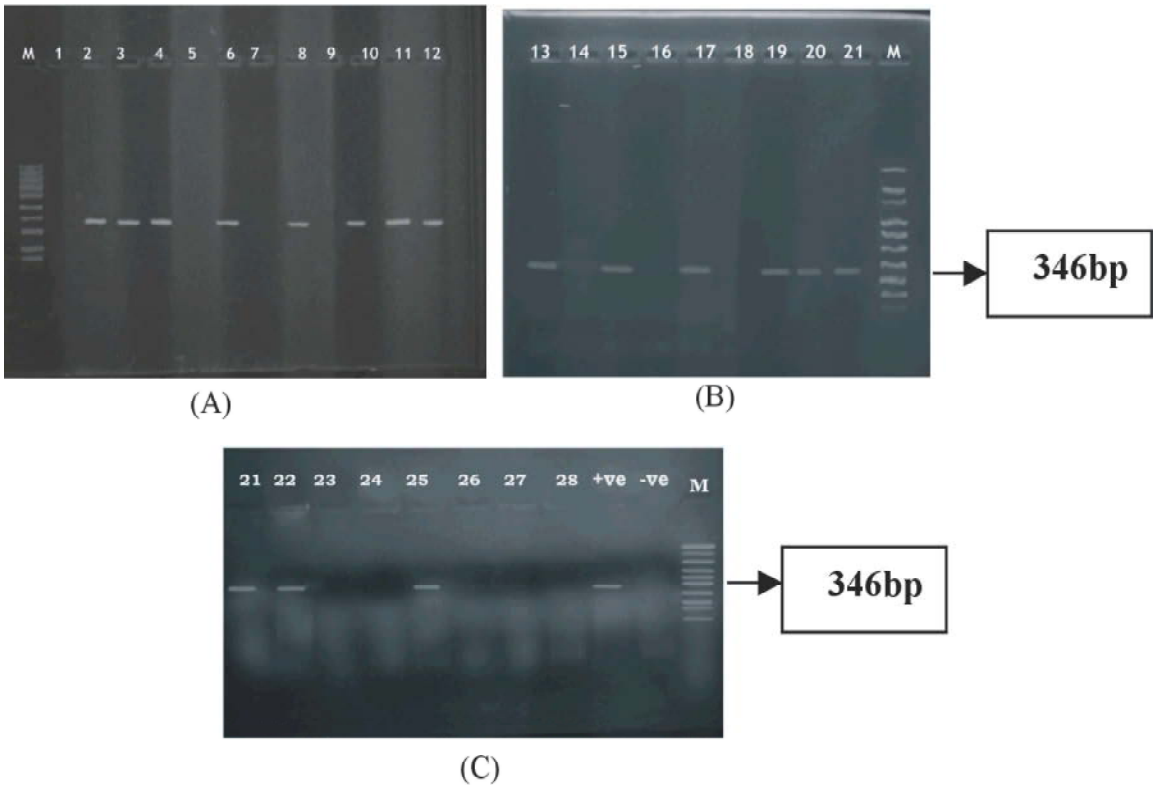
Table 2: Serotyping of E. coli isolated from the examined chichen, cattle and buffaloes samples

Sources of isolates	Types of samples	Serotypes
Buffaloes (n=3)	Fecal swabs	O157
Chicken (n= 12	Cloacal swabs	O2, O6, O8, O26, O27, O78, O86, O111, O128, O157 and O136
Cattle (n= 13)	Mastitic milk	O115, O157 and O159
	Raw milk	O111, O112, O114, and O124
	Meat	O78, O111, O112, O119 and O157
	Fecal swab	O119

Total (n= 28)



Photograph 1: Shows PCR analysis of *E. coli* strains for detection of *stx1*.
(A) Lane 1: O136 from diareheic chicken (-ve), Lane 2: O111 from diareheic chicken (-ve), Lane 3:O115 from cattle mastitic milk (+ve), Lane 4: O157 from diareheic buffalo (+ve), Lane 5: O27 from diareheic chicken (-ve), Lane 6: O119 from cattle meat (+ve), Lane 7: O157 from diareheic buffalo (+ve), Lane 8: O112 from cattle meat (-ve), Lane 9: O2 from diareheic chicken (+ve), Lane10: O114 from cattle milk (-ve), Lane11: O6 from diareheic chicken (-ve), Lane+ve: control +ve O157:H7 ATCC 35150 and Lane M: one kilo-base ladder marker (Stratagene,USA).
(B) Lane12: O111 from cattle meat (+ve), Lane13: O124 from cattle milk (-ve), Lane14: O78 from cattle meat (-ve), Lane15: O111 from cattle milk (+ve), Lane16: O8 from diareheic chicken (-ve), Lane17: O157 from chicken (-ve), Lane18: O157 from buffalo (+ve), Lane19: O8 from chicken (+ve), Lane20: O157 from cattle mastitic milk (-ve) Lane+ve: control +ve ATCC 35150 and Lane M: one kilo-base ladder marker (Stratagene,USA).
(C) Lane21: O159 from cattle mastitic milk (+ve), Lane22: O128 from diareheic chicken (+ve), Lane23: O86 from diareheic chicken (-ve), Lane24: O157 from cattle meat (-ve), Lane25: O112 from cattle milk (-ve), Lane26: O78 from diareheic chicken (+ve), Lane27: O26 from diareheic chicken (-ve), Lane28: O119 from diareheic cattle (-ve), Lane +ve: O157:H7 ATCC 35150 and Lane M: one kilo-base ladder marker (Stratagene,USA).

