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Risk of Zoonotic Verotoxigenic *Escherichia coli* Associated with Water Use in Egypt

[1]*Esraa Abdul-Majeed,* [1]*Khaled A. Abdel-Moein,*
[2]*Mohga F. Badawi and* [1]*Osman M. Hamed*

[1]Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[2]Department of Hygiene, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: Water is required for many agricultural operations, from livestock watering and dairy shed cleaning, to crop irrigation and even as an ingredient in food processing. Irrigation is necessary for farming operations; however, it could be contaminated with zoonotic water-borne pathogens from animal waste.The objectives of this study were to determine the occurrence and characteristics of verotoxigenic *E. coli* in different irrigation waters and farm animal feces in Egypt. For this purpose, twenty water samples (5 ground water, 11 surface water and 4 wastewater) were collected from irrigation ditches and streams in fresh produce farms from different localities in Egypt. Moreover, 62 fecal samples of farm animals (31 cattle, 10 buffalo, 8 sheep and 13 goats) from two farms were enrolled in the current study. Isolation of *E. coli* from water samples was performed using membrane filtration technique on Eosin Methylene Blue (EMB) medium while fecal samples were enriched in Tryptic Soya Broth at 37°C for 24 hours then the enriched samples were streaked on EMB plates. *E. coli* was identified through colonial characters; Gram's stain films and biochemical reactions. Multiplex PCR was performed to detect virulence genes (*vt1,vt2* and *eae*) to identify verotoxigenic *E. coli* (VTEC). VTEC strains were found in 40% of water samples whereas 22.58% of the examined farm animals were positive. The highest prevalence of VTEC was recorded in farm animals watered with surface water (28.5%) in contrast VTEC were not found in cattle watered with ground water.*vt2* gene was the predominate gene among VTEC water isolates. In conclusion, detection of VTEC in irrigation water and farm animal feces from the same farm highlights the potential role of animal waste in the contamination of irrigation waters with such dangerous pathogen a matter which has a great risk on public health.

Key words: Verotoxigenic *E. coli* · Irrigation water · Animal · Egypt

INTRODUCTION

Water is required for many agricultural operations, from livestock watering and dairy shed cleaning, to crop irrigation and even as an ingredient in food processing. The water sector in Egypt is facing many challenges including water scarcity and deterioration of water quality because of population increase and lack of financial resources [1]. Like other water-scarce countries, Egypt is facing fast growing demands versus limited waterresources. Agriculture consumes the largest amount of the available water In Egypt, with its share exceeding 85% of the total demand for water [2]. Irrigation is necessary for farming operations; however, it could be contaminated with zoonotic water-borne pathogens from animal waste.

Increases the need for freshwater sources to provide irrigation can be problematic especially for freshwater sources that come in contact with regions where there are large numbers of grazing animals. In addition, the application of animal wastes to agricultural soils can increase levels of foodborne pathogens originating from the manure of animal carriers [3].Animal manure is a well-recognized potential source of wide variety of infectious agents that can cause disease in humans directly or indirectly, particularly through consumption of contaminated water or food [4].

Verotoxin-producing *Escherichia coli* (VTEC) is an important zoonotic food-borne and waterborne pathogen causing diarrhea, hemorrhagic colitis and potentially fatal outcomes such as hemolytic uremic syndrome (HUS) in humans. The predominant VTEC serotype associated with

Corresponding Author: Esraa Abdul-Majeed. Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. Tel: +20 1120004866. E-Mail: Esraa_elshafiee@cu.edu.eg.

outbreaks and sporadic cases of serious VTEC illness is *E. coli* O157:H7 (VTEC O157) [5] and despite early recognition of non-O157 VTEC strains as human pathogens [6], VTEC O157 remains the major focus of clinical and food diagnostic laboratories in many locations. The use of faecally contaminated water for irrigation may disperse VTEC to vegetable and salad crops that act as secondary vehicles for human infection. Several potential sources of contamination during the production of fresh produce have been proposed including animal fertilizers, irrigation water and farm workers [7]. In several studies, irrigation watersheds heavily impacted by ruminant livestock appear to be linked to waterborne O157 and non-O157 VTEC infections [8].

