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EPA ENVIRONMENTAL RESEARCH BRIEF

Electron Microscopic Examination of *Giardia* Cultures for Viruses

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Abstract

Giardia lamblia is an important waterborne pathogen in the United States. Laboratory cultures of *G. lamblia* are maintained by USEPA researchers who conduct disinfection studies with this pathogen or who develop increasingly effective methods to detect it in the environment. Some *G. lamblia* cultures maintained in other laboratories have recently been shown to be infected with a virus. Researchers are concerned that infected cultures may react differently in laboratory studies. In this study, electron microscopy (EM) was used to examine USEPA cultures of *G. lamblia* for virus. Virus was demonstrated in one of four *G. lamblia* strains examined by this method. The infected strain will be used to determine the consequences of viral infection.

Introduction

Recently, investigators preparing a *G. lamblia* cDNA library discovered a virus infecting the protozoan (1). The identified virus is roughly spherical, 33-37 nm in diameter, and has a dsRNA genome 7 kb in size. The *G. lamblia* virus (GLV) bands in a CsCl gradient at a buoyant density of 1.368 g/ml. Analysis of the purified virus has revealed a single major protein species of 100,000 molecular wt (2).

GLV has been identified in *G. lamblia* cultures obtained from a variety of sources and has been found in isolates of both human and animal origin (3). Not all *G. lamblia* cultures are infected. Virus-free cultures have been demonstrated by various investigators (3,4). While some virus-free cultures are susceptible to infection with GLV, others appear to be resistant. GLV does not cause cell lysis, and infected cultures are not readily identifiable without special methods to detect the virus. The significance of GLV infection is not presently clear.

To determine if *G. lamblia* strains cultured and studied in EMSL-Cincinnati parasitology laboratories are infected with GLV or other viruses, preparations of these cultures were examined for the presence of virus particles using EM.

Materials and Methods

G. lamblia *cultures*. Axenic trophozoite cultures of four different *G. lamblia* strains were examined. These strains included Human-1-Portland (H-1-P), CDC:0284:1, ATCC 30957, and ATCC 50137. The specimens for EM examination were obtained as trophozoite suspensions in 1X Hanks' Balanced Salt Solution (HBSS). The suspensions were placed through three freeze-thaw cycles before

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examination. In one case (H-1-P), the crude freeze-thaw preparation was further processed to extract virus and remove cellular debris (after removing an aliquot of the crude preparation for EM examination). Virus particles which may have been entrapped in H-1-P cellular material were eluted with 3% beef extract and extracted with trichlorotrifluoroethane. After low-speed centrifugations to remove unwanted material (170 X g for 15 min and 3500 X g for 30 min), the possible virus component was pelleted by high-speed centrifugation at 110,000 X g for 2 hr. The resulting pellet was resuspended with 0.5 ml of HBSS and this suspension examined by EM.

Electron microscopy. Direct negative-stain examination of the four crude freeze-thaw preparations and single purified preparation was carried out as previously described (5). Briefly, a drop of one of the preparations was applied to an EM grid with carbon support film. After 1 min, excess material was removed from the grid with filter paper. The grid was rinsed with 1 or 2 drops of distilled water and then stained with 2% phosophotungstic acid, pH 7. Excess stain was removed with the filter paper. After drying, the grid was examined at 80 kV with a JEOL JEM-100CX electron microscope.

To optimize viral adsorption and distribution, some grids were pretreated with 0.1% poly-L-lysine (6) before specimen application. Each preparation was examined using both poly-L-lysine pretreated and untreated grids.

Cytopathic effect (CPE) in animal cell culture. Two 0.25 ml aliquots of the H-1-P purified preparation were used to inoculate two 6 oz culture bottles containing confluent monolayers of buffalo green monkey (BGM) kidney cells. The inoculated cell monolayers were examined daily for the development of CPE as compared to uninoculated control monolayers.

Plaque formation in bacterial culture. Serial dilutions of the H-1-P purified preparation were made and 0.5 ml aliquots of each dilution were assayed for plaque forming units according to a procedure described elsewhere (7). The plaque assay was performed in triplicate using three different *E. coli* strains as the host bacterium. The strains included *E. coli* C (ATCC 13706), *E. coli* C-3000 (ATCC 15597), and *E. coli* A-19 (An Hfr strain obtained from R. L. Ward, Gamble Institute for Medical Research, Cincinnati, OH).

