Presence of Cryptosporidium Oocysts in Sewage Effluent and Water Surface in Mosul Area

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Abstract
A new method for detection of cryptosporidium oocysts in sewage was modified to investigate oocysts prevalence. Surface water and sewage effluent were filtered, elutet from the filter and concentrated using centrifugation. The resultant sedemented pellet was then homogenized and placed on sucrose gradient to separate oocysts from the supernatant. Then examined by modified Ziehl-Neelson method. The filter of sewage effluents had significantly lower numbers of oocysts, sand filtration may reduce the concentration of this parasite in waste water. A large no. of oocysts were encountered in surface water. Since cryptosporidium oocysts are frequently present in environmental water, they could be responsible for waterborne outbreaks of disease.

Introduction
The coccidian protozom parasite cryptosporidium spp. Is now widely accepted as a cause of acute gastroenteritis [1]. It has been recognize as a parasite of both medical and veterinary importance. It is widely distributed in nature and has a short life cycle mainly in the intestinal epithelium [2,3].
Cryptosporidiosis is a disease which may produce symptoms of watery diarrhoea, nausea, vomiting, fever and malaise, is transmitted by oocysts that are shed in the feces of infected persons or animals. These oocysts must be ingested for this monoxenous parasite to continue its life cycle [3,4].
The parasite can readily cross mammalian species barrier, indicating the potential for zoonotic transmission [1,5].
The majority of cases of clinical cryptosporidiosis are probably as a result of direct transmission [6,7].it is possible that this parasite, like Giardia lamblia may be increasingly implicated in water borne outbreaks [8,9].
Sewage contamination of chlorinated water is responsible for the cryptosporidiosis outbreaks [8,10]. Cryptosporidium, like Giardia is more resistant than bacteria to most routine water disinfection and other commercially available disinfectants are ineffective in inactivating cryptosporidium spp. [10,11]. This parasite like Giardia may be implicated in waterborne outbreaks. Cryptosporidium has been found with other waterborne pathogens such as rotavirus, campylobacter, Aeromonas and Giardia in stools of persons with gastroenteritis [1,12,13].

The purpose of this study is to examine the water from sewage for the presence of cryptosporidium oocysts.

Material and Method

Water from sewage were collected from different places in Mosul province, collection of sewage water about 20-100 ml using clean dry wellcapped plastic containers and then the sewage samples transported to the laboratory for further investigation. 10 ml of each sewage water put in a centrifuge tube and centrifuged (1,200 g for 10 min) in order to concentrate the sediment to a single pellet. The pellet was divided in half, resuspended to 25 ml in a detergent solution (distilled water with 1% Tween 80 and 1% sodium dodecyle sulfate). The samples were then homogenized in water bath. The suspension was then washed with distilled water, centrifuged (1,200 g for 10 min), and resuspended to 25 ml with the detergent solution sheather's sucrose density centrifugation was used to separate oocysts from suspended matter in the pellet. 5 ml of each 25 ml samples was then mixed with 10 ml sheather's solution (500 gm sucrose, 320 ml distilled water and 9.7 ml phenol, 1.29 gm/ ml). Five tubes for each sample, left for 15 min, then, the scumed upper most layer were collected by bacteriological loop and the collected drop smeared on a clean slide, dried and then stained by a modified Ziehl–Neelson technique, after drying slides examined for detecting cryptosporidium oocyst, counting the no. of oocyst in a drop of the uppermost layer of the supernatant.

Ziehl–Neelsen Technique:
1. Dry smear at room temperature.
2. Fix in 96% methanol for 2 – 5 minutes.
3. Dry at room temperature.
4. Fix briefly in flame.
5. Stain with concentrated carbol fuchsin for 5 minutes.
6. Thorough rinsing in water.
7. Decolorization in 1% HCl in alcohol for 10 – 15 seconds.
8. Counter stain with 0.4 % malachite green for 30 seconds.
9. Rinse with water and dry.