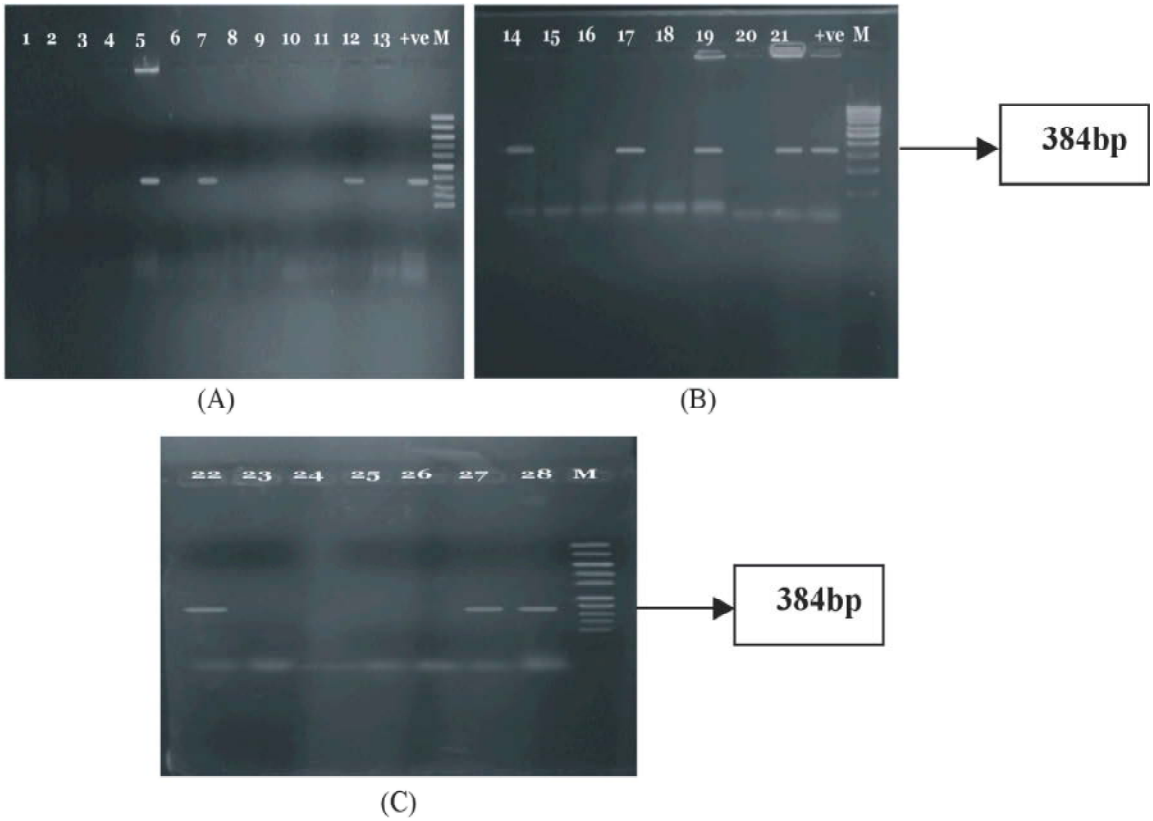


Photograph 2: Shows PCR analysis of *E. coli* strains for detection of *stx2*.
(A) Lane 1: O136 from diareheic chicken (-ve), Lane 2: O111 from diareheic chicken (+ve, Lane 3: O115 from cattle mastitic milk (+ve), Lane 4: O157 from diareheic buffalo (+ve), Lane 5: O27 from diareheic chicken (-ve), Lane 6: O119 from cattle meat (+ve), Lane 7: O157 from diareheic buffalo (-ve), Lane 8: O112 from cattle meat (+ve), Lane 9: O2 from diareheic chicken (-ve), Lane10: O114 from cattle milk (+ve), Lane11: O6 from diareheic chicken (+ve), Lane12: O111 from cattle meat (+ve) and Lane M: one kilo-base ladder marker (Stratagene,USA).
(B) Lane13: O124 from cattle milk (+ve), Lane14: O78 from cattle meat (-ve), Lane15: O111 from cattle milk (+ve), Lane16: O8 from diareheic chicken (-ve), Lane17: O157 from diareheic chicken (+ve), Lane18: O157 from diareheic buffalo (-ve), Lane19: O8 from diareheic chicken (+ve), Lane20: O157 from cattle mastitic milk (+ve) and Lane M: one kilo-base ladder marker (Stratagene,USA).
(C) Lane21: O159 from cattle mastitic milk (+ve), Lane22: O128 from diareheic chicken (+ve), Lane23: O86 from diareheic chicken (-ve), Lane24: O157 from cattle meat (-ve), Lane25: O112 from cattle milk (+ve), Lane26: O78 from diareheic chicken (-ve), Lane27: O26 from diareheic chicken (-ve), Lane28: O119 from diareheic cattle (-ve), Lane+ve:control +v O157:H7 ATCC 35150,Lane-ve:control-ve Lane M: one kilo-base ladder marker (Stratagene,USA).

showed the amplification of 384 bp specific for *eae* gene among 4, 5 and 1 strains of cattle, chicken and buffalo strains, respectively.

