NOTE Parasitology

Seasonal Change in the Number of Cryptosporidium parvum Oocysts in Water Samples from the Rivers in Hokkaido, Japan, Detected by the Ferric Sulfate Flocculation Method

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ABSTRACT. An epidemiological study was carried out in natural water supplies of Hokkaido, one of the largest dairy prefectures in Japan. To investigate the prevalence of Cryptosporidium parvum (C. parvum) oocysts water samples were collected from three rivers in the eastern area of Hokkaido from August 1999 to October 2001, and C. parvum oocysts were collected and purified by the ferric sulfate flocculation method. The oocysts were detected using the immunofluorescent assay test (IFAT) and 4′, 6-diamidino-2-phenylindole (DAPI) staining. The seasonal change in the number of oocysts detected was observed. Oocysts increased in numbers from the late summer to the early autumn (from August to November), thereafter, they exhibited a trend to decrease until December, when no oocysts could be detected in the three rivers was 3.50, 5.00 and 3.33 oocysts/l, respectively. The oocyst density in river water changed in relation to the season in 1999, 2000 and 2001. This report first cleared up the seasonal changes in C. parvum oocysts number in river water.

KEY WORDS: Cryptosporidium parvum oocyst, river water, seasonal change.


The protozoan parasite C. parvum is recognized as one of the common pathogenic protozoa in the gastrointestinal tract of humans and animals [1]. Several outbreaks of cryptosporidiosis have been reported in the last few decades [4, 10]. Humans are infected with the pathogen through the fecal-oral route, namely by ingestion of contaminated food or water [2]. Water is perhaps the major source of massive outbreaks of the infection, so that the contamination of raw or treated water with the pathogen results in the outbreaks [3]. Despite the difficulties in detecting procedures, the number of oocysts detected ranges from 0.005 to 252.7 oocysts/l of surface waters in different countries of the world [8, 11]. Information on Cryptosporidium oocysts in the drinking water supply systems is very limited in Japan compared to the other countries. Moreover, cryptosporidiosis has been shown to occur sporadically in cattle [5]. Two outbreaks of waterborne cryptosporidiosis were described in Japan: the first occurred in Kanagawa prefecture in 1994 with 461 cases and the second in Saitama prefecture in 1996 with 8,000 cases. Previously, we detected C. parvum oocysts in river water by morphological and immunological methods, and presumed that Cryptosporidium contamination might be linked to agricultural activities, especially, dairy farming [13]. Economic statistical data obtained from the Obihiro Chamber of Commerce and Industry indicated that the eastern area of Hokkaido had a high agricultural activity. The population of cattle was 292,150 in the Tokachi area, where the excrement and urine of domestic animals were inadequately treated despite the expansion of dairy and feedlot farming sectors, as shown by the previous studies [7]. As a result, sewage from farms caused a serious environmental contamination. August, September and November are the harvest months in Hokkaido.

Hitherto, the seasonal number of oocyst in environmental water has not been examined. In this study, we detected C. parvum oocysts in river water of the eastern area Hokkaido and continued to monitor the seasonal change in oocyst density from August 1999 to October 2001.

Oocysts were collected in three rivers in the Tokachi area. Oocysts in river water were collected and purified by the ferric sulfate flocculation method, which is well established and reliable [6, 13]. Oocysts were detected using the immunofluorescent assay test kit (Cellabs DIF, Cellabs Pty., Ltd. Sydney, Australia), and 4′, 6-diamidino-2-phenylindole, (DAPI) solution (2 mg/ml in DW). The samples were observed under a light microscope using UV-light.

Prior to harvesting of oocyst from raw water samples, the sensitivity of ferric sulfate flocculation method was determined for C. parvum oocysts in DW. C. parvum oocysts detected in fecal samples from cattle raised in the Tokachi area were used in this study. Six samples of 1 to 10 thousands of oocysts added to 2 liter of distilled water (DW) were prepared. Oocysts in each sample were purified by the ferric sulfate flocculation method and detected by IFA and
DAPI staining. The trials were repeated at least 6 times. The results are summarized in Table 1. The sensitivity of the ferric sulfate flocculation method in this study was the same as that in previous reports [6]. The detection rate of oocyst was identical when 10 or 100 oocysts were added to DW. It is most likely that the detection rate may be affected by the adhesion of oocysts to each other, especially when small numbers of oocysts were detected in water samples.

