Characterization of Diarrheagenic Escherichia Coli Serotypes Isolated from Poultry and Humans

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Abstract

Virulent strains of *Escherichia coli* cause colibacillosis, an important disease in poultry which also cause diarrheagenic episodes and deaths in humans. Here we present the prevalence and characteristics of *E. coli* strains isolated from different poultry management systems and in-contact humans. A total of 1,528 samples from broilers (908), ducks (253), backyard chickens (117) and in-contact humans (250) were collected, and isolates from these sources were tested to detect verocytotoxigenic and virulence genes stx_1, stx_2, *eae*, ST and LT. The prevalence of *E. coli* was 43.6 %, 57.7 %, 53.0 % and 7.2 % in broilers, ducks, backyard chickens and humans respectively (P < 0.05; X^2). Frequency ranged from 41.5 % (78/188) in unabsorbed yolk sacs to 49.9 % (188/371) in septicemic lesions (P > 0.05; X^2). Thirty of the 100 *E. coli* isolates tested were verocytotoxigenic, 31.7 % (26/82) and 22.0 % (4/18) from poultry and human samples respectively, with respective 20.0 %, 53.3 %, 26.7 %, and 46.7 % for stx_1, stx_2, stx_1/stx_2 and *eae* virulence genes (P < 0.05; X^2). Serogroups O2 (30.0 %), O128 (16.0 %), O125 (13.0 %) and O114 (11.0 %) were the predominant isolates in both poultry and human. Poultry species are potential reservoirs of human toxigenic *E. coli.*

Keywords: *E. coli;* VTEC strains; Serogroups; Virulence genes; Multiplex PCR

Introduction

Six categories of *E. coli* have been widely associated with diarrhea in several epidemiological studies. These groups include the enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC) which are generally regarded as Shiga toxin-producing *E. coli* (STEC) and diffusely adherent *E. coli* (DAEC) based on the pathogenic mechanisms [1].

Avian pathogenic *E. coli* (APEC) strains produce serious extra-intestinal lesions in poultry causing high morbidity and mortality in chickens and turkeys, leading to considerable economic losses [2]. In addition, *E. coli* is a common and an important pathogen that causes at least 5% of mortalities in poultry flocks [3], including air sacculitis, colisepticemia, synovitis, osteomyelitis and cellulitis [4]. Poultry has also been reported to be a reservoir for extra-intestinal pathogenic *E. coli* O45:K1:H7-B2-ST95 in humans [5] and the zoonotic potential of APEC strains is under consideration [6].

EPEC infections are associated with outbreaks of neonatal diarrhea in infants from developing countries with clinical presentations that can range from self-limiting diarrhea to chronic enteritis and wasting [1]. Shiga toxins (stx) produced by EHEC strains induce local damage in the colon resulting in hemorrhagic diarrhea, necrosis and intestinal perforation. In addition, stx target the kidneys where they damage the renal endothelial cells and occlude the microvasculature. The resulting nephritis leads to hemolytic uremic syndrome (HUS) which consists of acute renal failure, thrombocytopenia and micro angiopathic hemolytic anemia [1].

Although molecular methods for identifying specific virulence genes are valuable procedures, serotyping remains a useful tool for epidemiologic studies. Globally, numerous studies have been conducted to determine the *E. coli* serotypes most frequently associated with disease in poultry [7]. PCR has been developed to detect specific genes important in the virulence of microorganisms [8]. It has also been reported that the similarity of virulence factors between septicemic *E. coli* strains in humans and avian pathogens represents a significant zoonotic risk [9]. Studies by others have established similarities in the characteristics (serogroups, virulence genes and antibiograms) of *E. coli* strains isolated from poultry and poultry farm workers [10, 11, 12].

Therefore, the objectives of this study were to investigate the potential zoonotic spread of *E. coli* serogroups, and to compare the characteristics virulence genes of isolates from poultry and in-contact human beings, in addition the study assessed the usefulness of VCA in the detection of VTEC (vero toxigenic *E. coli*).

