

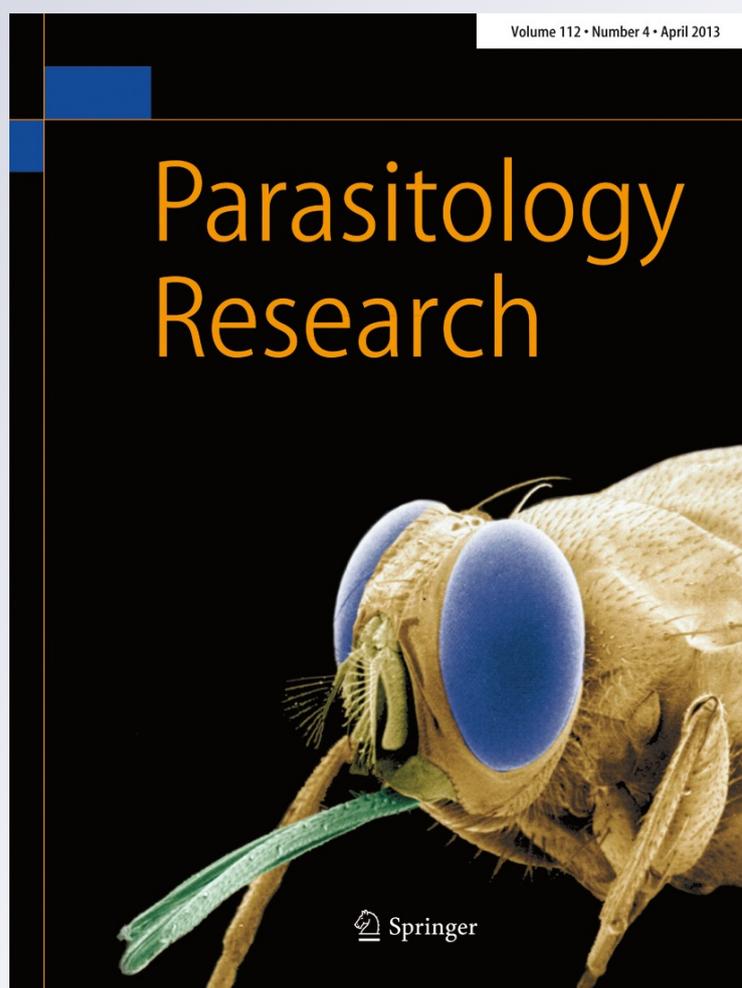
*Adhesive-tape recovery combined with molecular and microscopic testing for the detection of Cryptosporidium oocysts on experimentally contaminated fresh produce and a food preparation surface*

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# Adhesive-tape recovery combined with molecular and microscopic testing for the detection of *Cryptosporidium* oocysts on experimentally contaminated fresh produce and a food preparation surface

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**Abstract** A proof of concept study was conducted to determine if transparent double-sided adhesive tape could be used to recover and detect [by polymerase chain reaction (PCR) and immunofluorescence microscopy (IFA)] *Cryptosporidium parvum* oocysts on fresh produce and on a food preparation surface. Oocysts were applied on the surface of ten apples, ten peaches, eight cucumbers, and eight tomatoes within circles drawn with a permanent marker. Approximately 18 h later, skin excised from three uncontaminated and three contaminated circles from each piece of produce was subjected to PCR. Pieces of transparent double-sided adhesive tape were lightly pressed onto the surface of three other contaminated circles and examined by PCR. Other pieces of adhesive tape were pressed against the surfaces of three other circles and examined by IFA. At concentrations of 100 and 50 oocysts per circle, every produce item examined by PCR of contaminated excised skin was found positive, and every item examined by adhesive tape subjected to PCR and IFA was found positive, except one. At ten oocysts per circle, every produce item was found positive by PCR of contaminated excised skin, and all apples, cucumbers, and tomatoes were found positive by adhesive tape subjected to IFA. Detection of low numbers of oocysts on peaches by IFA examination of adhesive tape was

problematic because trichomes that cover peaches and impart the fuzzy surface partially restrict the tape from reaching some areas where oocysts adhere. Tape combined with IFA was successful in recovering and identifying oocysts from six areas of laminate countertop where the oocysts had been applied and allowed to dry for 30–60 min. These are the first findings to demonstrate that adhesive tape can be used to recover and identify a protozoan parasite from fresh produce and from a laminate food preparation surface.

