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ZOONOTIC AND MICROBIOLOGICAL ASPECTS OF *ESCHERICHIA COLI* O:157 WITH SPECIAL REFERENCE TO ITS RAPD-PCR

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SUMMARY

A total of 160 animals, water, food and human samples were collected and examined for the presence of *Escherichia coli* O:157. The samples included 53 fecal swabs of farm animals (19 sheep, 18 buffaloes, 16 cattle), 21 water (6 wells, 7 public and 8 River Nile), 46 food (14 raw milk, 12 chicken meat and 20 crayfish, *Procambarus clarkii*), 20 surgically excised appendices and 20 human fecal swab samples. *E. coli* O:157 was detected in 3 out of 18 (16.7%) buffalo fecal samples, meanwhile, *E. coli* O:157 was neither detected from any examined cattle nor sheep fecal samples. Only one River Nile water sample out of 8 (12.5%) was positive for *E. coli* O:157, but the well and public water samples were free from it. Moreover, two isolates were recovered from raw milk and chicken meat samples (one isolate, each) with the percentages of 7.1% and 8.3% respectively. On the other hand, *E. coli* O:157 was not detected from any examined crayfish samples. Regarding human samples, one isolate from an appendix and 2 from human stool were identified with the percentage of 5% and 10%, respectively. Moreover, hemolysis was observed in 6 out of 9 (66.7%) *E. coli* O:157 isolates recovered from different sources. Meantime, *E. coli* non-O:157 were also identified from farm animal fecal swabs, River Nile water, food and human samples with varying percentages. Histopathological examination of appendices which were positive to *E. coli* O:157 revealed inflammatory cells infiltration in mucosa and submucosa. Random Amplified Polymorphic DNA (RAPD)- Polymerase Chain Reaction (PCR) fingerprinting of the isolated strains from different sources were carried out using five multiplex arbitrary primers. Molecular reproducibility of RAPD-PCR assay for *E.coli* O:157 strains recovered from different sources produced many bands ranged from 1 to 6 in number and 212 to 2000 bp in molecular weight. The

molecular similarity coefficients of human, bovine, avian and water origin *E. coli* O:157 strains were assessed. Similarity coefficient was identical (100%) between human and buffalo strains. Clonal relatedness ranged from 58 to 92% among other strains. The zoonotic importance of the isolated *E. coli* O:157 was discussed.

INTRODUCTION

Escherichia coli O:157 infection is a zoonotic food borne disease (Nelson et al., 1998) and emerged as a major public health concern in North America, Europe, and increasingly in other parts of the world (Coia, 1998). It may cause haemorrhagic uremic syndrome (HUS), (Karmali , 1989), hemorrhagic colitis (Nelson et al., 1998), thrombocytopenic purpura (Padhye and Doyle, 1992) and acute renal failure in childhood (Robson, 2000). *E.coli* O:157 belongs to the potential Enterohemorrhagic E. coli (EHEC) group of pathogenic *E.coli* (Acheson and Keusch, 1994) and is common verocytoxin producing *E. coli* (Willshaw et al., 2001). As previously reported *E. coli* O:157 has a high virulence in human and at a very low infective dose which presents a major threat (Attenborough and Matthews, 2000). Outbreaks of *E.coli* O:157 infections were documented in at least 14 countries (Karmali, 1989). As previously reported, calf-to-human transmission of *E.coli* O:157 infections occurred by contact (Renwick et al., 1993). Cattle are tolerant to infection with *E. coli* O:157 (Pruimboom-Brees et al., 2000). *E.coli* O:157 became reportable in Wisconsin, USA on April, 2000 (Proctor and Davis, 2000). *E.coli* O:157 has been isolated from raw milk (Abdel-Khalek et al., 2001), salmon roe (Makino et al., 2000), appendix (Volinsky et al., 1998), chicken meat (Radu et al., 2001), water (Leclerc et al., 2002) and sheep (Chapman et al., 2001).