Restriction Enzyme Analysis of Genomic DNA of *E.coli* Strains: Twenty eight *E. coli* strains obtained from cattle (13) chicken (12) and buffaloes (3) were analyzed by restriction enzymes (HindIII and EcoRI). Analysis of shared fragments in HindIII and EcoRI digests of *E. coli* strains DNA as shown in Tables (3-5) and photos (4 and 5) revealed that EcoRI is more efficient (5-9 fragments) than HindIII (3-6 fragments).

Restriction Enzyme Analysis of Genomic DNA of *E.coli* Strains Using HindIII: Among chicken strains, 8 isolates had one or two bands at 590-344bp, 6 isolates had one or



Photograph 3: Shows PCR analysis of *E. coli* strains for detection of *eae*.
(A) Lane 1: O136 from diareheic chicken (-ve), Lane 2: O111 from diareheic chicken (-ve), Lane 3: O115 from cattle mastitic milk (-ve), Lane 4: O157 from diareheic buffalo (-ve), Lane 5: O27 from diareheic chicken (+ve), Lane 6: O119 from cattle meat (-ve), Lane 7: O157 from diareheic buffalo (+ve), Lane 8: O112 from cattle meat (-ve), Lane 9: O2 from diareheic chicken (-ve), Lane10: O114 from cattle milk (-ve), Lane11: O6 from diareheic chicken (-ve), Lane12: O111 from cattle meat (+ve), Lane13: O124 from cattle milk (-ve), Lane+ve:control +ve O157:H7 ATCC 35150 and Lane M: one kilo-base ladder marker (Stratagene,USA).
(B) Lane14: O78 from cattle meat (+ve), Lane15: O111 from cattle milk (-ve), Lane16: O8 from diareheic chicken (-ve), Lane17: O157 from diareheic chicken (+ve), Lane18: O157 from diareheic buffalo (-ve), Lane19: O8 from diareheic chicken (+ve), Lane20: O157 from cattle mastitic milk (-ve), Lane21: O159 from cattle mastitic milk (+ve), Lane+ve: control +ve, O157:H7 ATCC 35150 and Lane M: one Kilo-base ladder marker (Stratagene,USA).
(C) Lane22: O128 from diareheic chicken (+ve), Lane23: O86 from diareheic chicken (-ve), Lane24: O157 from cattle meat (-ve), Lane25: O112 from cattle milk (-ve), Lane26: O78 from diareheic chicken (-ve), Lane27: O26 from diareheic chicken (+ve), Lane28: O119 from diareheic cattle (+ve) and Lane M: one kilo-base ladder marker (Stratagene, USA).

two bands at4399-4047bp, 6 isolates had one band at 14215-19594 bp,5 isolates had one band at 2015-2169 bp and 5 isolates had one band at 719-688bp as shown in Photo 4. *E. coli* isolated from cattle 7 had one band at 1986-1962 bp, 5isolates had one band at 2015-2092 bp, 5 isolates had one band at 553-585 bp,4 isolates had one band at 3927-3777 bp, 4 isolates had one band at 4512-4240bp and 4 isolates had one band at 677-656 bp. While the 3 O157 strains collected from buffaloes, the first strain had 5 fragments (27603,4399,2064,1967 and 661bp) the 2nd strain had 3 fragments (34684,2039 and 703bp) and the 3rd one had 6 fragments (14656,4001,3677,1876,1798 and 556bp).

Table 3: Comparison of EcoRI and HindIII for fingerprinting diareheic chicken *E.coli* strains

	Serotypes			
Isolate No.	EcoRI	HindIII	Number of bands by	Isolates positive for:
1	O111	7	3	*stx2*
2	O157	7	3	*stx2* and *eae*
3	O136	7	6	-
4	O27	9	3	*Eae*
5	O6	8	3	*Stx2*
6	O8	7	4	*stx1, stx2* and *eae*
7	O128	7	4	*stx1, stx2* and *eae*
8	O86	8	4	-
9	O26	9	4	*Eae*
10	O2	6	4	*stx1*
11	O8	7	4	-
12	O78	5	4	*stx1*

