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ORIGINAL ARTICLE

Development of a Diagnostic Kit to Detect *Cryptosporidium parvum* and *Giardia lamblia*

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Cryptosporidium parvum, *Giardia lamblia*, immunochromatography, rapid diagnostic kit

Abstract
Objectives: This study aims to develop a high-sensitivity antibody diagnostic kit that will enable a rapid and accurate detection of *Cryptospofidium parvum* and *Giardia lamblia* in patients with diarrhea.
Methods: The cultivated *C. parvum* oocysts and *G. lamblia* cysts in each calf and dog were injected to mice to obtain antibodies, which were titrated. Spleen cells of the immunized mouse were separated and blended with myelomas to produce hybrid cell lines that form monoclonal antibodies. Using ELISA method, antibodies that specifically respond to *C. parvum* and *G.lamblia* were then selected. The cells were injected into the abdominal cavity of a BALB/c mouse to isolate hydrops abdominis containing high level of antibodies. The IgG antibody was purified using protein G gel.
Results: The detection limit of monoclonal antibodies for *Cryptosporidium parvum* and *Giardia lamblia* was 125 oocysts/mL and 1250 cysts/mL, respectively. In addition, during testing they did not show cross-reactivity to viruses ($n = 15$), bacteria ($n = 17$), and parasites ($n = 9$).
Conclusion: The rapid diagnostic antibody kit developed in this study, which specifically responds to *C. parvum* and *G. lamblia*, will be useful in detecting and monitoring diarrheal infections.

1. Introduction

Cryptosporidium parvum and *Giardia lamblia* have been recognized as the causative agents of diarrhea in humans worldwide [1]. These protozoans are transmitted by the fecal–oral route and most commonly by the consumption of contaminated food and water [2]. Infections are mostly seen in young children and immunocompromised patients. These infections are seen in both developing countries and developed countries.

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However, especially in developing countries, there is an increased risk of transmission, due to urban crowding and poor sanitation facilities [3].

Among the Korean population, *C. parvum* and *G. lamblia* account for less than 1% of diarrheal cases; however, the rate of *C. parvum* infection has been on the rise, and one case of infection by *G. lamblia* was reported in Jinan-gun, Jeollabuk-do Province, among people who drank water from a nearby valley [4,5].

Traditional diagnostic methods for treating these parasitic infections include testing fecal samples for the pathogen and must include concentration procedures along with specific staining techniques for proper microscopic detection and identification of the parasite [6]. These methods are laborious, take a long time, and require specialized and trained personnel. Although other techniques such as immunofluorescence microscopy improve sensitivity, they are expensive and laborious, and are not routinely available in all laboratories [7]. In addition, molecular techniques to detect *Cryptosporidium*, *Giardia* include polymerase chain reaction (PCR) and real-time PCR that provide high sensitivity and specificity, but these techniques are time consuming and require expensive specialized equipment [8,9].

Therefore, there is a need for a simple yet accurate method of detection for rapid and effective treatment of diarrheal infection. The aim of this study was to develop a new antigen diagnostic kit and evaluate its efficiency in detecting *C. parvum* and *G. lamblia* infections. In addition, the usefulness of this rapid diagnostic kit was compared with enzyme-linked immunosorbent assay (ELISA) and other diagnostic kits that are commercially available.

2. Materials and Methods

2.1. Preparation of immunogen

C. parvum oocyst was purchased from MEGACOR (MEGACOR Diagnostik GmbH, Hoerbranz, Vorarlberg, Austria) and orally injected to a month-old calf. From the 2nd day onward, the stool samples were examined with a Crypto-Strip (Coris BioConcept, Gembloux, Belgium). The samples were floated on saline solution, kept still for 2 hours, and the upper layer was extracted. The collected fluid was centrifuged and the precipitation was cleansed three times with sterilized saline solution to retrieve *C. parvum* oocysts.

G. lamblia cyst was purchased from American Type Culture Collection (Manassas, VA, USA; catalog number: PRA-242) and orally injected to a 2-month-old beagle dog. From the 2nd day onward, the stool samples were examined with a Giardia-Strip (Coris BioConcept). The cysts were retrieved similar to the procedure described for *C. parvum*.

2.2. Administration of adjuvant emulsions

Samples of *C. parvum* and *G. lamblia* were separately mixed with complete Freund's adjuvant (Sigma Aldrich). Approximately 200 μg of the emulsion was injected four times into the tail vein of a mouse at a 2-week interval. While complete adjuvant was used for the first injection, incomplete adjuvant was used for the rest of the injections. Overall, three intravenous injections were administered into the tail vein of the mouse.

2.3. Serum collection, titration, and cell fusion

A small amount of blood was drawn from the tail of the immunized mouse; subsequently, the serum was separated and ELISA was used for titration of the serum sample. The immunogen was adhered to the ELISA plate at a concentration of 1 μg/mL. An antiserum was then diluted in ten stages (10, 100, 1000 times, and so on) by adding 1% bovine serum albumin for reactivity test. Secondary reactivity test was conducted using the goat antimouse immunoglobulin G (IgG) peroxidase, and 3,3′,5,5′-tetramethylbenzidine substrate (Thermo Fisher Scientific Inc., Rockford, IL, USA) was added for color development. The cut-off rate was set at three times the absorbance level of a normal mouse serum. At a dilution factor greater than 1000, if the sample shows antibody titer above the cut-off value, cell fusion was performed. Spleen cells of the immunized mouse were separated and blended with myelomas to produce hybrid cell lines that form monoclonal antibodies. Using ELISA method, antibodies that specifically respond to *C. parvum* were then selected. The cell lines were cultured in a large

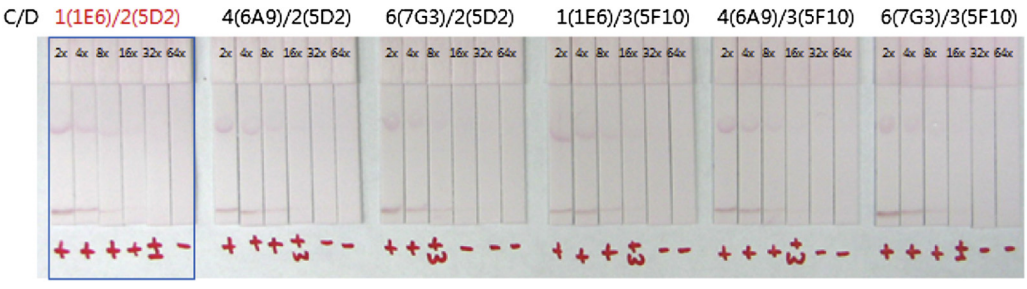

Figure 1. Selection of optimal pair for anti-*Cryptosporidium parvum* by the detection limit test.