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DETECTION OF ZOONOTIC PATHOGENS IN UNTREATED GROUND WATER IN RURAL EGYPTIAN COMMUNITIES

By

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ABSTRACT

The simplified individual water supplies that are created by rural agriculture population usually maximize the customers' exposure to impurity pathogens via diffused human and animal excreta or wastages. This study evaluated the frequency of zoonotic pathogens in infiltrated untreated ground water. A total of two hundred and seventy ground water samples were collected from various individual home water supplies at different rural Egyptian localities and analyzed for the detection of bacterial, fungal and parasitic zoonotic pathogens. The results recognized *Campylobacter jejuni*, *Campylobacter coli*, *Escherichia coli*, *Salmonella typhi*, *Shigella spp*. *Yersinia enterocolitica* and *Pseudomonas aeruginosa* at percentage of 10.37, 5.93, 27.41, 4.81, 8.15, 4.44 and 9.26, respectively. The fungal isolates were *Candida albicans* and *Cryptococcus neoformans* at a percentage of 6.66 and 1.48, respectively. Also, *Entamoeba histolytica and Giardia intestinalis* were detected in 5/270 (1.85%) and 20/270 (7.41%) of the examined untreated ground water, respectively. The results confirm human bio-hazards through rural individual water supplies, and reflect the need of public health education toward the accurate use of drinking ground water only after perfect treatment.

Keywords: Untreated ground water, zoonotic pathogens, human bio-hazards, rural Egyptian community.

INTRODUCTION

The renew impact of human waterborne zoonoses is connected to the significant epidemic and endemic worldwide incidence **(Slifco *et al.*, 2000)**. Poor conditions of water, sanitation, and hygiene serve up communication of waterborne pathogens at all points of water services; where people without access to improved water supplies are regarded as at risk groups. In Africa, Asia, and Latin America, at least 600 million urban and rural inhabitants live using unhealthy drinking

water that increase their susceptibility to diarrheal syndrome **(WHO, 2006).** Approximately 4 billion cases of diarrhea occur each year, leading to nearly 2 million deaths (**WHO, 1996)**.

Outbreaks of different infections following contamination of potable water from sewage, wastewater effluent, muck spreading, slurry spraying, etc., pose significant problems for both the developed and developing countries of the world **(Warburton *et al.*, 1998)**. High quality information on many aspects of waterborne zoonoses is not available. Up to 75% of significant numbers of emerging and re-emerging waterborne pathogens are zoonotic **(Warburton *et al.*, 2002)**. Zoonotic bacteria caused fewer outbreaks than zoonotic protozoa; **Bartram *et al*. (2001)** reported that 24 and 20% of the world wide waterborne outbreaks are caused by protozoan parasites and bacterial pathogens, respectively. *Campylobacter*, non-typhoid *Salmonella* and *E. coli* O157:H7 were identified in 10%, 8% and 6% of these outbreaks, respectively. **WHO (2006)** reported that bacteria cause 15%, 16%, and 21% of the outbreaks of identified etiology in community, non-community, and individual water systems, respectively. *Giardia* was identified in 66% of all drinking water outbreaks and in 70%, 62%, and 56% of the outbreaks in community, non-community, and individual systems, respectively **(WHO/UNICEF, 2000)**.

Minimizing public health hazards can be through accelerating efforts for excellent sanitation of water design and provision of a preventive and thus a cost-effective method for ensuring water safety **(Timothy and Darell, 2003)**.

The present study aimed to inspect considerable numbers of untreated ground water supplies from different rural Egyptian localities for the detection of bacterial, mycotic and parasitic microorganisms of zoonotic significance to imitate the degree of possible personal risks due to dinking or use of infiltrated untreated ground water.

II. Materials and Methods:

1. Water Samples:

A total of two hundred and seventy untreated ground water samples (2 liters each) were collected under complete aseptic precautions from the individual community water supplies at different Egyptian rural localities during the period 2009 -2010.