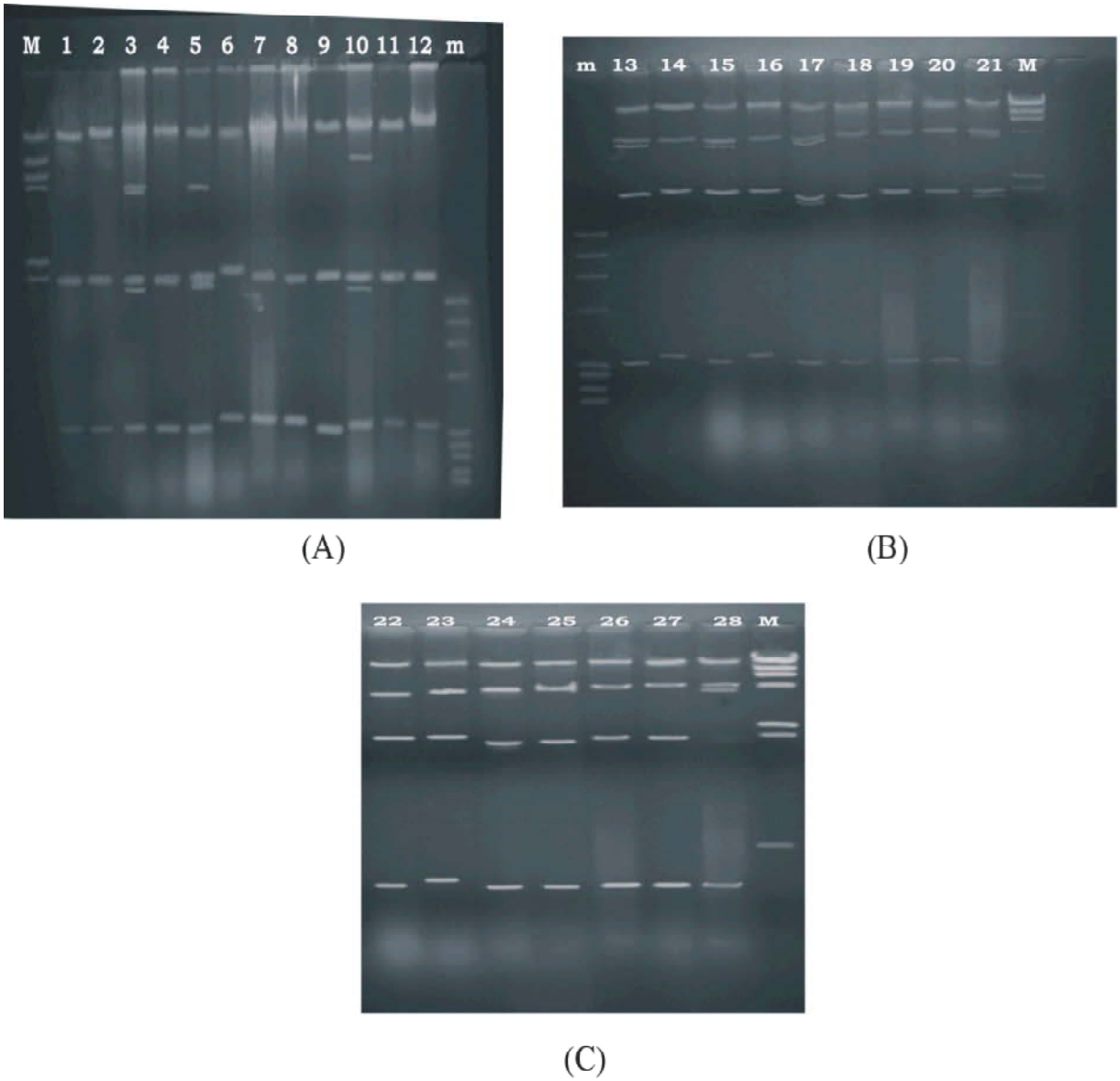
Table 4: Comparison of EcoRI and HindIII for fingerprinting cattle *E.coli* strains

			Number of bands by		
Isolate No.	Source of The strains	Serotypes	EcoRI	HindIII	Isolates positive for:
1	Mastitic milk	O115	6	3	*stx1* and *stx2*
2	Raw meat	O119	6	3	*stx1* and *stx2*
3	Raw meat	O112	6	3	*stx2*
4	Raw milk	O114	8	5	*stx2*
5	Raw meat	O111	8	3	*stx1, stx2* and *eae*
6	Raw milk	O124	9	5	*stx2*
7	Raw meat	O78	8	4	*eae*
8	Raw milk	O111	7	5	*stx1*and *stx2*
9	Raw meat	O157	9	4	-
10	Raw milk	O112	8	6	*stx2*
11	Feces	O119	7	4	*eae*
12	Mastitic milk	O157	5	4	*stx2*
13	Mastitic milk	O159	6	4	*stx1, stx2* and *eae*