Although serotyping is used as typing method for epidemiological purposes and surveys of pathogenic bacteria (Soto et al., 1999), it is not enough to further differentiate *E.coli* isolates (Maurer et al., 1998). Bovine isolates showed a clonal relatedness to *E.coli* O:157 strains isolated from patients in Germany and Czech Republic (Bielaszewska et al., 2000). Molecular typing methods were used to investigate transmission of *E.coli* O:157 from cattle to human (Louie et al., 1999). E.coli can be differentiated into genetically distinct isolates using procedures that identify differences in the genetic composition of microbial population (Samira et al., 2001). One of these techniques involves Random Amplified Polymorphic DNA (RAPD) (Welsh and McClelland, 1991). A number of studies have indicated that RAPD method is useful in genotype identification (Woods et al., 1993). Furthermore, no prior DNA sequence information is required. The resulting polymorphism provides a simple approach for performing DNA fingerprinting. This method randomly identifies genomic DNA fragments (Samira et al., 2001).

Because farm animals, water, food of animal origin and rural environment are potential sources for zoonotic *E.coli* O:157 infection, the present study was undertaken to determine the occurrence of this pathogen in different urban and rural niches at Zagazig, Egypt. Also application of Polymerase Chain Reaction (PCR) fingerprinting-RAPD to detect the clonal relatedness between the isolated strains was assessed.

MATERIAL AND METHODS

A) Sampling:

A total of 160 animals, water, food and human samples were collected from different localities at Sharkia Province. From animals, 53 individual fecal swabs (19 sheep, 18 buffaloes, 16 cattle) were taken from rectum. Fecal swabs were directly immersed into enrichment broth (Buffered peptone water supplemented with Vancomycin 8 mg/l and Cefsulodin 10 mg/l) (Karuniawati, 2001). From water, 21 samples comprising 6 wells, 7 public and 8 River Nile tributaries water were obtained. Water samples were collected in sterile colorless labeled glass bottles provided with a ground glass stopper. From food, 46 samples consisting of 14 raw milk, 12 chicken meat, and 20 freshwater crayfish (*Procambarus clarkii*) were collected. Individual raw milk samples were collected in sterile screw-capped bottles. Chicken meat samples were collected from different chicken markets in sterile polyethylene bags. Freshwater crayfish were collected from rice fields, agriculture drainage canals and River Nile tributaries. The fish samples were identified and packed separately in sterile polyethylene bags. Regarding human samples, 20 surgically excised appendices were obtained from appendectomies at Zagazig University Hospitals, Zagazig, Egypt. The patients ages ranged from 12 to 34 years; the total leucocytic counts of patients ranged from 4.5x103 to 14x103 /cc blood. Two appendiceal specimens were collected immediately from each operation; one was immersed in a disposable container containing enrichment broth for bacteriological isolation and the second was kept in formalin for histopathological examination. Moreover, 20 fecal swabs were collected from human suffering from gastrointestinal disorders, and admitted to some clinic medical laboratories at Zagazig city, Sharkia Province. Fecal swabs were directly immersed into 9 ml enrichment broth. All water, food, animal and human samples were transferred to Zoonoses and Microbiology Departments, Faculty of Vet. Med., Zagazig University, of sampling day, under complete aseptic conditions in sterile, clean and dry ice containers with a minimum of delay, and subjected to bacteriological examination.

B) Preparation and enrichment procedures (Karuniawati 2001).

Water, milk: Twenty five ml from each sample was mixed with 225 ml enrichment broth.

Chicken meat: Twenty five g of chicken meat were homogenized well with 225 ml of enrichment broth.

Crayfish: Each crayfish was washed and scrubbed under tap water. The surface of each crayfish was sterilized by dipping in 70% ethanol, and opened aseptically. Ten grams specimens were taken from edible parts of each crayfish in a sterile aluminum foil, then blended for 2 minutes in 90 ml enrichment broth.

Appendices: Portions (about 5 g) of each excised appendix was blended for 2 min in 45 ml enrichment broth.

Human and animal fecal swabs: Each fecal swab was directly transferred to 10 ml enrichment broth.

After preparation, all animal, water, food and human samples were incubated at 37°C for 6 hours.

C) Isolation of Enterohaemorrhagic E.coli (EHEC)(Karuniawati, 2001).