## Introduction

Parasites of the genus *Cryptosporidium* infect a wide range of vertebrates, and two species are of particular importance for human health: *Cryptosporidium hominis* which has a predilection for human infection but has been found infrequently in other animals, and *Cryptosporidium parvum* which is host-promiscuous, found in a wide range of vertebrates, especially domesticated and wild ruminants (Robertson and Fayer 2012). The environmentally robust oocyst stage is transmitted by the fecal–oral route. Following ingestion, development occurs in the small intestine accompanied by watery diarrhea. Contaminated water and food products serve as vehicles of transmission for oocysts. Billions of oocysts may be excreted per infection, but as few as ten can initiate infection in a susceptible host. Many food products have been found contaminated with oocysts, including fruit, vegetables, beverages, meat, and shellfish (Robertson and Fayer 2012). Food can be contaminated at production sites from contact with manure, from contaminated irrigation water, or from agricultural workers; contamination can also occur during the distribution and preparation chain from food handlers, wash water, food handling surfaces, equipment, and utensils (Nichols 2008; Stinson and Tiwari 1978).

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A variety of adhesive tape applications have been developed to recover pathogens on plants, animals, humans, environmental surfaces, and foods for detection by microscopic and culture methods. Adhesive tape has been used to capture fungi on leaf surfaces (Langvad 1980) and to capture fungi, mites, and fleas on the skin of humans and animals (McEwan 2001; Miranda and Silva 2005; Pinter 1999; Sberna 1957; Teplitsky et al. 2008). It has been used to capture bacteria on meat surfaces (Cordray and Huffman 1985; Fung et al. 1980). An adhesive tape-based method was combined with fluorescence in situ hybridization to test for *Salmonella* on artificially contaminated tomatoes; captured *Salmonella* could then be enriched either by placing tapes face down in selective agar or could be detected in liquid media via flow cytometry (Bisha and Brehm-Stecher 2009).

Adhesive tape has been used routinely to capture pinworms and other helminth parasites which have then been identified by microscopy (Beaver 1949; Tang and Luo 2003) or by a molecular-based method (Piperaki et al. 2011) after applying tape to the anus of suspected hosts, especially children. Modifications of this basic method have been used to test for the presence of helminth eggs in buses (Borges et al. 2009) and on toilet seats, internal and external door knobs, latches, faucet handles and discharge valves (push buttons or pulling strings) in public restrooms (Aidar Sobrinho et al. 1995). There are, however, no known reports of adhesive tape being used successfully to detect any protist parasites.

Oocysts of *Cryptosporidium* that had adhered to the surface of experimentally contaminated spinach leaves and apples (Macarasin et al. 2010a, b) were observed with the aid of scanning electron microscopy and laser scanning confocal microscopy. Both methods, although appropriate for demonstrating the presence of the parasite for research purposes, are time-consuming, labor-intensive, and require a high level of expertise and expensive equipment. The present study was designed as a proof of concept to determine if adhesive tape combined with immunofluorescence microscopy (IFA) or with PCR might be applicable as a rapid, accurate, and inexpensive method for recovery and detection of the protozoan parasite *Cryptosporidium* from fresh produce and from a laminate work surface typical of a kitchen countertop.

## Materials and methods

### Acquisition of produce and oocysts

Apples, peaches, cucumbers, and tomatoes were purchased from a local supermarket. Each item was hand washed twice using a liquid detergent and warm tap water, rinsed with clean water, then blotted dry with paper towels immediately before use. Oocysts of *C. parvum* were purchased from Waterborne, Inc. (New Orleans, LA) and used within 2 weeks of delivery.

Oocysts, suspended in deionized water, were enumerated by direct counting on three-well Teflon-coated microscope slides. Fluorescein labeled anti-*Cryptosporidium* antibody (Mer/Fluor™; Meridian Bioscience, Inc., Cincinnati, OH) was added to each well containing the oocyst suspension, a 50×22-mm coverslip was placed over the microscope slide, and the wells were examined by fluorescence microscopy (Zeiss Axioskop™ microscope equipped with a 560-nm filter for fluorescein). The number of oocysts in each of six wells in the final suspension was counted after each dilution until oocyst counts reached approximately 100, 50, or 10 oocysts per 4 μl, the quantity applied to produce for testing or 250 oocysts, the quantity applied to locations on a laminate countertop.