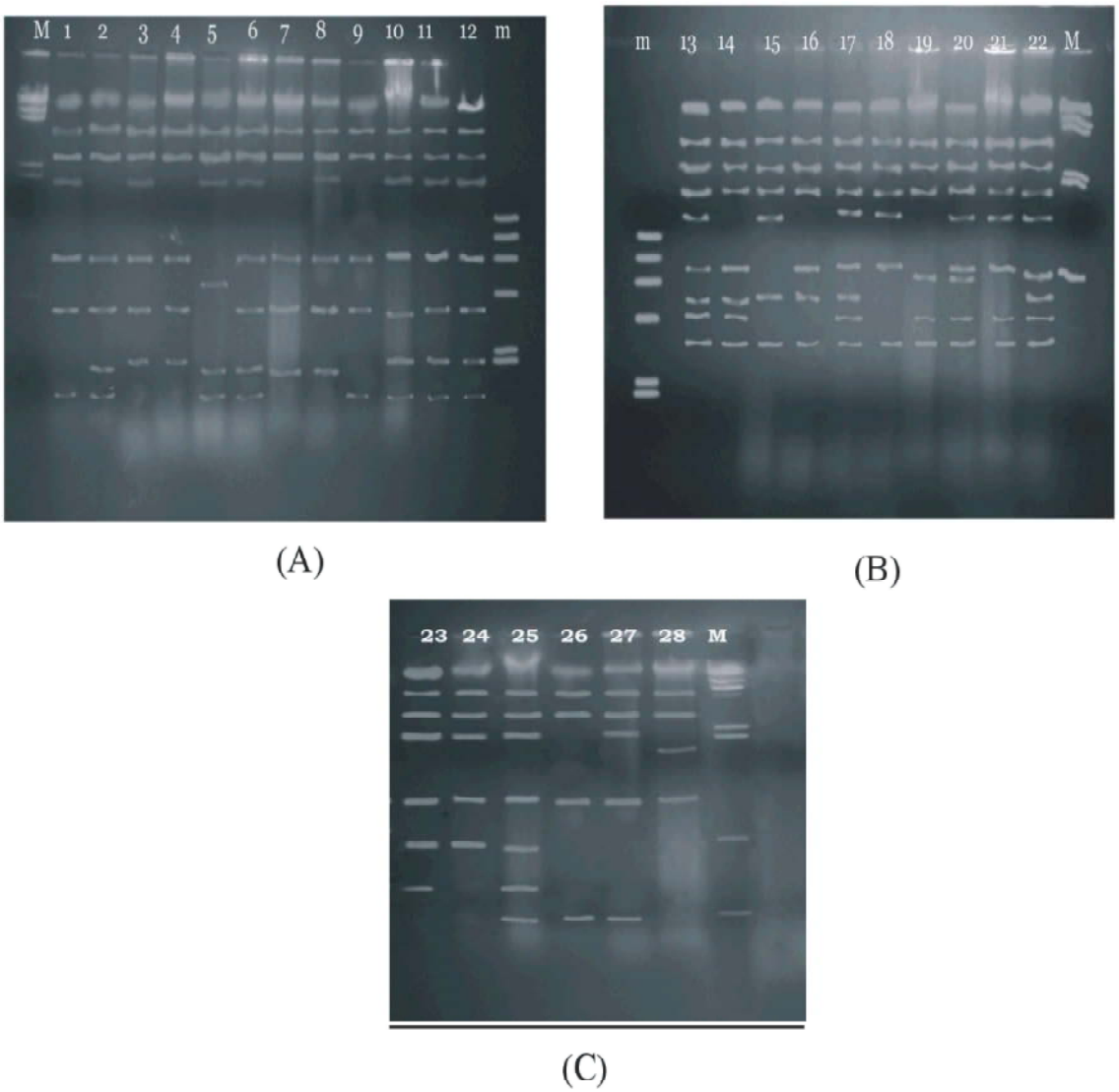
Table 5: Comparison of EcoRI and HindIII for fingerprinting diareheic buffaloes *E.coli* strains

		Number of bands by		
Isolate No	Serotype	EcoRI	HindIII	Isolates positive for:
1	O157	7	5	*stx1, stx2* and *eae*
2	O157	7	3	*stx1*
3	O157	9	6	*stx1*

Restriction Enzyme Analysis of Genomic DNA of *E.coli* Strains Using EcoRI: Among chicken strains 12 isolates had one band at 2839-2497bp, 9 isolates had one band at3751-3554bp, 8 isolates had one band at 1896-1699 bp,8 isolates had one band at 492-427 bp and 8 isolates had one band at 193-238bp as shown in Photo 5. Among cattle strains 12 had one band at 2898-2525 bp, 10 isolates had one band at 3713-3554 bp and 9 isolates had one band at 2031-1721 bp.



Photograph 4: Shows Ribotype patterns generated with HindIII digested genomic DNA of *E.coli* strains.
(A) Lane1: O111from diareheic chicken, Lane2: O157 from diareheic chicken, Lane3: O136 from diareheic chicken, Lane 4: O115 from cattle mastitic milk, Lane5: O157 from diareheic buffalo, Lane6: O27 from diareheic chicken, Lane7: O119 from cattle meat, Lane8: O157 from diareheic buffalo, Lane 9: O112 from cattle meat, Lane10: O114 from cattle milk, Lane11: O6 from diareheic chicken, Lane12: O111 from cattle meat and Lane M: one kilo-base ladder marker (Gibbco).
(B) Lane13: O124 from cattle milk, Lane14: O78 from cattle meat, Lane15: O111 from cattle milk, Lane16: O8 from diareheic chicken, Lane17: O157 from diareheic buffalo, Lane18: O128 from diareheic chicken, Lane19: O86 from diareheic chicken, Lane20: O157 from cattle meat, Lane21: O112 from cattle milk and Lane M: one kilo-base ladder marker (Gibbco).
(C) Lane22: O26 from chicken, Lane23: O119 diareheic from cattle, Lane24: O2 from diareheic chicken, Lane25: O8 from diareheic chicken, Lane26: O157 from cattle mastitic milk, Lane27: O159 from cattle mastitic milk, Lane28: O78 from chicken and Lane M: one kilo-base ladder marker (Gibbco).



Photograph 5: Shows Ribotype patterns generated with EcoRI digested genomic DNA of *E.coli* strains.
(A) Lane1: O111from diareheic chicken, Lane2: O157 from diareheic chicken, Lane3: O136 from diareheic chicken, Lane4: O115 from cattle mastitic milk,, Lane5: O157 from diareheic buffalo, Lane6: O27 from diareheic chicken, Lane7: O119 from cattle meat, Lane8: O157 from diareheic buffalo, Lane9: O112 from cattle meat, Lane10: O114 from cattle milk, Lane11: O6 from diareheic chicken, Lane12: O111 from cattle meat and Lane M: one kilo-base ladder marker (Gibbco).
(B) Lane13: O124 from cattle milk, Lane14: O78 from cattle meat, Lane15: O111 from cattle milk, Lane16: O8 from diareheic chicken, Lane17: O157 from diareheic buffalo, Lane18: O128 from diareheic chicken, Lane19: O86 from diareheic chicken, Lane20: O157 from cattle meat, Lane21: O112 from cattle milk, Lane22: O26 from diareheic chicken and Lane M: one kilo-base ladder marker (Gibbco).
(C) Lane23: O119 from diareheic cattle, Lane24: O2 from diareheic chicken, Lane25: O8 from diareheic chicken, Lane26: O157 from cattle mastitic milk, Lane27: O159 from cattle mastitic milk, Lane28: O78 from diareheic chicken and Lane M: one kilo-base ladder marker (Gibbco).