In smears stained by this technique, cryptosporidia appear as 3 – 6 µm large, densely stained red bodies, clearly distinguishable against a green background. (fig. 1)

Results

The raw sewage samples collected yielded 150-630 oocysts/ L. The type of samples and the no. of oocyst detected in each sewage sample shown in table one. The first two samples collected from the intake of a domestic sewage of a small suburban community. The 3rd sample came from the intake of a domestic sewage. The fourth sample containing the highest concentration of oocysts came from the effluent wastes of cattle slaughterhouse. Six surface water samples were collected and examined for cryptosporidium oocysts (Table II). These samples were taken from irrigation canals. One pass through agricultural field with cattle pastures and the other one was exposed to untreated sewage discharge. The oocyst concentration were 1,220 and 970 respectively. The third sample was collected from a river exposed to septic tank leakage and the fourth from a river exposed to small amount of effluent discharges.
The oocyst concentration were 155 and 5 respectively. The fifth sample from a river have an oocyst concentration of 97.

The last sample was from a stream had the small no. of the oocyst concentration only two oocyst. (Table II).

**Table I** Occurance of cryptosporidium oocysts in raw sewage (fig. 1)

<table>
<thead>
<tr>
<th>Sewage</th>
<th>Volume</th>
<th>No. of oocyst.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20 ml</td>
<td>150</td>
</tr>
<tr>
<td>II</td>
<td>30 ml</td>
<td>212</td>
</tr>
<tr>
<td>III</td>
<td>40 ml</td>
<td>305</td>
</tr>
<tr>
<td>IV</td>
<td>50 ml</td>
<td>630</td>
</tr>
</tbody>
</table>

**Table II** Detection of cryptosporidium oocyst in surface water

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample no.</th>
<th>source of contamination</th>
<th>No. of oocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canal water</td>
<td>I</td>
<td>Agricultural area (Cattle pastures)</td>
<td>1,220</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Untreated sewage effluent</td>
<td>970</td>
</tr>
<tr>
<td>River</td>
<td>III</td>
<td>Septic tank leakage</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Effluent discharge</td>
<td>5</td>
</tr>
<tr>
<td>streams</td>
<td>V</td>
<td></td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>VI</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

**Discussion**

In this survey cryptosporidium oocysts were found in both sewage effluents and surface water with a concentration as high 630 and 1,220 respectively, these data suggest that cryptosporidium oocysts are being passed into sewage effluent and surface water, by infected humans or animal.

Oocysts may be entering these waters in fecal material via sewage effluent discharges, septic tank leakage, agricultured runoff, or from soils exposed to infected feces [1, 2, 8, 9]. Sand filtration, may be beneficial helping to remove this parasite. Without additional treatment, poor removal in waste water can be expected, possibly releasing as many as 100 – 1000 oocysts/ L. into the environment [14, 15, 16, 17]. These oocysts could then contaminate surface waters used as a sources of drinking water.

If the drinking water facility does not use sand filtration, these oocysts may then contaminate the water supply.

Cryptosporidium oocysts retain viability when exposed to many commonly used disinfectants, including hypochlorite solutions with a concentration of 3% [10]. Then, chlorination as a sole means of disinfectants within water supplies may not be entirely effective in inactivating infective cryptosporidium oocysts.

Wastewater in the form of raw sewage and runoff from diaries and grazing lands has been identified as a likely source of oocysts that contaminate drinking and recreational water [14, 15]. The importance of agrianltural sources of oocyst contamination should not be taken lightly since infected calves and lambs can pass up to $10^{10}$ oocysts can enter the surface water system following a hard rain on apasture containing infected animals.
The studies reviewed as well as the prevalence data discussed and demonstrate that Cryptosporidium parvum is ubiquitous in the environment and that it is likely to be present as a waterborne pathogen, especially where standard of sanitation and water treatment technology are low. The data present in this study indicate that Cryptosporidium oocysts can be frequently isolated from a variety of water sources, suggesting that this protozoan could play a role in water borne disease outbreaks [18].

References
Figure1 Cryptosporidium oocysts (vright red colour) isolated from sewage.