After enrichment, 0.1 ml portion of cultured homogenate was streaked onto MacConkey sorbitol agar plates (Oxoid) supplemented with cefixime tellurite selective supplement (Oxoid SR172E), then were incubated at 37°C for 24 hours.

After incubation, sorbitol positive and negative colonies (up to 5 colonies each) were picked, purified by subculturing onto Standard-I-agar plates and incubated at 37°C overnight and subjected to the following identification tests:

1.Identification of *E. coli*:

The following tests (Gram`s stain, Lactose fermentation, Indole, Methyl red, Glucose fermentation, Four-Methyl-umbelliferyl-β-glucronidase (MUG) activity and Citrate utilization) were used for morphological and biochemical identifications of the suspected *E.coli* colonies (Szabo et al., 1986).

2- Enterohaemolysin production test:

The pure culture was streaked onto Enterohaemolysin agar plates (Heipha, Heidelberg, Germany) and incubated at 37°C. The colonies show (-haemolysis on enterohaemolysin agar after 4 hours, then enterohaemolysis after 18 hours are positive for enterohaemolysin (Karuniawati, 2001).

3. Serological identification of the *E.coli* isolates:

Serotyping was done by slide agglutination test using monovalent test sera for *E.coli* O:157 (Behring, Marburg) kindly gifted by Dr. W. Philipp, Animal Hygiene Institute, Hohenheim University, Germany.

Histopathological examination of appendices:

Small portions of excised appendix were taken for histopathological examination. They were fixed in 10% buffered formalin, embedded in paraffin wax blocks, serially sectioned at 5 μm and stained with haematoxyline and eosin (Culling, 1983).

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RAPD-PCR Fingerprinting (Samira et al., 2001):

RAPD-PCR fingerprinting was performed at Biotechnology Center, Faculty of Vet. Med., Cairo University, Egypt. RAPD-PCR fingerprinting was done on 6 *E.coli* O·157 strains recovered from different sources.

Genomic DNA extraction:

Chromosomal DNA was isolated from *E.coli* O:157 strains using proteinase K extraction (Bridge, 1996). Briefly, pure cultures of bacteria in trypticase soya broth (TSB) were streaked onto McConkey agar. The inoculated plates were incubated for 24 hours at 37°C. One single colony from the plate was subcultured into 300 ml of TSB and it was incubated for 24 hours at 37°C, at the end of incubation, bacteria were collected by centrifugation at 13.000 X g for 2 minutes and suspended in equal volume of TE (Tris-HCl, 10 mM; EDTA, 1 mM-pH, 8.0) buffer. The following solutions were sequentially added: 100 µl of lysozyme solution (final concentration 10 mg/ml), 100 µg of proteinase K enzyme (0.3 mg/ml) with 1% SDS (Sodium dodecyl sulphate). The lysate was extracted once with chloroform/ isoamyl alcohol (24:1 V/V), one with equilibrated pheno/ chloroform/isoamyl alcohol (25:24:1 V/V/V). The aqueous phase was mixed with 0.6 volume of isopropanol and incubated at -20 oC for 30 min. The precipitated DNA was spooled out, rinsed in 70% ethanol and dissolved in 0.5 ml of TE buffer.

Arbitrary primers (AP):

Five oligonucleotides primers as mentioned in Table (1) were obtained from (MWG-Biotech AG) and were used as pooling primers for RAPD amplification.

Table (1): Multiplex oligoneucleotides primers used for RAPD-PCR analysis of *E.coli* O157 recovered from different sources.

No.	Code	Primers sequence (5'3')	Size	Melting point	Guanine-Cytosine%
1	1247	5'AAGAGCCCGT3'	10-mer	32°C	60
2	1281	5'AACGCGCAAC3'	10-mer	32°C	60
3	1283	5'GCGATCCCCA3'	10-mer	34°C	70
4	1290	5'GTGGATGCGA3'	10-mer	32°C	60
5	D14803	5'AAACGGTTGGGTGAG3'	15-mer	47.8°C	53.3

RAPD-PCR Reaction (Soto et al., 1999):