Methodology

Sample collection

A total of 1,528 samples were collected from rural communities in Kom Hamada and El-Delengate centers, El-Behera Province, Egypt between May 2012 and April 2013. Overall, a total of 1278 cloacal samples were collected from poultry comprising 908 from broilers, 253 from ducks and 117 from backyard chickens. Litter samples were collected in sampling polyethylene bags, swabs were obtained from cloacae of live broilers or ducks and from septicemic lesions and unabsorbed yolk sac of recently dead birds, were dipped in buffered peptone water (BPW) (Oxoid Ltd., UK) and transported immediately to the laboratory of the Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Sadat University, Egypt.

Human stool samples were collected from the health centers and clinical laboratories serving the villages. For the 250 stool samples collected, history on the health status (diarrhea/no diarrhea) and contact with poultry (contact/no contact) was obtained. Overall, the distribution of the humans associated with the different management systems and poultry types are as follows: Broiler farm-related persons, 161; duck farm-related persons, 59 and backyard chicken associated persons, 30, comprising 64.4%, 23.6% and 12.0% respective of the 250 human samples collected. The stool samples were handled the same way as described for poultry samples.

Isolation and Identification of E. coli

One milliliter (1 ml) of homogenized BPW or 1 g of sample was transferred to Trypticase soy broth (TSB) and incubated overnight at 37oC. Loopful from incubated TSB was streaked for isolation on MacConkey agar plates and incubated for 24 h at 37oC. Lactose-fermented colonies were streaked on eosin methylene blue (EMB) agar plates and then incubated for 24 h at 37oC. The suspected purified colonies with metallic sheen were identified using standard biochemical tests [13].

Selection and Characterization of E. coli Isolates

One hundred (100) isolates comprising 50, 19, 13 and 18 *E. coli* isolates from broiler, ducks, backyards and humans respectively, were randomly selected for further characterization.

Vero Cell Assay (VCA) of E. coli Strains

The cytotoxicity of the *E. coli* isolates for Vero cells was determined according to the procedure described by Konowalchuk et al. [14].

Serotyping of E. coli Isolates

Serotyping of *E. coli* isolates was performed in the Serology Unit, Animal Health Research Institute, Doki, Egypt using commercially available antisera (Denka Seiken Co., LTD, Tokyo, Japan) by the slide agglutination test. Detection of stx1, stx2, eae, ST and LT genes in STEC isolates from fecal samples was performed using PCR as described by Paton and Paton [15].

Genomic DNA Extraction

Pure colonies of bacteria from MacConkey agar plates were sub-cultured into TSB and incubated for 12 h at 37oC. After incubation, bacteria were collected by centrifugation at 13000 rpm for 2 minutes and the sediment was suspended in equal volume of Tris-EDTA buffer. Thereafter, 100 µl of lysozyme solution (10 mg/L), 100 µl of proteinase K (0.3 mg/L) and 1% dodecyl sulphate were added. The DNA lysate was extracted once with chloroform/isoamyl alcohol (24:1 ratio by volume), and then extracted with phenol/chloroform/isoamyl alcohol (25: 24: 1 ratio). The aqueous phase was mixed with isopropanol alcohol and stored at -20oC for 30 minutes. The precipitated DNA was spooled out, rinsed in 70 % ethanol and dissolves in 0.5 ml of Tris EDTA buffer.

PCR Amplification

The primers used for PCR amplification are displayed in [Table 1]. PCR was performed in a thermal cycler model (Biometra, Germany). The PCR mix (50 µL) for each sample consisted the following: 10 µL extracted DNA, 5 µL primers mix (0.5 µL from every primer), 1 µL deoxynucleotide triphosphate (dNTP-mix), 5 µL 10x buffer, 1 µL Taq-DNA polymerase enzyme (5000 U/ml), 28 µL ultra-pure deionized water. The reaction mixture was overlaid with mineral oil and incubated in the thermal cycler as follows: The first initial cycle: 94oC for 4 minute (initial denaturation). The subsequent 35 cycles: 94oC for one minute (denaturation), 60oC for one minute (annealing) and 72oC for one minute and 30 seconds (extension). The final extension step at 72oC for 7 minutes then kept at 4oC as the hold temperature [15]. Expected fragments providing visible bands of appropriate sizes of 180 bp (stx1), 255 bp (stx2), 384 bp (eae), 170 bp (ST) and 322 bp (LT) were considered positive.

