References


Application of a Capture Enzyme Immunoassay in an Outbreak of Waterborne Giardiasis in the United Kingdom

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A capture enzyme immunoassay (ELA) for the detection of Giardia lamblia antigen was used to examine 136 fecal samples collected during an outbreak of waterborne giardiasis in a city in the UK. Six cases of Giardia lamblia infection were detected that had previously not been diagnosed by microscopy. The capture ELA provides an efficient means of processing large numbers of samples for prompt and accurate assessment of an epidemic. It may also facilitate rapid tracing of epidemic sources.

Giardiasis is a worldwide cause of diarrhoeal disease in both developing and developed nations, and is implicated as an important cause of childhood growth retardation in the tropics.

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Transmission of the causative agent, *Giardia lamblia*, is principally by faecal-oral contamination. Giardiasis is also spread by contaminated or insufficiently treated drinking water, and *Giardia lamblia* cysts are resistant to chlorine at concentrations used in water treatment (2). There have been well-documented water-borne outbreaks at ski resorts and other localities in the USA (3, 4), and among travellers to the USSR (5). It is strongly suspected that animal reservoirs may contaminate North American water sources (6, 7, 8).

The diagnosis of giardiasis by microscopy may require multiple examinations of concentrated faecal samples or jejunal aspirates (9). As one potential solution to this problem, the enzyme immunoassay (EIA) has been adapted for the detection of *Giardia lamblia* antigen in faeces (10, 11). A sandwich EIA using affinity purified antisera has been reported to have a sensitivity and specificity of 98% and 100% respectively as read by spectrophotometry or direct visual evaluation (11, 12). Here we describe the application of the EIA for antigen detection in faecal samples collected during an outbreak of water-borne giardiasis (13).

**Materials and Methods.** Faecal specimens were kindly provided by the Bristol Public Health Laboratory Service (PHLS). The samples were collected in the summer of 1985 at a time when there was a marked increase in the number of cases of giardiasis (13). One hundred and eight cases of giardiasis had been recognised by the Bristol PHLS between June and August 1985, of which 94% were adults. The shape of the incidence/time graph strongly suggested that the infections had originated from contamination in a single incident. Most of the individuals infected lived in an area to the south of Bristol supplied from a single water reservoir. Repairs had been carried out in July on two main pipelines carrying water to the locality (13). A total of 136 faecal samples were received for examination. Faeces were suspended in PBS at a concentration of 10 g% with a mortar and pestle. The suspensions were vortexed, filtered through wire mesh to remove large particulate matter and stored at -20°C in glass vials. In addition to the faecal specimens, four concentrated water samples from around the excavated water mains were also received. All 136 samples were examined by microscopy of formol ether concentrates stained by Lugol's iodine method (14). Stained and unstained centrifuge deposits of the concentrated water samples were also checked thoroughly by microscopy.

The preparation of affinity purified anti-*Giardia lamblia* rabbit antisera was as described in detail elsewhere (11). Rabbits were immunised with disrupted trophozoites grown in vitro or with disrupted cysts isolated from infected human faeces by centrifugation on 0.85 M sucrose. Antisera were affinity purified by column chromatography against a dialysed detergent extract of trophozoite protein bound to cyanogen bromide activated Sepharose-4B (Pharmacia, Sweden), and the affinity purified antibodies were conjugated to horseradish peroxidase (11). EIA plates (M129A, Dynatech, USA) were coated overnight at 4°C with the purified antibodies at a concentration of 10 μg/ml in sodium carbonate coating buffer of pH 9.6 (11). Plates were washed three times with PBS/0.05 % Tween, blocked for 1 h at 37°C with 50 % foetal calf serum (FCS)/coating buffer, washed again, and exposed for 2 h at room temperature to 200 μl of faecal suspension diluted 1:1 in FCS. After further washing, affinity purified rabbit anti-*Giardia lamblia* peroxidase conjugate was added at a predetermined optimal dilution in 50 % FCS/PBS Tween, for 2 h at room temperature. After washing, colour development was achieved with 0.04 % orthophenylenediamine (Sigma, USA) and 0.012 % hydrogen peroxide in phosphate citrate buffer pH 5 (11). Tests were performed in duplicate, and results read visually and by measuring optical density at 492 nm. Control wells were coated with 10 μg/ml non-immune rabbit immunoglobulin (although in this study this did not significantly enhance assay specificity). Suspensions from concentrated water samples were also tested in the capture EIA.

**Results and Discussion.** Faecal specimens from 63 individuals were found to contain *Giardia lamblia* cysts when examined by microscopy. No *Giardia lamblia* cysts were detected in faeces from the remaining 73 subjects, but six of them were known to have previously contributed microscopically positive samples to the Bristol PHLS. Thus a total of 69 out of 136 persons had parasitologically proven *Giardia lamblia* infections.

EIA results were expressed as the mean optical density for the duplicate test samples less the mean optical density for PBS/FCS controls. The cut-off point for a positive test was taken as three standard deviations above the mean result for a group of standard negative reference faecal samples. The negative reference samples were from individuals who had donated faeces on three separate occasions and on each occasion had been negative when tested by formol ether concentration and microscopy (11). A com-