The PCR reaction mix consisted of 0.25 mg/ml bovine serum albumin (BSA), 3nM MgCl2, 50 mM Tris (pH 8.3), 0.2 mM nucleotides, 0.1 mM primers and 0.5 unit Taq DNA polymerase. one microliter of sample DNA template (containing 100µg) was added 100 µl of the PCR reaction mix. The reaction mixture was overlaid with min-

eral oil, and was incubated in a thermal cycler as follows:

* The 1st initial cycle: 94°C for min, 35°C for 2 min and 72°C for 2 min.
* The consequent 35 cycles: 94°C for 30 seconds (denaturation), 35°C for 1 min (annealing) and 72°C for 2 min (extension).
* The final extension step at 72°C for 5 minutes then kept at 4°C (hold temperature).

After the amplification was completed, the amplified product was analyzed on agarose gel consisting 1.5% agarose (GIBCO-BRL; Life Technologies) and 0.5 μg of ethidium bromide per ml in 1x Tris acetic acid EDTA (TAE) buffer. The samples were electrophoresed at a constanbt 100 volts for one hour, visi;aized under ultraviolet and photographed.

Analysis of PCR-generated patterns:

Analysis of PCR-generated patterns were assessed as described previously by (Versalovic et al., 1994) who performed quantitative comparison of DNA fingerprint patterns by measuring fragment band position of the PCR product after electrophoresis. The similarity coefficient of *E.coli* O:157 strains recovered from different sources (Table, 8) was calculated according to simple matching coefficient previously described by (Muckendi, 1996), for closely related bacteria (from the same species).

This defines fingerprint similarity (s) as, $s = (a + d)/(a + b + c + d)$ where:

a= number of bands in both fingerprints being compared.

b= number of bands in the first fingerprint which do not appear in the second fingerprint.

c= number of bands in the second fingerprint which do not appear in the first fingerprint.

d= number of bands that do not appear in both fingerprints.

RESULTS

Table (2) shows the occurrence of *E.coli* O:157 in fecal samples of farm animal. *E.coli* O:157 was detected in buffalo with 16.7% (3/18), although it was not detected from any examined cattle nor sheep.

Table (3) shows the occurrence of *E.coli* O:157 in water samples. *E.coli* O:157 was recovered only from a River water samples with a percentage of 12.5 (1/8), but well and public water samples were free from it.

Table (4) shows the occurrence of *E.coli* O:157 in raw milk, chicken meat and crayfish samples. Two isolates were recovered from raw milk and chicken meat samples (one isolate, each) with the percentages of 7.1% and 8.3% respectively. On the other hand, it was not detected in any crayfish samples.

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Table (5) shows the occurrence of *E.coli* O:157 in human appendix and stool samples. One isolate from an appendix and 2 from human stool were recovered with the percentages of 5% and 10% respectively.

Table (6) shows frequency distribution of enterohemolysin producing *E.coli* O:157 isolates. Six out of 9 (66.5%) *E.coli* O:157 isolates were enterohemolysis positive. These six isolates were identified from buffalo feces, River Nile water, raw milk, chicken meat, human appendix and human stool (one isolate, each).

Table (7) shows the epidemiological data with reproducibility of RAPD-PCR for *E.coli* O:157 recovered from different sources. RAPD-PCR patterns showed 6 bands with each River Nile and appendix strains, while it showed only one band with each human and buffalo fecal strains.

Table (8) and Figure (2) show RAPD profile of *E.coli* O:157 strains generated by multiplex PCR. The molecular weight of reproduced bands ranged from 212 to 2000 bp.

Table (9) shows similarity coefficients of *E.coli* O:157 strains from different sources. It was identical (100%) between human and buffalo strains. Clonal relatedness (s) ranged from 58 to 92 in other strains.

Figure (1) shows histopathological examination of appendix which is positive for *E. coli* O:157. It revealed destruction and desquamation of the epithelial cells, metaplasia of the glandular epitheli-

Table (2): Occurrence of *E.coli* O:157 in fecal samples of farm animals.

Source of fecal samples	No.	*E.coli*		*E.coli* O:157		Non-*E.coli* O:157	
		No. of positive	%	No. of positive	%	No. of positive	%
Sheep	19	6	31.58	0	0	6	31.58
Cattle	16	1	6.25	0	0	1	6.25
Buffalos	18	8	44.44	3	16.66	5	27.77
Total	53	15	28.3	3	5.66	12	22.64

Table (3): Occurrence of *E.coli* O:157 in water samples.