Statistical Analyses

The prevalence of *E. coli* strains, occurrence of virulence markers and serotypes of the isolates from poultry and human sources were compared to detect statistically significant differences using the Chi-square test. The level of significance was set at an alpha 0.05.

Table 1: Primers used for PCR amplification

Target gene	Primer sequence	Amplicon size	Reference
Stx₁	F: 5'- ATAAATCGCCATTCGTTGACTAC-3' R: 5'- AGAACGCCCACTGAGATCATC-3'	180 bp	Paton and Paton (1998)
Stx₂	F: 5'- GGCACTGTCTGAAACTGCTCC-3' R: 5'-TCGCCAGTTATCTGACATTCTG-3'	255 bp	Paton and Paton (1998)
eae	F: 5'-GACCCGGCACAAGCATAAGC-3' R: 5'- CCACCTGCAGCAACAAGAGG-3'	384 bp	Paton and Paton (1998)
St	F: 5'- TCTTTCCCCTCTTTTAGTCAGTC-3' R: 5'-CCAGCACAGGCAGGATTAC-3'	170 bp	Rappelli et al. (2001)
Lt	F: 5'-TCTATGTGCATACGGAGC-3' R: 5'-ATACTGATTGCCGCAAT-3	322 bp	Rappelli et al. (2001)

Results

Prevalence of E. coli in Poultry and Human contacts

The prevalence of *E. coli* from three types of poultry and human beings is shown in [Table 2]. Overall, of a total of 1528 samples collected from poultry and human beings, 622 (40.7%) were positive for *E. coli*. The prevalence of *E. coli* was 43.6%, 57.7%, 53.0% and 7.2% for broiler, ducks, backyard chickens (including litters) and humans respectively and the differences were statistically significant (P < 0.05; X2). Amongst poultry samples, the prevalence of *E. coli* in broilers (43.6%) was statistically significantly (P < 0.05; X2) lower than found in either backyard chickens (53.0%) or ducks (57.7%).

Of the 140 humans who had contact with poultry (backyard and/or poultry farms), 13 (9.2%) were positive for *E. coli* compared with 5 (4.2%) of 110 humans without poultry contact. The difference was statistically significantly different (P < 0.05; X2). The prevalence of *E. coli* in apparently healthy humans (non-diarrheic) and in diarrheic humans was 5.3% (9 of 171) and 11.4% (9 of 79) respectively. The difference was statistically significantly different (P < 0.05; X2).

Frequency of Isolation of E. coli by type of Samples Collected

Overall, *E. coli* was isolated from 41.5% (78 of 188), 46.3% (193 of 417), 49.0% (148 of 302) and 49.9% (185 of 371) of unabsorbed yolk sacs, litter, cloacae and septicemic lesions respectively. The differences were however not statistically significant (P > 0.05; X2). [Figure 1] shows the isolation rates of E. coli from broilers, ducks and backyard chicken samples. For broilers, the range of isolation rates was from 39.1% (115 of 294) for litter samples to 47.8% (120 of 251) from for septicemic lesions (P < 0.05; X2). For duck samples, the lowest frequency of isolation of *E. coli* was 48.0% (12 of 25) which was originated from unabsorbed yolk sacs while the highest frequency was from litter samples, 65.1% (54 of 83) again the difference was not statistically significant (P > 0.05; X2). From backyard chickens, the frequency of isolation ranged from 46.7% (14 of 30) for septicemic lesions to 60.0% (24 of 40) for litter samples but the differences were not statistically significant (P > 0.05; X2).

The prevalence of *E. coli* in litters of backyard farms, 60% (24 of 40) and duck farms, 65.1% (54 of 83) were statistically
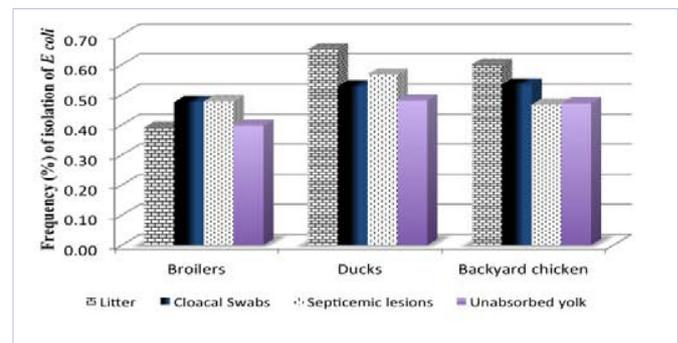

Figure 1: Prevalence of *E. coli* in different types of poultry sampled

significant (P < 0.05; X2) and higher than detected in litters from broiler farms, 39.1% (115 of 294).