### Contamination and sampling of produce and a food preparation work surface

A fine-tipped permanent marker was used to draw 12 circles, each with a 5-mm diameter, on the surface of each apple, peach, tomato, and cucumber. On each piece of produce, 4-μl aqueous suspensions of 100, 50, or 10 oocysts of *C. parvum* were pipetted within each of nine circles. Three circles remained as uncontaminated controls. The aqueous circles were allowed to dry for 3 h at room temperature (~22 °C) before the produce was placed in a plastic container with a lid and placed under refrigeration (5 °C) overnight, usually for 18 h. After the produce was removed from refrigeration and reached room temperature so that no aqueous condensate was present on the surface, samples were obtained. The skin containing three uncontaminated circles from each produce was excised with the aid of a scalpel, and each was placed in a 1.5-ml micro-centrifuge tube labeled “control.” Skin containing three contaminated circles was similarly removed, and each was placed in a micro-centrifuge tube labeled “no tape.” Three pieces of transparent double-sided adhesive tape (Skilcraft permanent double-sided tape with dispenser, 1/2×450 in.) approximately 10 mm long were each placed over the surface of three other circles, pressed lightly, and removed. Each tape was folded into a cylinder with the putative oocysts on the outer side and placed within a micro-centrifuge tube labeled “tape.” Three pieces of adhesive tape approximately 10 mm long were each pressed against the surface of each of the three remaining circles. The back side of each tape was applied to a glass microscope slide, the side exposed to the produce was flooded with 20 μl of Mer/Fluor™ reagent, a 50×22-mm coverslip was placed over the microscope slide, and the tape was examined by fluorescence microscopy at ×400 with a Zeiss Axioskop epifluorescence microscope equipped with a fluorescein isothiocyanate-Texas Red dual wavelength filter. The presence or absence of oocysts on each tape was recorded for each of the three circles per produce item examined (Tables 1, 2, and 3).

**Table 1** Produce contaminated with 100 oocysts per encircled area

Produce contaminated	PCR		IFA
	Tape	Excised skin	Tape
Apple 1	3 <sup>a</sup>	3	3
Apple 2	3	3	ND
Peach 1	3	2	3
Peach 2	2	2	ND
Cucumber 1	3	3	3
Cucumber 2	1	3	ND
Tomato 1	3	3	3
Tomato 2	3	3	ND

ND not done

<sup>a</sup>Number of encircled areas found positive per three examined

Six 5×9-cm rectangles were drawn on the surface of a laminate countertop, and the entire area of each was flooded with deionized water containing 250 oocysts. Between 30 and 60 min after the water had evaporated and none one could be seen on the countertop, a glass microscope slide with a 50-mm strip of adherent double-sided adhesive tape was gently pressed three times onto areas within the rectangle to recover oocysts. The surface of the tape was flooded with 20 µl of MeriFluor™ reagent, a 50×22-mm coverslip was placed over the microscope slide, and the tape was examined by fluorescence microscopy at ×400.

Photomicrographs of oocysts on the surface of adhesive tape were obtained using a Zeiss™ 710 confocal laser scanning microscope to simultaneously capture fluorescence and differential interference contrast images. Those images were observed with a Zeiss Axio Observer™ inverted microscope equipped with a 40×1.2 NA water immersion planapochromatic objective, and a photomultiplier tube captured the light from a 488-nm argon laser with a 3.7-µm pin hole passing through a MBS 488 filter with detection limits set between 492 and 543 nm for fluorescein. Images were recorded using Zeiss Zen™ 2009 software.