Healthy cattle and other ruminants are the major animal reservoirs of many VTEC, carrying these organisms in their gastrointestinal tracts and shedding them in manure at levels ranging from 10 to _105 CFU/g. Although contaminated ground beef has been considered the most frequent source of human exposure, recent investigations have increasingly identified numerous outbreaks and sporadic cases/clusters linked to nonmeat sources [9]. Among these sources, rural water supplies, crop irrigation water and contaminated municipal water supplies in agricultural areas have been implicated [10].

Verocytotoxin/Shiga toxin (VT/Stx)-producing *Escherichia coli* (VTEC/STEC) are characterised by the production of potent cytotoxins that inhibit protein synthesis within eukaryotic cells. These toxins are synonymously either termed verocytotoxins (VT), because of their activity on Vero cells, or Shiga toxins (Stx) because of their similarity with the toxin produced by *Shigelladysenteriae* [11].

This led us to conduct the current study to investigate the occurrence of VTEC in different irrigation waters and farm animals.

MATERIALS AND METHODS

A total of 20 water samples including different irrigation watersheds (ground water (n=5), surface water (n=11) and wastewater (n=4) were collected from fresh produce farms from different localities in Egypt in sterile 250 ml polyethylene bottles. In addition, 62 rectal swabs were taken from farm animals (31 cattle, 10 buffalo, 8 sheep and 13 goats) in 2 livestock farms located near the irrigation water sources (surface water and ground water). All samples were sent to laboratory in an ice box.

Isolation and Identification of *E. coli*: Upon arrival, all water samples were filtered using membrane filtration method which was carried out accordingto American Public Health Association [12]. In this procedure, 100 ml of water samples were allowed to drawn through vacuum pump filter apparatus containing sterile filter membrane with pore size (0.45 µm) which retained bacteria. After filtration, the membranes containing bacteria were spread on Eosin Methylene Blue (EMB) Agar (Oxoid, UK) and incubated for 24 hours at 37°C. Rectal swabs were taken from animals and aseptically transferred to tryptic soya broth (TSB)and incubated for 24 hours at 37°C. Tryptic soya enrichment was then streaked onto EMB agar that was incubated for a further 24 hours at 37°. Suspected *E. coli* colonies were subcultured onto nutrient agar plates and tentatively identified according to morphological features, Gram's stain and biochemical characters [13].*E. coli* were biochemically identified using RapID ONE test (Oxoid-remel USA).

Molecular Identification of VTEC: The presence of genes associated with virulence in VTEC (*eaeA*, *vt1* and *vt2*) was verified by multiplex PCR according to methods described by Aranda *et al.* [14].

DNA Extraction: A rapid boiling procedure was used to prepare template DNA from bacterial strains according to Reischl *et al.* [15]. Briefly, 2 to 5 loops of *E. coli* isolates were picked from the nutrient agar plate and suspended in 200 µl of RNA DNA free water. After boiling for 10 min, the suspension was centrifuged for 2 minutes and the supernatant was aspirated to be used as DNA template in the subsequent reaction.

Amplification Step: *E. coli* O157:H7 possessing all *vt1*, *vt2* and *eae*was used as positive control while water was used as negative control. The positive control, *E. coli* O157:H- was kindly supplied from Poultry Disease Department (Faculty of Veterinary Medicine, Cairo University, Egypt). DNA was stored at-20 °C. *Vt1*, *vt2* and *eae* genes were amplified using sets of specific primers as described in Table (1). All primers were synthesized by (Metabion, Germany).

The reaction were carried out using a total volume of 25 µl containing 3 µl of DNA as template, 5 pmol of each primer and 5 µl of 1X of PCR master mix (Jena bioscience).The PCR mixtures were then subjected to the following cycling conditions:50°C (2 min, 1 cycle); 95°C (5 min, 1 cycle); 40 cycles of 95°C (45 s), 50°C (1 min) and 72°C (1 min); and 72°C (7 min, 1 cycle) in a thermal cycler (Perkin-Elmer, USA) [14].. Amplification products entered electrophoresis step in 1.5% agarose gel and visualized under ultraviolet light.