2. Microbiological Analysis:

100 ml of each water sample were filtered through membrane filters (0.45µm pore size and 47 mm diameter) **(APHA, 1998)** and these membrane filters were inoculated onto different selective and differential media for the detection of the following:

2.1. Detection of yeast: The membranes were inoculated onto Sabouraud's dextrose agar (Difco) plates containing chloramphenicol 0.05 mg per ml. The plates were incubated aerobically at 30ºC for 24 hours, then for 48hours at 37ºC with daily examination for growth **(Barwick *et al*., 2000)**.

2.2. Detection of *Salmonella, E. coli, Shigella* and *Yersinia*: The membranes after filtration were inoculated onto SS agar, MacConkey agar and ***Yersinia*** selective agar plates and incubated at 37ºC for 48 hrs for detection of ***Salmonella*** & ***Shigella***, ***E. coli*** and ***Yersinia.*** Suspected colonies appearing on the different media were identified according to **Holt *et al*. (1994)**.

2.3. Detection of *Campylobacter*: Aspirations of 1 ml from water samples were cultured in thioglycolate broth at 37ºC under microaerophilic conditions (5% O2, 10% CO2 and 85% N2). All samples were examined after 24 hrs under phase contrast microscope for the characteristic ***Campylobacter*** motility **(Ledergerber *et al*., 2003)**. The positive samples for ***Campylobacter*** were filtered through 0.45 µm Millipore filter and these membranes were cultured in the brain heart infusion agar plate with 10% sheep R.B.Cs and ***Campylobacter*** selective supplement. All plates were incubated at 37°C for 48hrs in microaerophilic condition. Suspended colonies were identified based on their motility and biochemical tests according to **Acha *et al*. (2004)**.

2.4. Detection of *Pseudomonas aeruginosa*: The membranes were inoculated onto the m-PA-D agar **(selective for *P. aeruginosa***) and incubated at 37°C for 48 hrs. Suspected colonies were identified according to **de Vicente *et al*. (1986).**

3. Detection of free living amoebae: Free-living amoebae were detected in the collected water samples according to the method of **Al-Herrawy (2001)**. Briefly, water samples (1 liter each) were filtered through membrane filters (1.2 µm pore size and 142 mm diameter). The filter holder was washed with 10 ml sterile distilled water and the membrane was inverted face to face on the surface of non-nutrient (NN) agar plates previously seeded with 0.1 ml live ***E. coli***. The inoculated plates were incubated at 22ºC for 7 days with daily microscopic examination for the presence of any amoebic growth **(Al-Herrawy, 1992)**.

III &IV. Results &Discussion:

Bio-surveillance of waterborne zoonoses signifies important aspect of public health practice. Although not all countries do so, there is a growing recognition for detecting and quantifying the occurrence of cases and outbreaks.

The international standards recommended that drinking water should be free from total and fecal coliforms and other pathogens that cause health hazards to human **(APHA, 1998).**

Most emerging zoonoses will have some similarities to existing pathogens and thus may be adequately controlled by current management strategies, technologies, and/or infrastructure **(Ying, *et al.*, 2003)**. This study indicated to how extent the zoonotic bio-hazards are due to individual supplies of untreated ground water.