**Table 2** Produce contaminated with 50 oocysts per encircled area

Produce contaminated	PCR		IFA
	Tape	Excised skin	Tape
Apple 3	3 <sup>a</sup>	3	3
Apple 4	3	3	3
Peach 3	3	3	3
Peach 4	1	3	1
Cucumber 3	3	3	3
Cucumber 4	2	2	3
Tomato 3	3	2	3
Tomato 4	0	1	3

<sup>a</sup>Number of encircled areas found positive per three examined

**Table 3** Produce contaminated with 10 oocysts per encircled area

Produce contaminated	PCR		IFA
	Tape	Excised skin	Tape
Apple 5	2 <sup>a</sup>	1	2
Apple 6	2	2	ND
Apple 7	1	2	2
Apple 8	0	1	ND
Apple 9	1	1	3
Apple 10	1	1	2
Peach 5	1	1	0
Peach 6	0	1	ND
Peach 7	0	2	0
Peach 8	0	2	ND
Peach 9	1	2	0
Peach 10	2	1	1
Cucumber 5	1	2	1
Cucumber 6	1	2	2
Cucumber 7	1	3	3
Cucumber 8	1	2	2
Tomato 5	0	2	3
Tomato 6	1	3	2
Tomato 7	1	2	2
Tomato 8	0	2	0

ND not done

<sup>a</sup>Number of encircled areas found positive per three examined

Molecular methods of detection

Molecular detection and identification was performed on the specimens placed in 1.5-ml micro-centrifuge tubes and subjected to DNA extraction with a DNeasyTissue Kit (Qiagen, Valencia, CA). ATL buffer and proteinase K (20 mg/ml) were pipetted into the tubes which were incubated overnight at 55 °C before AL buffer was added. The protocol then followed the manufacturer's instructions except, to recover more DNA, the nucleic acid was eluted in 100 µl of AE buffer. A two-step nested PCR protocol was used to amplify an 830-bp segment of the SSU rRNA gene. Primers 5'-TTCTAGAGCTAATACATGCG-3' and 5'-CCCATTT CCTTCGAAACAGGA-3' for primary PCR and 5'-GGAAGGGTTGTATTT-ATTAGATAAAG-3' and 5'-AAGGAGTAAGGAACAACCTCCA-3' for secondary PCR were used (Xiao et al. 1999). The primary and secondary PCR mixtures were identical to those described (Fayer et al. 2010). PCR products were placed on 1 % agarose gel for electrophoresis and stained with ethidium bromide to identify positive and negative reactions.

Scanning electron microscopy

Additional produce were similarly purchased, contaminated with 1,000 *C. parvum* oocysts within a 5-mm circle and

examined with the aid of a low-temperature scanning electron microscope. Observations of produce skin and adhesive tape containing oocysts removed from produce were performed using an S-4700 field emission SEM (Hitachi High Technologies America, Inc., Pleasanton, CA) equipped with a Quorum CryoPrep PP2000 (Quorum Technologies Ltd., East Sussex, UK) cryotransfer system. To prepare specimens, skin was excised from produce and placed on flat specimen holders consisting of 16×30-mm copper plates that contained a thin layer of Tissue Tek (OCT Compound, Ted Pella, Inc., Redding, CA), which acted as a cyro-adhesive upon freezing. Adhesive tape was pressed onto the skin of produce; the back of the tape was placed on specimen holders with Tissue Tek as described above. The samples were frozen conductively, in a Styrofoam box, by placing the plates on the surface of a pre-cooled (−196 °C) brass bar with the lower half submerged in liquid nitrogen. After 20–30 s, the holders containing the frozen samples were transferred to a liquid nitrogen Dewar for future use or cryo-transferred under vacuum to the cold stage in the pre-chamber of the cryo-transfer system. Removal of any surface contamination (condensed water vapor) took place in the cryo-transfer system by etching the frozen specimens for 10–15 min by raising the temperature of the stage to −90 °C. Following etching, the temperature was lowered below −130 °C, and a magnetron sputter head equipped with a platinum target was used to coat the specimens with a 10-μm layer of platinum. The specimens were transferred to a pre-cooled (−140 °C) cryostage in the SEM for observation. An accelerating voltage of 5 kV was used to view the specimens. Images were captured using a 4pi Analysis System (Durham, NC).

#### Statistical analysis

This study was designed as a proof of concept to determine if adhesive tape can recover oocysts from fresh produce and a food preparation surface based on validation of their presence on the tape by either PCR or IFA. The results are either positive or negative and consequently are not subject to statistical applications.