Results

Results of the EM examination of the *G. lamblia* cultures are presented in Table 1. Virus particles were visualized in both the crude freeze-thaw preparation and the purified preparation of strain H-1-P (Figure 1; A, B, and C). The virus particles were visualized using both untreated EM grids and grids pretreated with poly-L-lysine. The particles appeared to be more abundant in the crude preparation than in the purified preparation. Massive aggregates of virus particles were observed in the crude preparation (Figure 2 and Figure 3). These massive aggregates were not apparent in the purified preparation. Such aggregates were likely removed or dispersed during the further processing of the crude preparation. No virus particles, similar or of other type, were detected in the crude preparations of the other three *G. lamblia* strains.

The virus particles appeared generally spherical with only slight indication of an icosahedral nature. Most of the particles were observed to be penetrated by the negative stain. The mean diameter of 91 particles ± SD was 38 ± 2 nm. The particles revealed no capsomeric detail, and no distinctive structural features were evident.

Table 1. Results of EM Examination of *Giardia* Cultures for Viruses

G. lamblia Strain	Type of Preparation	Virus Particles Visualized: Untreated Grids	Virus Particles Visualized: Pretreated* Grids
H-1-P	Crude	+	+
H-1-P	Purified	+	+
CDC:0284:1	Crude	-	-
ATCC 30957	Crude	-	-
ATCC 50137	Crude	-	-

*EM grids pretreated with 0.1% poly-L-lysine before sample application.

CPE was not observed in the two BGM cultures that were inoculated with the virus-particle-containing H-1-P preparation. Inoculated monolayers appeared intact and identical to uninoculated control monolayers through day 21 post inoculation.

Plaques were not detected in the bacterial cultures of *E. coli* C, A-19, and C-3000 that were inoculated with the H-1-P preparation.

Discussion

The virus particles visualized in the H-1-P culture preparations appear to represent the same virus (GLV) that was detected by Wang and Wang (1986). Although those investigators originally reported particles 33 nm in diameter (1), they later reported a diameter of 37 nm for GLV (2). This latter figure corresponds well to the mean diameter of 38 nm found for the particles observed in the present investigation. Additionally, published electron micrographs of GLV reveal particles readily penetrated by negative stain. This was also observed in the present investigation. It should be noted that these stain-penetrated particles should not be considered defective in lacking their nucleic acid component. Similar yeast virus particles, which also appear to be readily stain-penetrated, have been shown to have their nucleic acid present (8,9).

Cells (BGM) routinely used to detect viruses of the human gastrointestinal tract did not develop CPE after being inoculated with the H-1-P particles. Similar negative findings were reported by Wang et al. (1988) using two human intestinal cell lines. The particles also caused no lysis of bacterial cultures using E. coli strains known to be susceptible to small isometric bacterial viruses such as MS2 and ϕX174.

Mention has been made of a morphological resemblance between GLV and minirotavirus particles (1). Minirotavirus particles have been described as being 32 nm in diameter. They exhibit an "irregular margin, at times resembling a palisade of very small capsomeres" (11). Such characteristics are consistent with the "small round structured virus" (SRSV) classification proposed by Caul and Appleton (1982). In the present investigation, the visualized particles exhibited no such characteristics (and SRSVs have often been observed in this laboratory using the same negative-stain procedures). These particles can best