Source of water	Source of samples	No. of positive	%
Well	6	0	0
Puvlic	7	0	0
River	8	1	12.5
Total	21	1	4.76

Table (4): Occurrence of *E.coli* O:157 in raw milk, chicken meat and crayfish samples.

Source of food samples	No. of samples	*E.coli*		*E.coli* O:157		Non-*E.coli* O:157	
		No.	%	No.	%	No.	%
Raw milk	14	2	14.2	1	7.1	1	7.1
Chicken meat	12	3	25	1	8.3	2	16.7
Cray fish	20	5	25	0	0	5	25
Total	46	10	21.74	2	4.35	8	17.39

Table (5): Occurrence of *E.coli* O:157 in human appendix and stool samples.

Source of samples	No. of samples	*E.coli*		*E.coli* O:157		Non-*E.coli* O:157	
		No.	%	No.	%	No.	%
Appendix	20	3	15	1	5	2	10
Stool	20	5	25	2	10	3	15
Total	40	8	20	3	7.5	5	12.5

Table (6): Frequency distribution of enterohemolysin producing *E. coli* O:157 isolates.

Source	Total isolates	Enterohemolysin positive	
		No.	%
Buffalo feces	3	1	33.3
River Nile water	1	1	100
Raw milk	1	1	100
Chicken meat	1	1	100
Human appendix	1	1	100
Human stool	2	1	50
Total	9	6	66.6

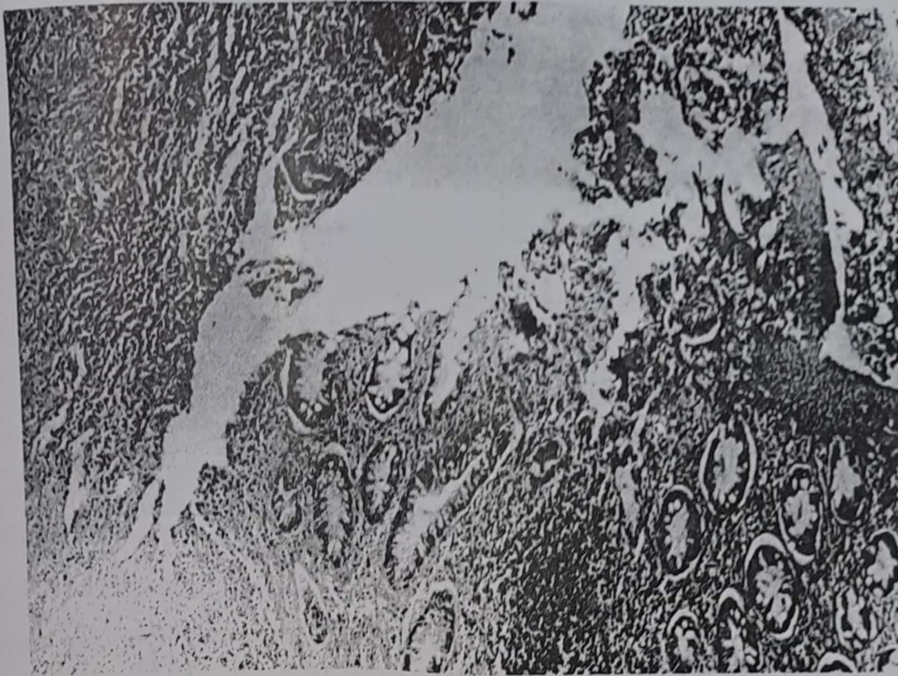

Fig. (1): Appendix is positive for *E.coli* O:157 serotype showing desquamation of the lining epithelium and inflammatory cells infiltration.

Table (7): Epidemiological data of *E.coli* O:157 strains and RAPD-PCR products.