A comparison of the prevalence of *E. coli* in feces of birds (without inclusion of their litters) revealed a prevalence of 45.8% (281 of 614), 54.1% (92 of 170), and 49.4% (38 of 77) for broilers, ducks and backyard chickens respectively. The differences however were not statistically significant (P > 0.05; X2). For poultry fecal samples, the prevalence of *E. coli* was 47.7% (411 of 861) which was ssignificant (P < 0.05; X2) and highe than the 7.2% (18 of 250) found in humans.

Serogroups of E. coli Isolates

The predominant serogroups of *E. coli* isolated were O2, O128, O125, and O114. Out, of the 100 isolates of *E. coli* tested, 30 (30.0%) belonged to serogroup O2 which are EPEC strains and of these, 24 (80.0%) and 6 (20.0%) were recovered from poultry and humans respectively [Table 3]. Sixteen (16.0%) isolates belonged to serogroup O128 which were ETEC strains and of these 13 (81.3%) were recovered from poultry and 3 (18.7%) from humans. Serogroup O125 was detected in 13 isolates which were ETEC strains and of these 10 (76.9%) and 3 (23.1%) originated from poultry and humans respectively. Eleven (11.0%) isolates belonging serotype O114 were EPEC strains and of these 9 (81.8%) were recovered from poultry and 2 (18.2%) from humans.

Of the total 10 different serogroups detected in this study, 10 (100.0%), 9 (90.0%), 8 (80.0%) and 8 (80.0%) were found in *E. coli* isolates from broilers, ducks, backyard chickens and humans respectively. Serogroups O19 and O1 found in poultry isolates were not detected in the 18 isolates obtained from the human samples. EPEC strains were most predominant constituting 41.0% (41 of 100), followed by EHEC strains 30.0% and ETEC strains 29.0%.

Frequency of Detection of Virulence Genes in E. coli Isolates

The frequency of virulence genes amongst VTEC strains isolated from poultry and human sources as detected by VCA is displayed in [Table 4]. Overall, of the 30 VTEC/STEC strains from poultry and human isolates, 6 (20.0%), 16 (53.3%), 8 (26.7%) and 14 (46.7%) were positive for stx1, stx2, stx1/stx2 and eae respectively. Of the isolates positive for stx genes, stx2 was most

Table 2: Frequency distribution of *E. coli* isolation in poultry and human samples

Source	Type of poultry and humans	No. of samples	No. (%) positive for *E. coli*
Poultry	Broiler	908	396 (43.6)
	Duck	253	146 (57.7)
	Back yards chicken	117	62 (53.0)
	Sub-total	1278	604 (47.3)
Human beings	In-contact humans	250	18 (7.2)
	Grand total	1,528	622 (40.7)

frequently detected (57.7%) in poultry isolates while stx1 was most frequently detected (50.0%) in human isolates. A similar frequency of eae was detected in poultry and human isolates of *E. coli*, 46.2% (5 of 7) and 50.0% (2 of 4) respectively.

The frequency of detection of the selected virulence genes in *E. coli* strains isolated from poultry and human sources by serogroups is shown in [Table 5]. Serogroups O78 and O111 from poultry sources showed a relatively high frequency of STEC strains, 26.9% (7 of 26) and 23.1% (6 of 26) respectively. The 4 serogroups (O126, O111, O26 and O78) detected from human isolates of *E. coli* each had a frequency of 25.0 % (1 of 4) for STEC strains. [Figure 2] shows the presence of verocytotoxic genes stx1, stx2 and attachment gene eae, from different serotypes O119, O126 and O111 from poultry, and [Figure 3] shows serotypes O125 and O128 isolated from human carrying ST and LT genes.