Table 1: Primer sequences for *vt1*, *vt2* and *eae* produced byverotoxigenic *E. coli* (VTEC):

Primers target	sequence	Amplified product size	Reference
vt1	ATAAATCGCCATTCGTTGACTAC		
	AGAACGCCCACTGAGATCATC	180bp	Aranda *et al.* [14].
vt2	GGCACTGTCTGAAACTGCTCC		
	TCGCCAGTTATCTGACATTCTG	255bp	
eae	CTGAACGGCGATTACGCGAA		
	CCAGACGATACGATCCAG	917bp	

RESULTS

VTEC were recovered from 40% of water samples (8 out of 20). The occurrence rates showed variation between different irrigation waters 20, 36.4 and 75%, in ground, surface and wastewater, respectively (Table 2). Furthermore, 22.58% of the examined farm animals were positive. The highest prevalence of VTEC was recorded in farm animals watered with surface water (28.5%) in contrast tocattle watered with ground water (VTEC were not found)- (Table 3).Moreover, the results of multiplex PCR confirmed the presence of twenty VTEC isolates relying on the targeted genes *vt1*, *vt2* and*eae*genes which were present in different prevalence among the isolates 59.1, 40.9 and 36.4% respectively (Table 4).

DISCUSSION

In the current study, VTEC were recovered from 40% of irrigation water samples (8 out of 20) collected from fresh produce farms located in different localities in Egypt(Table 1). The occurrence rates showed variation between different irrigation waters type ranging from20% to 36.4% and 75%, in ground, surface and waste water, respectively. There is presently little data on the occurrence of these pathogens in irrigation waters in Egypt. But this is in agreement with Steele and Odumeru [16] who reported that Groundwater is generally of good microbial quality, unless it is contaminated with surface runoff; wastewater is usually of very poor microbial quality and requires extensive treatment before it

Table 2: Occurrence of verotoxigenic *Escherichia coli* in irrigation waters:

Type of irrigation waters	No. of Samples	No. of VTEC positive samples (%)
• Surface water	11	4(36.36%)
• Ground water	5	1(20%)
• Wastewater (irrigation waste)	4	3(75%)
• Total	20	8(40%)

Table 3: Occurrence of verotoxigenic *Escherichia coli* in farm animals:

No. of farms	Type of samples	Type of drinking and irrigation water	No. of Samples	No. of VTEC positive samples (%)
Farm (1)	• Cattle	Surface water	18	5(27.7%)
	• Buffalo		8	2(25%)
	• Sheep		10	2 (20%)
	• Goat		13	5(38.5%)
Farm (2)	• Cattle	Ground water	13	0 (0%)
	Total		62	14(22.58%)

Table 4: Virulence profiles of VTEC (n=22) recovered from irrigation waters and animal samples:

		Virulence genes (%)		
Sources	No. of isolates	*vt1*	*vt2*	*eae*
Irrigation waters	8	4(50%)	5(62.5%)	2(25%)
Farm animals	14	9(64.3%)	4(28.6%)	6(42.9%)
Total	22	13(59.1%)	9(40.9%)	8(36.4%)

can be used safely to irrigate crops [16]; surface water is of variable microbial quality. Hence, the high occurrence of VTEC in wastewater may be due to the poor microbial quality and lack of treatment. Surface water contamination with VTEC could be due to the irrigation water examined in this study was potentially affected by a range of agricultural activities, including the production of livestock. Importantly, ruminants are the most important reservoir of the zoonotic VTEC which are transmitted to humans through the ingestion of food or indirectlythrough contamination of the agricultural environment, including surface waters [17]. River Nile, the primary source of fresh water in Egypt, is also the primary receptor of wastewater and drainage generated by different activities [18]. So that, the surface water in Egypt became of poor microbial quality due to sewage pollution which carry highly pathogenic microorganisms. Groundwater contamination also may be induced by different practices in the management of domestic wastewater and livestock manure and from surface water infiltration [19].

Megan and Stefan [20] explained that developing countries use untreated wastewater for irrigation as alternative water source to compensate the water shortage and lack of financial resources.

This confirmed that wastewater reuse in irrigation without treatment poses a potential risk to human due to presence of highly pathogenic microorganisms including VTEC which entered through the feces of animals or humans hosts.

It is well documented that animals and, in particular, ruminants can carry a range of potentially harmful pathogens, including verocytoxigenic*Escherichia coli* (VTEC), in their gastrointestinal tract- [21].Table (3) demonstrated the occurrence rate of VTEC in fecal samples of farm animal which was 22.58% (14 out of 62). VTEC can reportedly survive for several months in the environment, in feces and in soil, which allows for the recycling of VTEC among food animals and wildlife and prolonged environmental contamination. So that- irrigation water sources (surface water and unprotected groundwater) may becomecontaminated with VTEC from livestock effluent which poses potential risks to human when such watersare used to irrigate ready to eat crops. In addition, the application of these animal wastes to agricultural soils as fertilizers can increase the levels of foodborne pathogens [3].