No. of lane	code	Isolation date	Source	Amplicon bands (No.)
1	RW	15.12.2002	River water	6
2	App	28.12.2002	Appendix	6
3	HS	10.01.2002	Human stool	1
4	RM	02.01.2003	Raw milk	2
5	BF	05.01.2003	Buffaloe feces	1
6	CM	09.01.2003	Chicken meat	4

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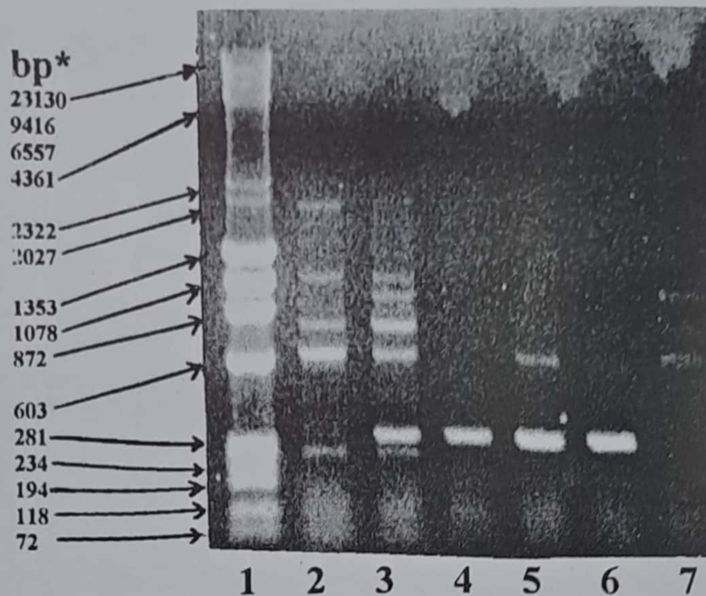

Fig. (2): Ethidium bromide-stained agarose gel electrophoresis showing RAPD-PCR profiles of *E.coli* O:157 genotypes by 5 random primers. Lane 1, *Hind III* digest& *Hae III* digest marker; lane 2, RW; lane 3, App; lane 4, HS; lane 5, RM; lane 6, BF and lane 7, CM were described in Table (7). * bp=base pair.

Table (8): RAPD profile of *E.coli* O:157 strains generated by multiplex PCR.

Amplified bands No. (Amplicon)	Amplicons size* (pb)	RWx1	RWx2	RWx3	RWx4	RWx5	RWx6
1	2000	+	-	-	-	-	-
2	1155	+	+	-	-	-	-
3	1096	-	-	-	-	-	+
4	1011	-	+	-	-	-	-
5	927	+	-	-	-	-	-
6	857	-	-	-	-	-	+
7	828	+	+	-	-	-	-
8	651	+	+	-	+	-	+
9	325	-	+	+	+	+	-
10	255	+	+	-	-	-	-
11	212	-	-	-	-	-	+

1XRW = River water
2XApp = Appendix
3XHS = Human stool
4XRM = Raw milk
5XBF= Buffalo feces
6XCM = Chicken meat
* Molecular weight

Table (9): Similarity coefficients of *E.coli* O:157 strains from different sourced

	% of similarity				
	App	HS	RM	BF	CM
RW	73	61	69	61	58
App		69	69	69	58
HS			92	100	69
RM				92	73
BF					69

um of the intestinal glands to goblet cells which appear vacculated and ruptured. Lymphocytic infiltration to lamina propria and submucosa with neutrophils, hyperplasia of lymphoid follicles, intermascular edema, necrotic enteritis, fatty infiltration and hyperplasia of lymphoid follicles were also showed.