In the present investigation, we isolated ***C. jejuni***, ***C. coli***, ***E. coli***, ***S. typhi***, ***Shigella spp.***, ***Y. enterocolitica*** and ***P. aeruginosa*** at percentages of 10.37, 5.93, 27.41, 4.81, 8.15%, 4.44 and 9.26, respectively (Table 1). These findings agreed with **Averham and Keith (2006)**. ***C. jejuni*** is a major cause of pediatric diarrhea in developing countries, where free-range chickens are presumed to be a common source. Analysis of 156 human and 682 avian strains demonstrated identical strains in chickens and humans in 70.7% of families, and 35–39% of human isolates from diarrheal and non - diarrheal cases were identical to a household chicken isolate **(Oberhelman *et al.*, 2003)**. ***E. coli*** have been found to survive in biofilms in distribution systems even when high residual chloramine concentrations are present **(Williams and Braun-Howland, 2003)**.Confirming our results, an Indonesian surveillance study conducted over a 2-year period among 6760 patients with debilitating diarrheal diseases, revealed that 587 (9%) of stools were positive for the following bacteria: ***Shigella flexneri*** (39%), ***Salmonella*** spp. (26%), ***Vibrio*** spp. (17%), ***Shigella sonnei*** (7%), ***C. jejuni*** (4.4%), ***S. typhi*** (3%), and ***Shigella dysenteriae*** (2.3%) and 48% of specimens of river water used for drinking contained ***Salmonella*** **(Graham *et al.*, 2000).*Giardia intestinalis*** and ***E. histolytica*** have become significant waterborne pathogens in the developed world for three reasons: First, ***Giardia intestinalis*** and ***E. histolytica*** are indigenous infectionswith a low infectious dose; second, densities of environmental contamination with infective cysts and oocysts are sufficient to pollute the aquatic environment; and third, oocysts are small enough to penetrate water treatment processes and are less sensitive to the disinfectants commonly used in water treatment **(Bartram *et. al.*, 2001)**. In current study; free living amoebae (FLA) were detected in 12/270 (4.07%) of the examined untreated ground water samples (Table 1). The isolated FLA were non-pathogenic as proved by the pathogenicity test. ***E. histolytica*** and ***G. intestinalis*** were detected in 5/270(1.85%) and 20/270 (7.41%) of the analyzed water samples, respectively (Table 1). Parasitic infestation might be due to the contamination of ground water in rural localities via human and animal excreta and wastages. Also, ***E. histolytica*** and ***Giardia intestinalis*** are both persistent environmentally and extremely resistant to chlorine. A study in Japan showed that ***E. histolytica*** and ***Giardia intestinalis*** cysts

were detected in 35and 12% of filtered water samples. Most (71%) outbreaks of giardiasis occurred in systems using surface water, whereas most (53%) outbreaks of ***E. histolytica*** occurred in groundwater systems **(AWWA, 1999)**. In this study, infiltrated untreated ground water identified ***Candida albicans*** *and* ***Cryptococcus neoformans*** by 6.66% (18/270) and 1.48 (4/270), respectively (Table 1). The presence of these fungal zoonotic pathogens indicate public health hazards specially when contaminated water is used as a shower, washing or other modes that connect fungus with skin particularly abraded one. Also, drinking the fungus polluted water is of medical worry especially with infants, children and immunosuppressed persons. The facts confirmed our results that in developing countries, fungal infection due to poor nutritional status render people, predominantly children to be more susceptible **(Nwachcuku and Gerba, 2004).**

It can be concluded that further efforts are needed from the Egyptian public health authorities and the other ministries in concern, to develop water networks and facilitate regional cooperation that lead towards international harmonization for replacement of the rural individual supplies. Also, Egyptian authorities must be rigid enough to adapt the legal application of HACCP (Hazard Analysis and Critical Control Points) which is found to be of control significance, enhance the systematicapproachforidentification,evaluation,andcontrolwater health hazards during production, processing and manufacturing. **(Codex Alimentarius Commission 1997)**.

(Table 1): Comparative percentages of isolated zoonotic pathogens from the untreated ground water samples.

No. of water samples	Type of Microbes	No. of positive (%)
270	**Bacterial agents:**	
	Campylobacter jejuni	**28 (10.37%)**
	Campylobacter coli	**16 (5.93%)**
	E. coli	**74 (27.41%)**
	Salmonella typhi	**13 (4.81%)**
	***Shigella* spp.**	**22 (8.15%)**
	Yersinia enterocolitica	**12 (4.44%)**
	Pseudomonas aeruginosa	**25 (9.26%)**
270	**Fungi :**	
	Candida albicans	**18 (6.66%)**
	Cryptococcus neoformans	**4 (1.48%)**
270	**Parasitic agents:**	
	***Amoeba* spp.**	**12(4.07%)**
	Entamoeba histolytica	**5(1.85%)**
	Giardia intestinalis	**20 (7.41%)**

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