DISCUSSION

E. coli plays an important role in maintaining intestinal physiology. However, there are pathogenic strains that cause distinct syndromes of diarrheal disease [14]. Typical and atypical EPEC strains appear to constitute two distinct groups of *E. coli*. Atypical EPEC strains appear to be more closely related to STEC strains in their serotypeprofiles, animal reservoirs, the toxins that they produce and other genetic characteristics and as such are considered emerging pathogens.Although typical EPEC strains have only been recovered from humans, ruminants are a recognized source of atypical EPEC strains [15]. Furthermore, cattle and sheep are major reservoirs of STEC strains capable of causing severe infections in humans, such as HC and HUS. In the present investigation 28 *E. coli* isolates were collected from chicken (12 isolates), cattle (13 isolates) and buffaloes (3 isolates) to study their diversity. O2, O6, O8, O26, O27, O78, O86, O111, O128, O136 and O157 were typed from chicken. O78, O111, O112, O114, O115, O119, O124, O157 and O159 were identified from cattle while O157 were typed from buffaloes.

The collected strains were analyzed by PCR to detect *stx1*, *stx2* and *eae* genes. The ability of STEC strains to cause serious disease in human is related to the production of one or more shiga toxins (*stx1*, *stx2* and variants of *stx2*), encoded by lysogenic bacteriophages. Several other additional markers that might contribute to the pathogenicity of STEC have been described. These virulence markers include intimin, the product of the *eae* (for *E. coli* attaching and effacing) gene [16]. Photos (1-3) revealed that 12 out of 28 strains of *E. coli* isolated from different sources were positive to *stx1* with an incidence of 42.9% and 16 out of 28 strains of *E. coli* were positive to *stx2* with an incidence of 57.14% while, 10 out of 28 *E. coli* strains were positive in *eae* with an incidence of 35.71%. PCR analysis of 49 *E. coli* O157:H7 and 209 non-O157 isolates recorded by Oporto *et al.* [17] showed a different distribution of virulence genes, all *E. coli* O157:H7 were *stx*(2) gene-positive*, eaeA* was detected in 95.9% and the toxigenic profile *stx*(2)/*eaeA/E-hlyA* was present in 75.5% of the isolates, among the non-O157 STEC, prevalence of *eaeA* was significantly lower (5.3%) and *E-hlyA* was present in 50.2% of the isolates but only sporadically associated with *eaeA*.

The present data revealed the amplification of 130, 346 and 384bp specific for *stx1, stx2* and *eae* genes among 4,5 and 5 chicken strains respectively. Although genetically diverse, avian *E. coli* isolates share several genes or traits associated with virulence in chickens [18]. *E. coli* isolates were recovered by Cook *et al.* [19] from the skin-off chicken breasts, 33 (33%) of 99, than from the skin-on chicken breasts, 77 (41%) of 187 (P = 0.204) and VTEC was detected on a single skin-off chicken breast. Verotoxigenic *Escherichia coli* (VTEC) are a specialized group of *E. coli* that can cause severe colonic disease and renal failure. Their pathogenicity derives from virulence factors that enable the bacteria to colonize the colon and deliver extremely powerful toxins known as verotoxins (VT) or Shiga toxins (Stx) to the systemic circulation [20]. In cattle, Zschock *et al.* [21] observed that 45.8% strains possessed the *stx2* and 42.7% presented both *stx1* and *stx2* combination while the lowest percentage corresponded to strains harboring the *stx1* gene only (11.5%). In accordance to these studies, it is clear that 76.92% of cattle isolates had *stx2* and 38.46% possessed both *stx1* and *stx2*, also *stx1* was detected from 38.46% of the examined cattle strains.as shown in Table(4). Several authers have underlined the strong association between the carriage of *eae* and the capacity of STEC strains to cause severe human disease [12]. Amplification of 384bp fragment of the intimin gene could be carried out on bacterial cultures, did not require a long PCR protocol and was detected in 30.76% among cattle strains. In cattle population that we studied, only 2 of STEC strains were positive for the *eae* gene. Similar prevalence values were reported by others in healthy cattle [21, 22]. It is clear that O157 strain of chicken origin had *stx2* and *eae* genes, O157 from mastitic milk had *stx2* gene while the O157 buffaloes strains had *stx1* (100%), *stx2* (33.34%) and *eae* (33.34%) genes. *E. coli* O157:H7 causes hemorrhagic colitis and hemolytic uremic syndrome in humans [23].