Detection of EAE Genes in EPEC Strains

Out of the 100 *E. coli* isolates tested, 41 (41.0 %) were positive for eae genes. The prevalence of EPEC strains (eae gene-positive) in poultry isolates was 40.2 % (33 of 82) and 44.4 % (8 of 18) in human isolates. The difference was not statistically significant (P > 0.05; X2). The 2 serogroups (O2 and O114) tested were

Table 5: Occurrence of selected virulence genes in VTEC strains isolated from poultry and humans by serogroups

Source of samples	Serogroup	No. of *E. coli* isolates	No. (%) of isolates positive:			
			stx_1	stx_2	stx_1 & stx_2	eae
Poultry	O119	2	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)
	O126	2	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)
	O111	6	1 (16.7)	3 (50.0)	2 (33.3)	4 (66.7)
	O78	7	1 (14.3)	4 (57.1)	2 (28.6)	5 (71.4)
	O26	4	1 (25.0)	2 (50.0)	1 (25.0)	0 (0.0)
	O1	5	0 (0.0)	3 (60.0)	2 (40.0)	2 (40.0)
	Sub-total	26	4 (15.4)	15 (57.7)	7 (26.9)	12 (46.2)
Human	O126	1	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
	O111	1	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
	O26	1	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
	O78	1	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
	Sub-total	4	2 (50.0)	1 (25.0)	1 (25.0)	2 (50.0)
	Grand-Total	30	6 (20.0)	16 (53.3)	8 (26.7)	14 (46.7)

Table 3: Serological characterization of *E. coli* isolates from poultry and humans

Serogroup	No. of isolates of *E. coli*					Characterization of strain
	Broiler	Duck	Backyard	Human	Total	
O119	1	1	0	0	2	EHEC
O126	1	1	0	1	3	EHEC
O78	4	2	1	1	8	EHEC
O111	4	1	1	1	7	EHEC
O2	15	6	3	6	30	EPEC
O26	3	0	1	1	5	EHEC
O1	3	1	1	0	5	EHEC
O128	8	3	2	3	16	ETEC
O114	5	2	2	2	11	EPEC
O125	6	2	2	3	13	ETEC
Total	50	19	13	18	100	--

Table 4: Frequency of virulence genes amongst VTEC strains detected by VCA

Source of samples	No. of VTEC* isolates	No. (%) of isolates positive for:			
		stx_1	stx_2	stx_1 & stx_2	eae
Poultry	26	4 (15.5)	15 (57.7)	7 (26.9)	12 (46.2)
Human	4	2 (50.0)	1 (25.0)	1 (25.0)	2 (50.0)
Total	30	6 (20.0)	16 (53.3)	8 (26.7)	14 (46.6)
*VTEC: Verocytotoxigenic *E. coli*					

Figure 2: This figure shows the shiga toxin Stx1 and 2 (180bp, 225bp) and attachment eae (384bp) genes; Lane (M): MW marker = 100 bp DNA ladder (Promega). Lane 1: negative control. Lane 2: positive control (*E. coli* O157H7 provided by Animal Health research Institute, Egypt, (Stx1, Stx2 and eae genes)) Lane 3, 4 (Stx2): *E. coli* O119 from poultry. Lane 6 (Stx2, eae), 7 (Stx1) *E. coli* O126 from poultry, and Lane 5 (eae, Stx1), 8 (Stx2), 9 (eae, Stx2): *E. coli* O111 isolates from poultry
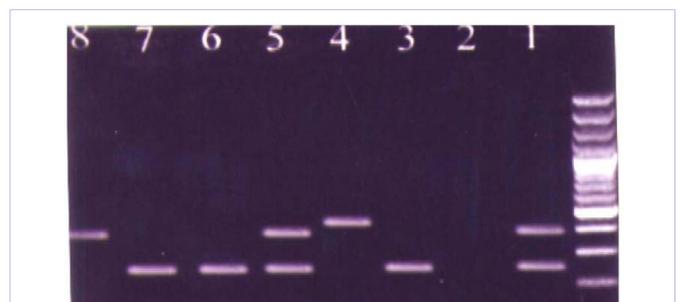