Notably, the occurrence of VTEC in farm animals differs according to the type of drinking waters (Table 3). The occurrence rates of VTEC in farm (1) that use surface water for drinkingwere 27.7, 25,20 and 38.6% from cattle, buffalo, sheep and goat respectively with overall (28.5%). These results indicate poor quality of the drinking water. In contrast, no evidence of VTEC in farm (2)those using cattle only for grazing which indicates good quality of ground water and good agricultural and hygienic measures used in this farm. Contaminated drinking water in livestock farmshas a role in the transmission of VTEC among animals [22] and large numbers of animals can be infected over a short period [23]. On many farms in Egypt, irrigation waters are not only used for irrigation but also for watering of grazing animals or even livestock located near such farm. Hence, continual recycling of VTEC among animals and irrigation water may occur. This finding is in line with Hrudey *et al.* [8] who found thatirrigation watersheds heavily impacted by ruminant livestock appeared to be linked to waterborne O157 and non-O157 VTEC infections.

Virulence genes of VTEC isolates (n=22) were detected using multiplex PCR that targets *vt1*, *vt2* and *eae* genes, which were in 59.1, 40.9 and 36.4% of the isolates respectively (Table 4). These results are of great public health concern; as the carriage of such genes and particularly *vt2* has been linked with more severe *E. coli* infection [24].

Furthermore, *vt2* gene was the predominant among VTEC irrigation water isolates (62.5%). While the predominant gene among VTEC animals isolates was *vt1* gene (64.3%). Thus, Fecal contaminated irrigation water with animal waste might pose a potential risk to human when used for irrigation of fresh produce (vegetables and fruits) as it is mainly eaten raw without heat treatment. All *E. coli* isolates showed low prevalence of *eae* gene (36.4%) which is responsible for the attachment of *E. coli* to the intestinal cells. Importantly, VTEC lacking the *eae* gene but capable of producing Verotoxin 2 encoded by the *vt2* gene have been of great public health concern since the implicated *E. coli* strains(O104:H4) in a large outbreak in Germany in 2011 were lacked the *eae*gene but were capable of producing VT 2 which is more toxic to human renal microvascular endothelial cells than VT 1 [7].

In conclusion, detection of VTEC in irrigation water and farm animal feces from the same farm highlights the potential role of animal waste in the contamination of irrigation waters with such dangerous pathogen a matter which has a great risk on public health. Contaminated drinking water has a role in the transmission of VTEC among animals which led to continual recycling of VTEC among animals and irrigation water.