In this study we used the collected 28 *E. coli* isolated from cattle, buffaloes and chicken obtained in the same geographical area to perform a detailed analysis of the molecular epidemiology of O157 and non O157 strains using RE analysis for identifying general similarities and differences in the genetic composition of *E. coli* populations. Among EcoRI between 5 to 9 fragments were obtained with digested genomic DNA of chicken and cattle and 7 to 9 fragments of buffalo isolates as shown in Tables (3-5) and Photo (5). Pradel *et al.* [24] indidiacted that PFGE had the greatest discriminatory power for non O157:H7 isolates. Although digestion of *E. coli* with either HhaI or HaeIII that was studied by Jenkins *et al.* [25] gave similar results, restriction patterns produced by HhaI showed no partially digested DNA fragments and better contrast between background and the bands of DNA. The SDS profile analysis of O157 strains obtained

with REs was indistinguishable between strains. O157 isolated from chicken, cattle and buffalo had 5-9 and 3-6 fragments when digested with EcoRI and HindIII respectively. Studies using molecular fingerprinting methods have revealed significant genomic diversity among O157:H7 isolates possessing the same known virulence determinants [26]. Recently, Dowd and Williams [27] provided additional data to support the existence of two diverse lineages of *E. coli* O157:H7, one of which may have lower pathogenic potential in relation to human hosts.

The three O111 strains (one from chicken, 2 from cattle) analyzed by HindIII and EcoRI. had one or two bands at 3777-2015bp. *E. coli* O111 can result in the very serious hemolytic uremic syndrome (HUS), in the United States annually, these serious types of *E. coli* outbreaks sicken approximately 110,000 people and cause about 90 deaths, says the CDC [28]. As many as 28 distinct RE patters were observed for 28 clinical *E. coli* isolates using HindIII and EcoRI enzymes as shown in Tables (3-5) and Photos (4 and 5). Most of the strains belonging to the same serotype produced identical ribotype patterns. The result indicates a distant relationship and ribotyping could reveal only minor differences in non O157:H7 strains belonging to the same serotype. Pradel *et al.* [24] findings indicated that the combination of stx_2-RFLP, stx_2 variant and plasmid profile analyses is as powerful as PFGE for molecular investigation of STEC diversity. Using two enzymes, Pradel *et al.* [24] identified eight different profiles for 25 isolates belonging to four serotypes, a total of 19 of 25 isolates could not be differentiated including six of seven O91:H10 isolates, six of seven O91:H21 isolates and 7 of 11 OX3:H21/H-isolates. This indicates that ribotyping which can be a useful tool for epidemiological investigation, was not able to discriminate between STEC isolates belonging to the same serotype The STEC strains of the same serogroup showed high genetic diversity [29]. A combination of serotyping and genotyping is the best way to identify an *E. coli* strain and is recommended in epidemiological studies [30].

Generaly the results obtained here in this work are promising and could be useful tool tracing *E. coli* infection and for epidemiological studies therfore, it is highly recommended that advenced DNA and protein sequence comparison tools have been tried for *E. coli* in order to obtain further discrimination required in addition, for outbreak studies as this pathogen has arisen as a major concern of food safty allover the world.

REFERENCES

1. Dwight, C., N. Hirsh, M. James and L. Richard, 2004. Veterinary Microbiology. 2nd Ed. Blackwell Publishing, pp: 61-68.
2. Borgattaa, B., N. Kmet-Lunacekb and J. Relloc, 2012. *E. coli* O104: H4 outbreak and haemolytic uraemic syndrome. Med. Intensiva. Jan. 11 Doi., 10.1016/j.medin.2011.11.022
3. Johnson, R.P., R.C. Clarke, J.P. Wilson, S.C. Read, K. Rahn, S.A. Renwick, K.A. Sandhu, D. Alves, M.A. Karmali, H. Lior, S.A. McEwen, J.S. Spika and C.L. Gyles, 1996. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *E. coli*. J. Food Prot., 59: 1112-1122.
4. Tarr, P.I. and M.A. Neil, 1996. Perspective problem of non-O157 *Shiga* toxin (verotoxin)-producing *E. coli*. J. of Infect. Dis., 174: 1136-1139.
5. Blanco, M., J.E. Blanco, J. Blanco, E.A. Gonzale, M.P. Alanso, H. Maas and W.H. Jansen, 1996. Prevalence and characterization of human and bovine verotoxigenic *E. coli* strains isolated in Galicia north western Spain. Eur. J. Epidemiol., 12: 13-19.
6. James, C.S., K.N. Stanley, H.E. Allison, H.J. Flint, C.S. Stewart, R.J. Sharp, J.R. Saunders and A.J. Maccarthy, 2001. Lytic and lysogenic infection of diverse *E. coli* and *Shigella* strains with a verocytotoxigenic bacteriophage. Appl. Environ. Microbiol., 67: 4335-4337.
7. Smith, R.A., D.D. Griffin and D.A. Dargatz, 2000. The risks and prevention of Contamination of beef feedlot cattle. Rev. Sci. Tech., 16: 359-368.
8. Koneman, E.W., S.D. Hllen, W. Janda, P. Schreckenberer and J.R. Winner, 1997. Diagnostic Microbiolgy. Sth Ed. J.B. Lippincott Co. Newyork.
9. Quinn, P.J., B.K. Markey, M.E. Carter, W.J.C. Donnelly and F.C. Leonard, 2002. Veterinary microbiology and microbial diseases. Black Well Scientific ublications, Oxford, London.
10. Sojka, W.J., 1965. *E. coli* in Domestic Animals and Poultry. 1st Ed. Commonwealth Agriculture.Bureau, Farnham, Royal Bucks, England.
11. Pollard, D.R., W.M. Johnson, H. Lior, S.D. Tyler and K.R. Rozes, 1990. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J. Clin. Microbiol., 28: 540-545.