Figure 3: Lane (M): MW marker = 100 bp DNA ladder (Promega). Lane 1: positive control *E. coli* O125 containing ST and LT genes of sizes 170 and 324bp respectively. Lane 2: negative control, Lanes 3 (ST), 4 (LT), 5 (ST and LT) are *E. coli* O125 isolates from human; Lane 6 (ST), 7 (St), 8 (Lt) are *E. coli* O128 isolates from humans

positive for eae genes. Of a total of 30 EPEC strains that belonged to serogroup O2, 24 (80.0 %) and 6 (20.0%) were originated from poultry and humans respectively. For the 11 EPEC strains in serogroup O114, 9 (81.8 %) were poultry isolates while 2 (18.2 %) were recovered from humans.

Frequency of detection of ETEC strains

29 of the 100 *E. coli* isolates of tested (29.0 %) were ETEC strains, comprising 23 (28.0 %) of the 82 poultry isolates and 6 (33.3 %) of the 18 human isolates [Table 6]. The difference was not statistically significant (P > 0.05; X2). ETEC strains were detected in 2 serogroups, O128 and O125 human isolates. Occurrence of LT gene was most common in poultry isolates (43.5 %) but ST gene (50.0 %) in human isolates of *E. coli* (P > 0.05; X2).

Discussion

The study was conducted using the 'One Health' concept which encompasses the interaction of animals, humans and the environment in the transmission of diseases [16]. The primary goal was to study the similarities in the prevalence and characteristics of toxigenic *E. coli* strains in rural communities in Egypt where there is a high animal-human-environment interaction. This is primarily due to the fact that backyard chickens are in very close contact with humans in the households, the exposure of poultry farm workers to *E.coli* from broilers and ducks and finally, the consumption of poultry products from these villages. These exposures have been reported by other researchers and it is well established that chickens could serve as reservoirs of toxigenic *E. coli* as a result of poor hygienic practices in rural communities which may pose food safety concerns [6, 11, 17].

The carriage rate of *E. coli* in feces of poultry which ranged from 45.8 % to 54.1 % in the current study is lower than the 75.5 % reported for apparently healthy layers and their environments in India [18] and the 78% reported in ducks by Adzitey et al. [19]. Considerably lower prevalence of 39.4 % was however detected in broilers feces by Abhilasha and Gupta [20]. The isolation of *E. coli*, a normal flora of the intestinal tracks of animals and humans [21], is an indication that poultry could be potential sources of

the microorganism to contaminate the environment and humans due to poultry meat consumption. Poultry have been recognized as reservoirs of pathogenic organisms such as *Salmonella* spp., *Campylobacter* spp., *E. coli*, amongst others [22].

In our study, although the prevalence of *E. coli* in the poultry species was significantly higher than found in human feces tested, frequencies of isolation of *E. coli* was reported to vary across countries, 60.0 % [23] and 62.5 % in Bangladesh [24]. On the other hand, the frequency of isolation or detection of *E. coli* by various methods ranged from 0.4 % to 22 % in stool samples from human clinical laboratories in 10 European countries [25].

The prevalence of *E. coli* in humans in contact with poultry (9.2 %) was significantly higher than found in those without poultry contact (4.2 %), showing important zoonotic implications. A much higher frequency (73.0 %) of isolation of *E. coli* was detected in 30 healthy animal farm workers elsewhere [26]. Several studies have reported the similarity of isolates of *E. coli* regarding the genes and antibiotic sensitivity, recovered from poultry and their human contacts [10, 12]. Riccobono et al. [11] had however suggested that cross-transmission between children and home raised chickens could not represent a major spreading mechanism for resistant *E. coli* in households of resource-limited settings with high human-animal interaction. It was also of zoonotic significance to have detected the four serogroups of *E. coli* (O126, O111, O26 and O78) isolated from humans were also recovered from poultry sources in the current study. It would however be necessary to apply more robust genetic tools, such as the pulse-field gel electrophoresis (PFGE) [27], to confirm the relatedness of the isolates. It is however pertinent to mention that the serogroups detected in the current study have also been isolated from poultry and humans elsewhere [28].