The monitoring results of oocyst numbers in water samples are shown in Fig. 1. The same pattern of oocyst numbers was observed in all the three rivers. C. parvum oocysts were detected only in August, September, October and November in 1999, 2000 and 2001. Before August, no oocysts could be detected. In August and September, oocyst numbers increased to the maximum levels. The maximum number of oocysts in the three rivers was 3.50, 5.00 and 3.33 oocysts/l, respectively. Thereafter, the number of detected oocysts decreased gradually. No water samples were collected from the rivers during the period from January to March due to heavy frost and snow.

We demonstrated seasonal changes in oocyst numbers in river water. To our knowledge, our data first showed the seasonal changes in the density C. parvum oocyst detected in water samples from the rivers in Hokkaido. Recent epidemiological reports on C. parvum, showed that oocysts were detected in polluted farms of many foreign countries in [12, 14]. Oocysts were not consistently detected in river water all the year round. Lack of consistent detection of oocyst may be indicative of the fact that other factors play a role in regulating the inflow of C. parvum oocysts in rivers.

The assumption that the pollution of river water with oocysts may be caused by the direct inflow of animal feces from dairy and feedlot farms, prompted us to select water collection points at the downstream of the rivers.

Previous reports of cryptosporidiosis in Tokachi [9] first showed that C. parvum oocysts were detected in the fecal samples of young cattle, under 1 month of age, raised at the private farms in the Tokachi area. The infection rate with C. parvum in cattle under one month of age was high in the world, as shown in previous reports [12, 14], and secondly, that, C. muris oocysts were detected in the fecal samples of adult cattle raised in the public farms situated in the upstream of all rivers under research. In view of these results, it is likely that C. parvum oocysts may originate from private farms. The fecal materials of domestic animal were used as compost or manure in the private farms in the Tokachi area. August, September and November are the harvest months in Hokkaido. The wheat is reaped in August, then, a lot of feces are heaped in fields as manure for various crops. The inflow of domestic animal feces from farms into rivers becomes a crucial and controversial issue in Hokkaido [7]. The number of cattle under one month of age is supposed to be maximal in August, because the number delivery in cows was maximal in July, according to the data obtained from Livestock Improvement Association of Japan, INC. (personal communication). Our findings suggest that the number oocyst as a contamination source increased in August and that the apparently higher rate of

### Table 1. Sensitivity of the ferric sulfate flocculation method indicated by the number of C. parvum oocysts detected in two liter of distilled water containing different numbers of oocysts

<table>
<thead>
<tr>
<th>No. of Oocysts in DW</th>
<th>$1 \times 10^4$</th>
<th>$1 \times 10^3$</th>
<th>$1 \times 10^2$</th>
<th>$1 \times 10^1$</th>
<th>$1 \times 10^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td>129</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>152</td>
<td>20</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exp. 3</td>
<td>130</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Exp. 4</td>
<td>145</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Exp. 5</td>
<td>123</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Exp. 6</td>
<td>132</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
delivery in cow in July than in the months may be one of the causes of seasonal changes in oocyst numbers in river water. Moreover, much rainfall was recorded in August and September by the Meteorological Observatory in Obihiro. Heavy rainfall would be an important factor accelerating the flow of compost or manure containing C. parvum oocysts into rivers. Farming systems and rainfall patterns may mainly cause the fluctuations of the density of C. parvum oocysts in river water. In conclusion, the fecal accumulation as compost in fields in August may be important to regulate oocyst numbers in river water. Further studies need to be carried out on the relationship between compost storage and the seasonal change of C. parvum oocysts.

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REFERENCES