## Results

Data demonstrating the proof of concept that adhesive tape subjected to PCR or IFA could detect and identify the presence of *C. parvum* oocysts on apples, peaches, cucumbers, and tomatoes are shown in Tables 1, 2, and 3. Oocysts were also captured by adhesive tape and observed by IFA from each of the six contaminated rectangular areas on a laminate countertop. All circles established as controls for

possible natural contamination were found negative by PCR on all produce used in this study.

At the concentration of 100 oocysts per circle, one to three contaminated circles were found positive on every fruit and vegetable when PCR was used to detect DNA on adhesive tape and on excised skin where adhesive tape was not applied (Table 1). Examination of adhesive tape by IFA revealed oocysts on each variety of produce that was experimentally contaminated.

At the concentration of 50 oocysts per spot, one to three contaminated circles were found positive on two apples, two peaches, two cucumbers, and one tomato when PCR was used to detect DNA on adhesive tape and on excised skin where adhesive tape was not applied (Table 2). On the second tomato, detection was limited to only one circle on the skin where adhesive tape was not applied. Examination of adhesive tape by IFA revealed oocysts on two of each variety of produce that was experimentally contaminated.

At the concentration of ten oocysts per circle, one to three circles were positive for all four cucumber replications when PCR was used to detect DNA on adhesive tape, and on excised skin where adhesive tape was not applied (Table 3). For all four varieties of produce in every replication of PCR in which skin was excised, one to three circles were found positive by PCR. PCR negative results were obtained for adhesive tape in one of six apples, three of six peaches, and two of four tomatoes. Examination of adhesive tape by IFA revealed oocysts on four of four apples, one of four peaches, four of four cucumbers, and three of four tomatoes.

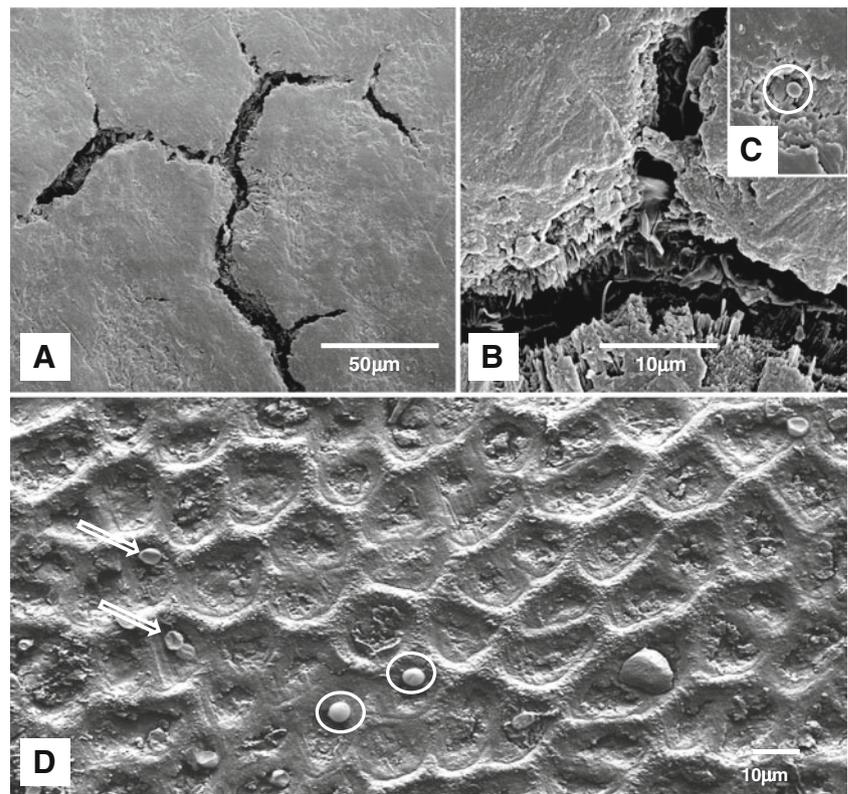
Scanning electron microscopy revealed oocysts adhered to produce and to adhesive tape used to remove oocysts from produce as shown in Figs. 1 and 2. Oocysts were located in microscopic fissures in apple skin (Fig. 1a–c), slight depressions in cucumber skin (Fig. 1d), and on the surface of peach skin beneath the trichomes (Fig. 2a). Of the few oocysts found on adhesive tape removed from peaches, oocysts were empty and surrounded by trichomes and other debris from the surface of the peaches (Fig. 2b).