The detection of *E. coli* (39.1 % to 60.0 %) in the litters of poultry farms (broilers and ducks) and backyard chicken environments is considered high and the organism has been recovered in the litters of poultry houses by others [29]. The significantly lower prevalence of *E. coli* in the litters on broiler farms compared with those from duck farms and backyard chicken environment may reflect a difference in management systems in which human contact is more prevalent on the duck farms and backyard chicken environment than the broiler farms. When changes of litters were completed on the duck farms and backyard chicken environment a higher exposure by humans to fecal matter resulted in a higher prevalence of E. coli, suggesting a higher risk factor for human infections by toxigenic E. coli [12].

The fact that *E. coli* was isolated at a significantly higher frequency from diarrheic humans (14.2 %) than from non-diarrheic (5.3 %) humans may be an indication that the microorganism was responsible either alone or in combination with other pathogens for the episodes. The pathogenic and toxigenic strains of *E coli* are an established human pathogens [2-4, 30].

From epidemiologic, virulence and pathogenic point, out of the 100 isolates tested, 30 (30 %) were confirmed by PCA as VTEC strains and all possessed stx1, stx2 and stx1/stx2 genes

Table 6: Occurrence of selected virulence genes (ST and LT) in ETEC strains from poultry and human samples

Source of isolates	Serogroup	No. of ETEC* isolates	No. (%) positive for:		
			ST	LT	ST & LT
Poultry	O128	13	3 (23.1)	6 (46.2)	4 (30.8)
	O125	10	4 (40.0)	4 (40.0)	2 (20.0)
	Sub-total	23	7 (30.4)	10 (43.5)	6 (26.1)
Human	O128	3	2 (66.7)	1 (33.3)	0 (0.0)
	O125	3	1 (33.3)	1 (33.3)	1 (33.3)
	Sub-total	6	3 (50.0)	2 (33.3)	1 (16.7)
	Grand-total	29	10 (34.5)	12 (41.4)	7 (24.1)

*ETEC: Enterotoxigenic *E. coli*

making them all STEC strains. Other researchers have reported the detection of VTEC from animal sources, for example, in Côte d'Ivoire only one isolate of *E.coli* was determined to be a STEC strain [31] while Amézquita-Lópe et al. [32] reported isolating STEC strains from cattle, chickens and sheep. However, isolates of *E. coli* from chickens have been reported to be negative for STEC strains in Greece [33] and the USA [34]. Although it has been established elsewhere that STEC strains are responsible for hemorrhagic gastroenteritis [35] in humans, only 4% of the isolates from humans were STEC strains. This was, higher than the 0.4% prevalence Shiga toxin strains in diarrheic humans reported by others [[33], however only oone isolate was reported as 0157:HNM positive from human diarrheal stool specimens in Côte d'Ivoire [31]. In our study, a comparatively high prevalence (26 %) of STEC was detected in poultry and their litters, which was considerably higher than the 6% reported for chickens in Burkina Faso [36].

This is the first documentation of STEC strains from poultry sources in Egypt, an indication that poultry could be important reservoirs of STEC strains for human infections [36, 37].

Overall in our study, VCA detected 30 % of the isolates tested to be VTEC strains, at a frequency of 31.7 % and 22.2 % in chicken and human isolates respectively. Comparatively lower frequencies of VTEC strains have been documented by others, 11% [3] and 9.1% [39] from chickens. The frequency of verocytotoxin producers amongst isolates from Egyptian villages in the current study (22%) is considerably lower than the 96.6% reported by Ananias and Yano [38], also using VCA.

The strategy employed in the current research, which used VCA followed by multiplex PCR successfully confirmed the presence of verotoxin (stx1, stx2, stx1/stx2) STEC strains as VTEC strains. Although the sensitivity of Vero cells to stx was first reported by Konowalchuk et al. [14], the cytotoxicity for this cell line remains the "gold standard" for confirmation of putative stx-producing isolates [15]. In our study, there was a 100 % correlation between VCA and multiplex PCR results. However, the only inference that can be made on VTEC-positive (by VCA) and verotoxin gene-positive to classify them as STEC strains because they were not serotyped specifically for O157 strains, which is a limitation of the current study. It is known that both O157 and non-O157 STEC strains exist that are VTEC [32].