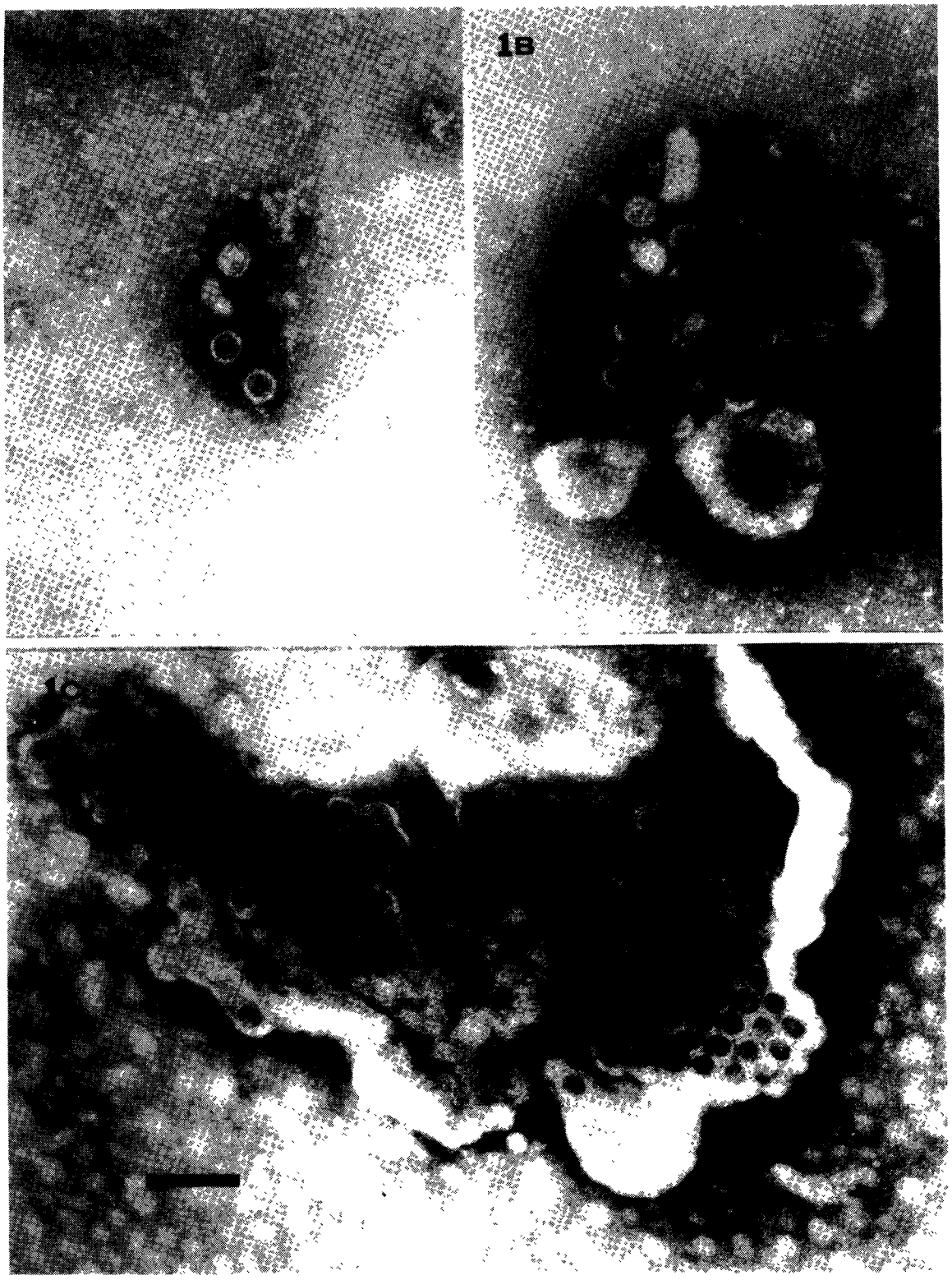
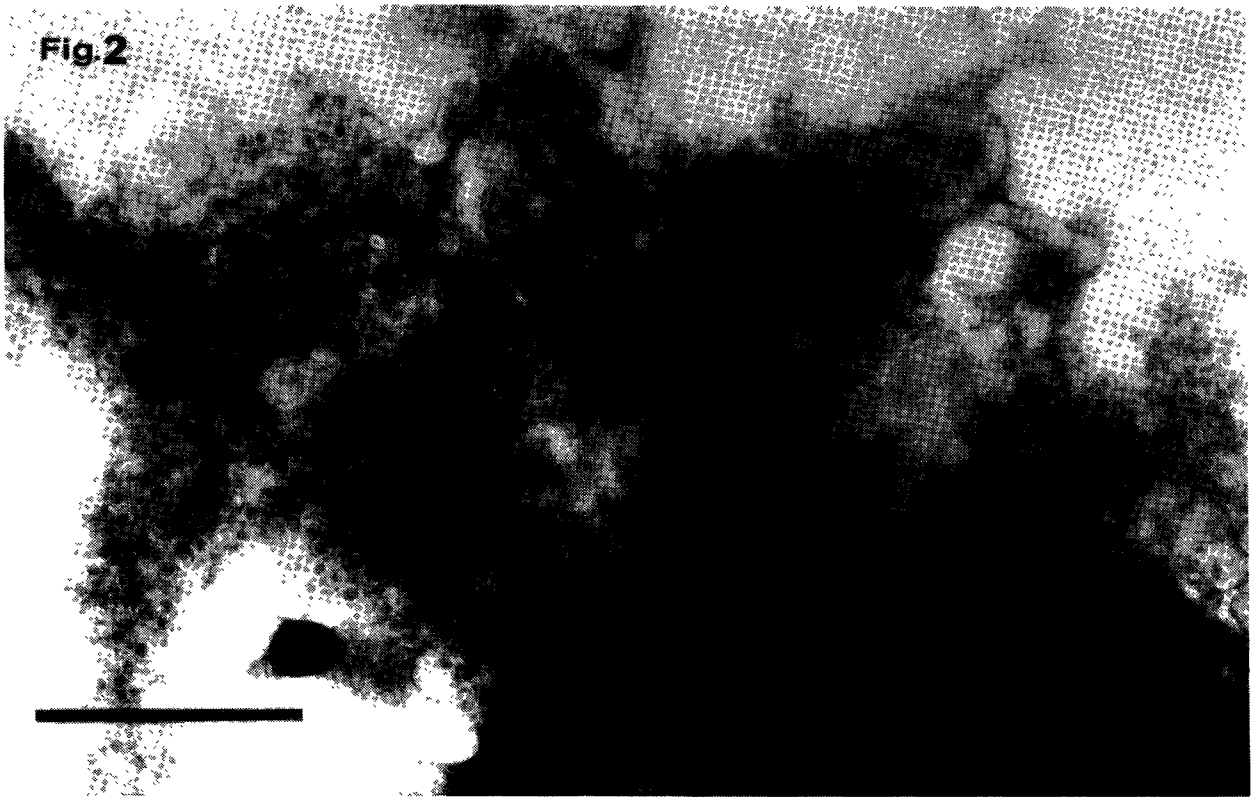
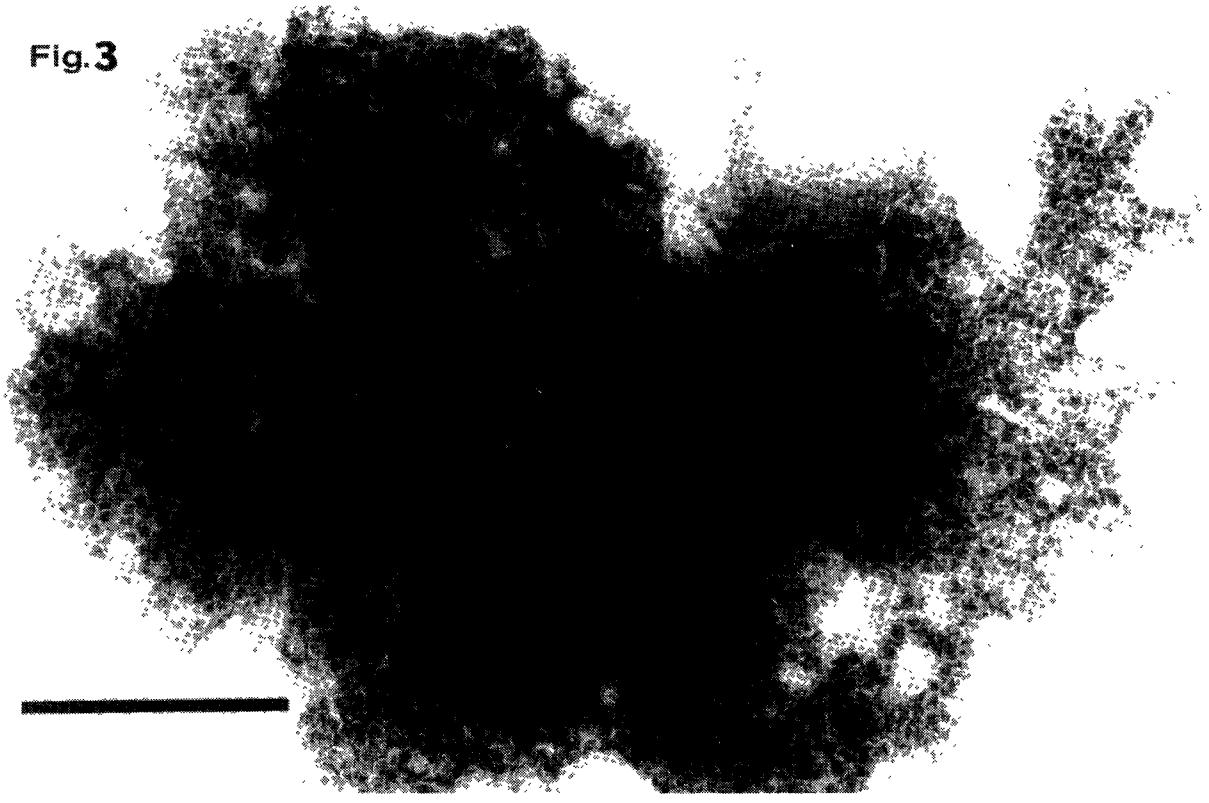


Figure 1. (A and B) Virus particles observed in the negatively stained crude freeze-thaw preparation of *G. lamblia* H-1-P. (C) Membrane-associated particles, some in hexagonal array, in the purified H-1-P preparation. Bar = 100 nm for A, B, and C.





Figures 2 and 3. Massive aggregates of virus particles observed in the crude preparation of *G. lamblia* H-1-P. Bar = 0.5 μ.

be described as being "featureless" (12) with regard to morphological appearance.

Acknowledgment

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