NOTE Parasitology

Detection of a Small Number of Cryptosporidium parvum Oocysts by Sugar Floatation and Sugar Centrifugation Methods

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ABSTRACT. Detection rates from the samples including a small number of Cryptosporidium parvum oocysts were compared between the sugar floatation and the sugar centrifugal floatation methods. As the results, the oocysts were detected from 70 and 80 of 100 samples including $6.0 \times 10^2$ and $1.0 \times 10^3$ oocysts per 1 ml by the floatation method, respectively, whereas from 52 and 53 of the same samples by the centrifugal floatation method. Therefore, it was considered that the floatation method is the most suitable method for the detection from samples including a small number of Cryptosporidium oocysts. It is also suggested that results of the sugar floatation method were reliable for samples including more than $1.0 \times 10^3$ oocysts /ml.

KEY WORDS: centrifugal floatation method, detection of oocysts, floatation method.

Cryptosporidium parvum is a protozoan parasite causing watery diarrhea in human and calves, and the maximum of oocyst number per gram of feces from infected laboratory animal is $10^4$ [8]. An outbreak of diarrhea caused by drinking water contaminated with C. parvum oocysts is a serious problem throughout the world [1, 4, 6, 11]. For the detection of oocysts from drinking water, drains and river water, a floatation method or a centrifugal floatation method using sugar solutions has been generally used. It is well known that the color of oocysts immersed in some concentrations of sugar solutions changes to pink under a light microscope and then their observation becomes easier. Matsui [7] used the former method using a sugar solution with a 1.266 of specific gravity (SG) which has been usually applied for the detection of coccidial oocysts because this solution can keep pink color of the oocysts for a long time. While, Iseki [5] recommended the latter method using a sugar solution with 1.2 of SG because concentration of oocysts and removal of impurities by centrifugation makes microscopic observation easier.

Both methods are able to detect C. parvum oocysts from the samples including a large number of oocysts but detection rate from the samples including a small number of oocysts has not been cleared yet. Detection from the sources including only a small number of oocysts might be important, because it was reported that the infection was realized in most human and mice by ingestion of $10^2$ oocysts of C. parvum or C. muris [2, 9]. Therefore, we attempted to investigate and compare the detection rates of these two methods under the presence of a small number of oocysts.

C. parvum oocysts used were isolated from the feces of naturally infected cattle. They were supplied by Dr. K. Shimura, the National Institute of Animal Health (Tsukuba, Japan), and were maintained by passage in SCID mice. Feces of the mice were collected, suspended in distilled water, stirred for 1 hr, filtered through double wire-screen (50- and 100 mesh), and centrifuged at 550 g for 7 min. The precipitates were suspended in 2% (w/v) potassium dichromate solution and then stored at 4°C. The sample solutions including $3.0 \times 10^2$, $6.0 \times 10^2$ and $1.0 \times 10^3$ oocysts per 1 ml were prepared after washing with distilled water to remove potassium dichromate solution. The solution including $3.0 \times 10^2$ oocysts/ml was used in order to determine a suitable sugar solution.

Sugar solutions used for two detection methods were the A solution (SG 1.266; 128 g sucrose and 100 ml distilled water), which usually applies for detection of coccidian oocysts, and the B solution (SG 1.203; A solution: distilled water = 3 : 1).

One ml of the sample solutions was added to each sugar solution in the small-sized test tubes and mixed thoroughly. For the floatation method, sugar solution was added again up to the brim of the tubes as to form a convex surface at the mouth of the tubes. The samples were picked up from the surface of solutions 30 min after, and examined by means of a light microscope. For the centrifugal floatation method, the mixed solutions were centrifuged at 550 g for 7 min, then sugar solution was added again up to the brim of the tubes same as the floatation method. Five minutes after, the samples were picked up from the surface, and examined under a microscope [5].

Photomicrographs of the oocysts detected by the floatation method using the A and B solutions are shown in Fig. 1. The A solution made oocysts deeper pink color than B solution, so the detection was easier when the A solution was used. The examination to determine a suitable sugar solution showed that oocysts could be detected from 9 and 6 of 100 samples by the floatation method using the A and B solutions, respectively. Nevertheless, by the centrifugal floatation method using B solutions, oocysts could not be detected at all. Therefore, it was considered that the A solution was the most suitable solution to detection a small num-
Fig. 1. Cryptosporidium parvum oocysts detected by the floatation method using the A and B solutions. a: A solution (specific gravity: 1.266); b: B solution (specific gravity: 1.203). The A solution made oocysts deeper pink color than B solution.

Table 1. Comparison of detected Cryptosporidium parvum oocysts by a floatation and a centrifugal floatation methods using the A sugar solution (SG: 1.266)

<table>
<thead>
<tr>
<th>Number of oocysts</th>
<th>Methods</th>
<th>N</th>
<th>Positive number</th>
<th>1</th>
<th>2</th>
<th>3≤5</th>
<th>6≤10</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0 × 10^2</td>
<td>SF</td>
<td>100</td>
<td>70</td>
<td>27</td>
<td>31</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>SCF</td>
<td>100</td>
<td>52</td>
<td>37</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1.0 × 10^3</td>
<td>SF</td>
<td>100</td>
<td>80</td>
<td>31</td>
<td>33</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>SCF</td>
<td>100</td>
<td>53</td>
<td>35</td>
<td>11</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

N: Number of samples; SG: Specific gravity; SF: Sugar floatation method; SCF: Sugar centrifugal floatation method.

As the results of the examination on the detection rates by a floatation and a centrifugal floatation methods using the A solution for the samples including a small number of oocysts, the rate by the floatation method was higher than that of the centrifugal floatation method. The low detection rate in the centrifugal floatation method may be caused by the loss of floating oocysts by centrifugation. In addition, it seems to be very important for efficient detection that the procedure of the floatation method without centrifugation is simple. Therefore, it is considered that the floatation method with the A solution is the most suitable method to detect from samples including a small number of Cryptosporidium oocysts.

The sugar floatation method was reliable for the samples including more than 10^3 oocysts (mL) in the present study, but the number of oocysts detected was 2 or less in the most samples, indicating that oocyst positive samples by this method actually include several hundred times of detected oocysts. However, it is reported that the results of the floatation method were neither affected by duration of floatation nor the number of oocysts included in Eimeria kriegsmanni infection [10]. Thus, the sugar floatation method is useful just for an examination whether oocysts are included or not, although the result of the proportion varies according to the number of oocysts in the materials. It is known that the infection of Cryptosporidium parvum is caused by 10^2 oocysts [2], so we should exactly understand the sensitivity of examination methods.

REFERENCES