Our finding that 26.7% of the 30 STEC isolates were positive for both stx1 and stx2 genes is in agreement with published studies [43, 44]. The frequencies of detection have also been variable according to reports, with lower frequency of 10% for stx1 and 16.5% for stx2 genes [45] and a higher frequency than found in our study by others [46]. In our study, a total of 6 (20 %) isolates were positive for only stx1 gene and 16 (53.3 %) for only stx2 gene, a slight variance from the reports of others [39] who stated that *stx1* was more frequently detected in STEC strains isolated from human patients with diarrhea.

The detection of 50% human isolates tested positive for the eae genes, which are usually associated with EPEC strains and known to be responsible for gastroenteritis [40], indicates possible pathogenic significance.

It is also pertinent to mention that 46.2% of the isolates from poultry from Egyptian villages were positive for EPEC strains and therefore could serve as potential sources for human infections. This by far is higher than reported elsewhere, 37% in chickens in Burkina Faso [35], 30.0% in diseased chickens in the USA [7] and 9.52% from chickens in India [43]. Four serogroups (O126, O111, O78 and O1) of *E. coli* from poultry were *eae* genes positive and therefore EPEC strains. These strains have been isolated from poultry by others [41].

The prevalence of 8 % for EPEC strains detected in humans in our study compares favorably with the 7.6 % reported for Melbourne infants [42] and 5.9 % found in Berlin infants [42]. However, relatively lower prevalence of 4.57 % for EPEC strains was reported in humans in while considerably higher prevalence has been reported in diarrheic (36.8%) and non-diarrheic (29.8%) humans by Garcia and others [44].

ETEC strains producing LT and ST have long been known to be responsible for diarrhea in both humans and animals [23, 25]. The prevalence of 28 % for ETEC strains in chicken isolates in our study is higher than the 5 % reported for chicken isolates in Burkina Faso [36] but comparative to the 22.8 % and 38.71 % detected in broilers and layers respectively in Bangladesh [23].

Regarding the frequency of detection of ETEC strains from human isolates in our study, 33 % is considerably higher than a range of 0.1% to 4.2% reported from human diarrheic patients in ten European countries [25]. This is not a surprise because it has been reported that EPEC strains and other pathogenic and toxigenic strains of *E. coli* are more prevalent in developing countries where poor hygienic practices are more prevalent than in developed countries [25, 45]. Serogroups O128 and O125 detected in chicken and human isolates in this study were also reported among ETEC strains [46]. The serotypes and the frequency of detection of both LT and ST genes, albeit at different frequencies, in *E. coli* isolates recovered from chickens and humans in rural communities in Egypt; indicate that they might play an important role as a cause of diarrhea in those areas.

Conclusion

Poultry (broilers, chickens and ducks) sampled from rural communities in Egypt displayed a significantly higher prevalence of *E. coli* than humans sampled in the same villages. However, the similarities detected amongst the strains of *E. coli* from poultry and human sources regarding the serogroups, EHEC, VTEC, ETEC and EPEC indicate that poultry in these villages have the potential to serve as important reservoirs of infection for humans. It is however imperative to use molecular methods such as the PFGE or MLST for future studies of this nature. This confirms the genetic relatedness of the *E. coli* strains isolated for poultry and humans in the Egyptian communities studied, which is considered a limitation of this study. It is also noted that although the sample size of 100 used in the current study which was primarily due limited resources, the study design which applied several serological and genetic methods successfully established

significant similarities in occurrence and characteristics between human and poultry isolates studied. Finally, to the best of our knowledge the present study is the first report that studied the relationship of poultry-human transfer of diarrhegenic *E. coli* in rural communities in Egypt.

It is recommended that future studies should consider the use of PFGE and/or MLST to definitely confirm the relatedness of *E. coli* isolates from both poultry and human isolates in rural Egyptian communities.

Acknowledgements

Ethical Approval

All procedures performed in this study including collection of humal fecal samples and animals were in accordance with the Egyptian ethical standards of the national research committee. All human subject gave their consent for the collection of the fecal samples, with the agreement that any identifying details of the individuals should not be published.

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