REFERENCES

1. Abdel Kader, A.M. and S.M. Abdel Rassoul, 2010. Prospects of Water Conservation in Egypt (Special Reference to Wastewater Reuse). In the procceding of the Fourteenth International Water Technology Conference, IWTC (14: 2010). Cairo, Egypt.
2. El Gamal, F., H. Mostafa and A. Shalby, 2005. Reuse of low quality water in Egypt. In: Non-conventional water use: WASAMED project. Eds., A. Hamdy, (ed.), F. El Gamal, N. Lamaddalena, C. Bogliotti and R. Guelloubi. Bari: CIHEAM / EU DG Research, pp: 93-103.
3. Irene, B.H., J.D. Nutt and S.C. Ricke, 2009. Salmonellosis Outbreaks in the United States Due to Fresh Produce: Sources and Potential Intervention Measures. Foodborne Pathogens And Disease, 6(6): 635-648.
4. Guan, Y., B.J. Zheng, Y.Q. He, X.L. Liu, Z.X. Zhuang, C.L.L.S.W. Cheung, P.H. Li, L.J. Zhang, Y.J. Guan, K.M. Butt, K.L.C.K.W. Wong, W. Lim and K.F. Shortridge, 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science, 302(5643): 276-278.
5. Karmali, M.A., V.P. Gannon and J.M. Sargeant, 2010. Verocytotoxin-producing *Escherichia coli* (VTEC). Vet. Microbiol., 140: 360-370.
6. Johnson, R.P., R.C. Clarke, J.B. Wilson, S.C. Read, K. Rahn, S.A. Renwick, K.S. 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 verotoxigenic *Escherichia coli*. J. Food Prot., 59: 1112-1122.
7. Grant, M.A., C. Hedberg, R. Johnson, J. Harris, C.M. Logue, J. Meng, J.N. Sofos and J.S. Dickson, 2011. The significance of non-O157 Shiga toxin producing *Escherichia coli* in food. Food Protection Trends, 31: 32-45.
8. Hrudey, S.E., P. Payment, P.M. Huck, R.W. Gillham and E.J. Hrudey, 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. Water Science And Technology, 47(3): 7-14.
9. Soderstrom, A., P. Osterberg, A. Lindqvist, B. Jonsson, A. Lindberg, S. Blide-Ulander, C. Welinder-Olsson, S. Lofdahl, B. Kaijser, B. De Jong, S. Kuhlmann-Berenzon, S. Boqvist, E. Szanto, S. Andersson, G. Allestam, I. Hedenstrom, L. Ledet Muller and Y. Andersson, 2008. A large *Escherichia coli* O157outbreak in Sweden associated with locally produced lettuce. FoodBornePathogen. Dis., 5: 339-349.
10. Olsen, S., G. Miller, T. Breuer, M. Kennedy, C. Higgins, J. Walford, G. McKee, K. Fox, W. Bibb and P. Mead, 2002. A waterborne outbreak of *Escherichiacoli* O157: H7 infections and hemolytic uremic syndrome: implications forrural water systems. Emerg. Infect. Dis., 8: 370-375.
11. Gill, A. and C.O. Gill, 2010. Non-O157 verotoxigenic *Escherichia coli* and beef: A Canadian perspective. The Canadian Journal of Veterinary Research, 74: 161-169.
12. APHA,(American Public Health Association), 2005. Standard methods for the examination of water and wastewater, 21st ed. APHA, Washington, DC.
13. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger and W.C. Winn, 1994. Introduction to diagnostic microbiology. JB Lippincott Company, pp: 117-123.
14. Aranda, K.R.S., U. Fagundes-Neto and I.C.A. Scaletsky, 2004. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic Escherichia coli and Shigella spp. J. Clin. Microbiol., 42: 5849-5853.
15. Reischl, U., M. Pluz, W. Ehret and H. Wolf, 1994. PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. Bio. Techniques, 17: 844-845.
16. Steele, M. and J. Odumeru, 2004. Irrigation water as source of foodborne pathogens on fruits and vegetables.J. Food Prot., 67: 2839-2849.
17. Cooley, M.B., B. Quiñones, D. Oryang, R.E. Mandrell and L. Gorski, 2014. Prevalence of Shiga toxin producing *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes*at public access watershed sites in a California Central Coast agricultural region. Frontiers in Cellular and Infection Microbiology, 4: 30.doi: 10.3389/fcimb.2014.00030.
18. Abo-State, M.A., M.M. Hesham, S.M. Ezzat, H.A. Essam and A.E. Mostafa, 2012. Antimicrobial Resistance Profiles of Enterobacteriaceae Isolated from Rosetta Branch of River Nile, Egypt. World Applied Sciences Journal, 19(9): 1234-1243.
19. Magali, D. and S. Emmanuel, 2007. Assessment of source water pathogen contamination. Journal of Water and Health, 05.Suppl 1.

20. Megan, E.G. and S. Stefan, 2012. Microbiological assessment of river water used for the irrigation of fresh produce in a sub-urban community in Sobantu, South Africa Food Research International, 47: 300-305.
21. Duffy, G. and E. McCabe, 2014. Veterinary public health approach to managing pathogenic verocytotoxigenic *Escherichia coli* in the agri-food chain. Microbiol Spectrum, 2(5): EHEC-0023-2013.
22. Hancock, B., 1998. An Introduction to Qualitative Research.Nottingham, UK: Trent Focus Group, Division of General Practice, University of Nottingham.
23. Le Jeune, J.T., T.E. Besser, D.H. Rice and D.D. Hancock, 2001. Methods for isolation of water borne Escherichia coli O157.Letters In Applied Microbiology, 32: 316-320.
24. Fuller, C.A., C.A. Pellino, M.J. Flagler, J.E. Strasser and A.A. Weiss, 2011. Shiga toxin subtypes display dramatic differences in potency. Infect. Immun., 79: 1329-1337.