DISCUSSION

The present study investigated the epidemiology of *E.coli* O:157 strains in farm animals, water, food and human samples. For this purpose, bacteriological, serological and molecular biological examination of the *E.coli* O:157 were carried out. The results of occurrence of *E.coli* O:157 in farm animal fecal samples are shown in Table (2). Three buffalo fecal samples (16.7%) were positive to *E.coli* O:157, while it was not detected in cattle nor sheep fecal samples. These results are supported by Hancock et al. (1997) who found low prevalence in cattle, ranged from 0 to 5.5%. However, Schakespeare (2002) reported that 1.7% of sheep fecal samples are positive to *E.coli* O:157. Moreover, the obtained results reflect the role of the buffalo as a potential reservoir of *E.coli* O:157. The recorded variation of its frequency could be interpreted as intermittent fecal shedding in cattle as recorded by Zhao et al. (1995) and Faith et al. (1996). From the zoonotic point of view, we need to reduce carriage shedding of *E.coli* O:157 by ruminant to decrease the incidence of zoonotic infections as mentioned by Synge (1997). Since 1983, there have been numerous reports on the occurrence of *E.coli* O:157 in ruminants. Links to human infection have been described and they may be the result of direct contact with animals or their feces or of contamination of the human food-chain (Stewart and Flint, 1999). On the other hand, the isolation of *E.coli* O:157 from cattle and sheep with the frequency of 15.7 and 2.2% was previously recorded by Chapman (1999).

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It was evident from the results achieved in Table (3) that *E.coli* O:157 was isolated from one of River Nile water sample with a percentage of 12.5%.. Following a water-borne outbreak in Africa, *E.coli* O:157 was isolated from 18.4% of River-water samples and a domestic water-storage drum. The contamination of water was thought to have occurred by cattle carcasses and manure into surface waters (Isaacson et al. 1993). Moreover, an outbreak of infections associated with swimming in lake water was previously reported by Keene et al. (1994). The examined public and well water samples were free from *E.coli* O:157 (Table 3) which reflect the efficiency of municipal water disinfection by chlorination and the absence of underground fecal pollution in examined well water. The obtained results were supported by the results of Taha (2002) who failed to detect *E.coli* O:157 in water. However, this result disagrees with that found by Raina et al. (1999) who isolated *E.coli* from well water implicated in gastrointestinal illness.

The occurrence of *E.coli* O:157 in raw milk, chicken meat and crayfish samples are summarized in Table (4). Two *E.coli* O:157 isolates were recovered from raw milk and chicken meat samples (one isolate, each) with the percentages of 7.1 and 8.3%, respectively, meantime, it was not detected in crayfish samples. This result was supported with the findings of Chapman et al (1993) and Padhye and Doyle (1991). Meanwhile, a lower percentage (2.4%) in milk were reported by Abdel-Khalek et al. (2001). Several outbreaks of infections linked with untreated milk were recorded (Chapman et al., 1993; Upton and Coia, 1994). The results recorded in Table (4) showed that *E.coli* O:157 was not detected in crayfish samples, whereas, *E.coli* non-O:157 was recovered from five samples (25%). This result agreed with that of Sanahth et al. (2001) who found that *E.coli* non O:157 is more common in fresh fish and clams. The public health importance of *E.coli* O:157 in fish was mentioned by Makino et al. (2000) who recorded an outbreak in Japan caused by salmon roe contaminated with *E.coli* O:157. Consumption of fish appeared to be a risk factor for infection although consumption of shellfish showed a negative association, corresponding to a decrease in risk. (Pierard et al. 1999). Elmossalami and Emara (1999) isolated *E.coli* from 40% of crayfish samples.

From the results achieved in Table (2,3,4), one could conclude that *E.coli* O:157 is frequently isolated from animals, water and food samples. The presence of virulent strains of *E.coli* O:157 in animals, water and food may play an important role as a reservoir and source of human infections.

Concerning the occurrence of *E.coli* O:157 in human appendix and stool samples. Table (5)

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showed that one out of 20 (5%) examined excised appendices and two out of 20 (10%) stool samples were positive for *E.coli* O:157. Volinsky et al. (1998) recorded a hemorrhagic uremic syndrome (HUS) caused by *E.coli* O:157 after perforated appendix. Moreover, Puskar et al. (1997) studied the public health significance of *E.coli* in appendix which could spread from appendix to the retroperitoneal space and may invade the urinary tract causing infection in patients with acute appendicitis. Saxen et al. (1996) described that colonization of the gut by virulent *E.coli* may be a prerequisite for the development of appendicitis. Taha (2002) recovered *E.coli* O:157 from 9.1% human stool. From the public health point of view, detection of *E.coli* O:157 in excised inflamed appendix and from human stool may reflect its potential role in appendicitis. Moreover, its occurrence in stool may represent a potential source of *E.coli* O:157 infection for human.