12. Paton, A.W. and J.C. Paton, 1998. Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex-PCR assays for stx_1, stx_2, eaeA, enterohaemorrhagic E. colihlyA, rfb_{O111} and rfb_{O157}. J. Clin. Microbiol., 36: 598-602.
13. Sambrook, J., E. Fritsh and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Ed., Spring Harborlaboratory Press.
14. Gibotti, A., T.L. Tanaka, V.R. Oliveiria, C.R. Taddi and M.B. Martinz, 2004. Molecular characterization of enteroinvasive *E. coli IPA* genes by PCR-RFLP analysis. Brazilian J. Microbiol., 35: 74-80.
15. Trabulsi, L.R., R. Keller and T. Gomes, 2002. Typical and atypical enteropathogenic *E. coli*. Emerg. Infect. Dis., 8: 508-513.
16. Meichtri, L., E. Miliwebsky, A. Gioffre, I. Chinen, G. Chillemi, E.C. Beatriz, O. Marcelo, A. Masana-Cataldi, H.R. Rodriguiz and M. Rivas, 2004. Shiga toxin-producing *E. coli* in healthy young beef steers from Argentina: prevalence and virulence properties. Internat. J. of Food Microbiol., 96: 189-198.
17. Oporto, B., J.I. Esteban, G. Aduriz, R.A. Juste and A. Hurtado, 2008. *Escherichia coli* O157:H7 and non-O157 shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in Northern Spain. Zoon. Pub. Hlth., 55: 73-81.
18. Ngeleka, M., J.K. Kwaga, D.G. White, C. Whittam, R. Riddeel, A.A. Goodhope, B. Potter and Allan, 1996. *E. coli* cellulitis in broiler chickens: clonal relationships among strains and analysis of virulence associated factors of isolates from diseased birds. Infect. Immun., 64: 3118-3126.
19. Cook, A., J. Odumeru, S. Lee and F. Pollari, 2012. *Campylobacter, Salmonella, Listeria monocytogenes,* Verotoxigenic *Escherichia coli* and *Escherichia coli* Prevalence, Enumeration and Subtypes on Retail Chicken Breasts with and without Skin. Journal of Food Protection, 75: 34-40.
20. Goldwater, P.N. and K. Bettelheim, 2012. Treatment of Enterohemorrhagic Escherichia coli (EHEC) infection and hemolytic uremic syndrome (HUS) BMC Medicine. 10:12 doi:10.1186/1741-7015-10-12
21. Zschock, M., H.P. Hamann, B. Kloppert and W. Wolter, 2000. Shiga toxin producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properaties. Lett. Appl. Microbiol., 31: 203-208.
22. Pradel, N., V. Livrelli, C. De Champs, J.B. Palcoux, A. Reynaud, F. Scheutz, J. Sirot, B. Joly and C. Forestier, 2000. Prevalence and characterization of shiga toxin producing *Escherichia coli* isolated from cattle, food and children during a one year prospective study in France. J. Clin. Microbiol., 38: 1023-1031.
23. Padhye, N.V. and M.P. Doyle, 1992. *E. coli* O157:H7: epidemiology, pathogenesis and methods for detection in food. J. of Food Protect., 55: 555-565.
24. Pradel, N., K. Boukhors, Y. Bertin, C. Forestier, C. Martin and V. Livrelli, 2001. Heterogeneity of shiga toxin producing *E. coli* strains isolated from hemolytic uremic syndrome patients, cattle and food samples in central France. Appl. Environ. Microbiol., 67: 2460-2468.
25. Jenkins, C., A.J. Lawson, T. Cheasty, G.A. Willshaw, P. Wright, G. Dougan, G. Frankel and R. Smith, 2003. Sutyping intimin genes from enteropathogenic *E. coli* associated with outbreaks and sporadic cases in the United Kingdom and Eire. Mol. Cell. Probs. J., 17: 149-156.
26. Preston, M.A., W. Johnson, R. Khakhria and A. Borezyk, 2000. Epidemiologic subtyping of *E. coli* serogroup O157 strains isolated in Ontario by phage typing and pulsed field gel electrophorasis. J. Clin. Microbial., 38: 2366-2368.
27. Dowd, S.E. and J.B. Williams, 2008. Comparison of shiga-like toxin II expression between two genetically diverse lineages of *Escherichia coli* O157:H7. J. Food Prot., 71: 1673-1678.
28. CDC Centers for Disease Control and Prevention, 2008. Investigation of Multistate Outbreak of E. coli O157: H7 Infections.
29. Varela, G., I. Chinen, P. Gadea, E. Miliwebsky, M. Mota, S. vGonzález, G. González, M.J. Gugliada, C.C. Carbonari, G. Algorta, M. Bernadá, R. Sabelli, L. Pardo, M. Rivas and F. Schelotto, 2008. Detection and characterization of shiga toxin-producing *Escherichia coli* from clinical cases and food in Uruguay. Rev Argent Microbiol., 40: 93-100.
30. Lipman, L.J.A., D. Nijsa, T.J.G.M. Lam and W. Gaastra, 1995. Identification of *E. coli* strains from cows with clinical mastitis by serotyping and DNA polymorphism patterns with Rep and Eric primers. Vet. Microbiol., 43: 13-19.