It was evident from the results achieved in Table 6 that 6 (66.6%) out of 9 *E.coli* O:157 were enterohemolysin positive which is supported by the results of Karuniawati (2001) and Mohamed (2002).

The results recorded in Tables (2-5) showed that *E.coli* non-O:157 were also identified from animal fecal swabs, River Nile water, food and human samples with varying percentages.

The histopathology of the appendix which was positive for *E.coli* O:157 revealed destruction and desquamation of the epithelial cells, metaplasia of the glandular epithelium of the intestinal glands to goblet cells which appear vacculated and ruptured. Lymphocytic infiltration to lamina propria and submucosa with neutrophils, hyperplasia of lymphoid follicles, intermascular edema, necrotic enteritis, fatty infiltration and hyperplasia of lymphoid follicles were also shown (Fig.2).

Table (7) illustrates the epidemiological data with reproducibility of RAPD-PCR for *E.coli* O:157 recovered from different sources. RAPD-PCR patterns showed 6 bands with each River Nile and appendix strains, while it showed only one band with each human and buffalo fecal strains.

Moreover, the molecular weight of reproduced bands ranged from 212 to 2000 bp (Table, 8 and Fig. 2). RAPD-PCR is a very exciting development in the field of molecular biology and it offers several advantages over other methods used for studying genetic variability because it is not limited to a single locus but theoretically detects polymorphisms across the whole genome (Baruffi et al., 1995). In addition, the amplified bands of avian serotypes ranged from 2101 to 165 bp using the same multiplex primers (Samira et al. 2001), which were nearly similar to the molecular weight of our PCR product (2000 to 212). On the

other hand, the obtained RAPD amplicon (Tables, 6,7) is lower than the reproduced profile of *E.coli* obtained by Samira et al. (2001) whose results ranged from 1 to 10 bands. This may be referred to difference in origin and she examined different serotypes but in the present study, we tested only one serotype.

The obtained genotypes of *E.coli* O:157 are supported by Navas-Nacher et al. (2001) who characterized E.coli by RAPD fingerprinting. Radu et al. (2001) used RAPD to compare *E.coli* O:157 using single primer, and reported that this method aids in the epidemiological clarification of the *E.coli* O:157. Grif et al. (1998) subtyped *E.coli* O:157 into 2 clusters from different sources using RAPD using pair primers. Maurer et al. (1998) identified genetic differences among avian *E.coli* by RAPD whereas conventional bacteriological methods failed to differentiate these isolates. In addition, Bando et al (1998) characterized *E.coli* using RAPD-PCR. Makino et al. (2000) analyzed *E.coli* O:157 by RAPD tests among all clinical isolates and clarified a homologous origin of contamination. Kurazono and Makino (1997) used RAPD test to investigate the diversity among *E.coli* O:157.

The similarity coefficients of *E.coli* O:157 strains from different sources are shown in Table (9). The clone of BF and HS was identical (100%) in similarity which may be due to its emergence from a heterogenous population of ancestor *E.coli* O:157 strains. The high genomic homogeneity (92%) between RM with HS and BF with RM may prospect the role of raw milk and buffalo feces as potential zoonotic sources. The obtained results denote that RAPD test may allow discrimination of different strains. So it is useful genetic marker for tracing the sources of *E.coli* O:157 infections. It could be concluded that, *E.coli* O:157 could be detected in buffalo feces, River Nile water, raw milk, chicken meat, appendix and human stools. The isolation of E. coli O:157 from human, animal, food and water sources may represent a potential public health hazard. The molecular typing reveals that some human strains are related to animal strains especially buffaloes origin which may reflect the major role played by buffaloes in Sharkia Province. The applied RAPD technique to discriminate between different strains of the same serotypes and its reproducibility was effective in molecular comparison. Fecal contamination during the recovery and processing of food derived from animals should be prohibited. Also rapid cooling and sufficient treatment, avoid post processing contamination are paramount in controlling food-borne O:157 infections. Further studies are needed to assess the period of communicability of *E.coli* O:157 in both rural and urban niches to achieve biosecurity together with cutting the food-chain cycle.

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