Oocysts that adhered to adhesive tape were readily recognized with the aid of immunofluorescence microscopy compared with brightfield or differential interference microscopic observations. Oocysts observed by immunofluorescence and differential interference microscopy on the surface of adhesive tape removed from an apple and a peach are shown along with a photograph that combines the two foregoing images (Fig. 3).

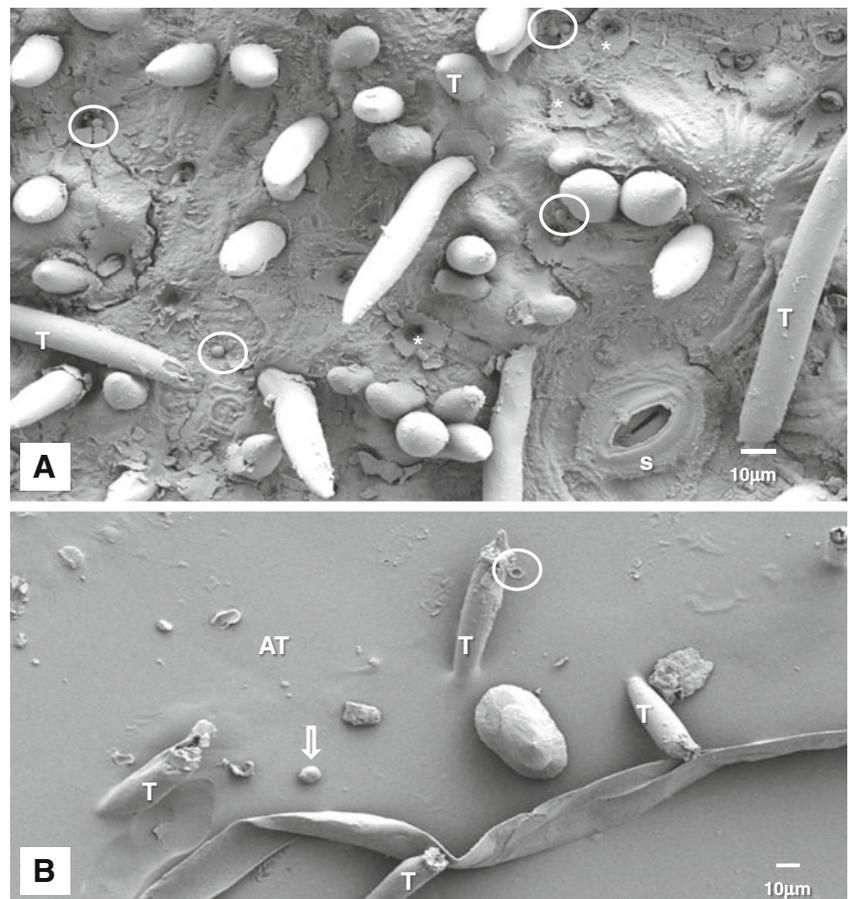
## Discussion

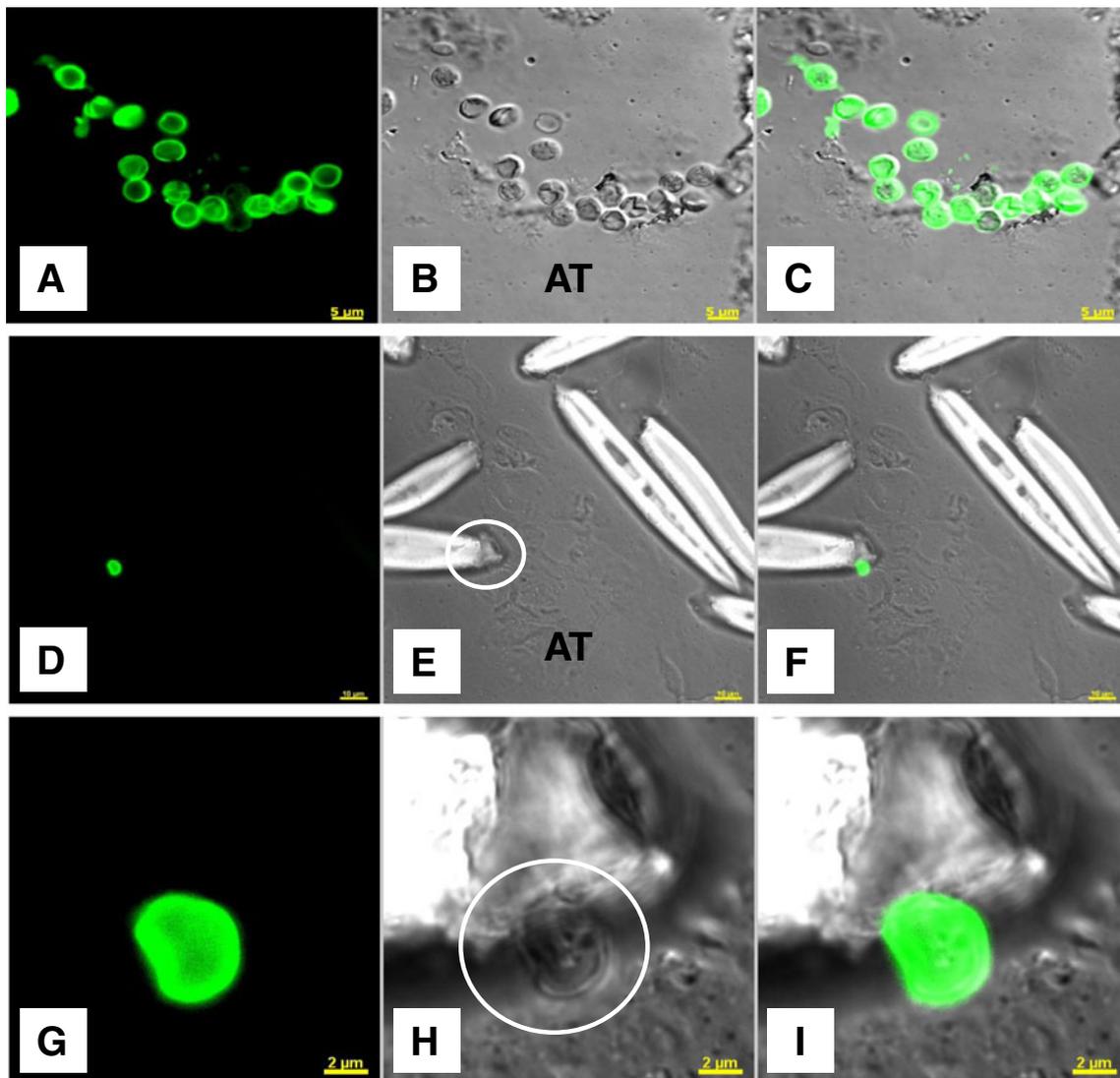
The present study is the first to demonstrate the concept that double-sided transparent adhesive tape when used in

**Fig. 1** Scanning electron micrographs. **a, b** An apple with multiple naturally occurring cracks. **c** An oocyst (within the circle) is located within a crack. **d** A cucumber with intact oocysts adhered (within circles) and collapsed or empty oocysts (open arrows)



**Fig. 2** Scanning electron micrographs. **a** Oocysts (within circles) on the surface of a peach amidst numerous hair-like trichomes (*T*) and sites (asterisk) where other trichomes had been attached to the surface of the peach. **a** Stomata (*S*) is also present. **b** A collapsed oocyst (circle) on the surface of adhesive tape (*AT*) removed from the surface of a peach. Trichomes (*T*), yeast (open arrow), and other debris are present





**Fig. 3** Adhesive tape (AT) containing adherent oocysts. **a–c** show AT removed from an apple, **d–f** show AT removed from a peach with an oocyst adhering to the base of a hair-like trichome (**e**, circle), **g–i** show a higher magnification of **d**, **e**, and **f**, respectively, with the oocyst (circle) appearing more prominently. **a**, **d**, and **e** show oocysts by

immunofluorescence microscopy (IFA); **b**, **e**, and **h** show the same oocysts by differential interference microscopy (DIC); and **c**, **f**, and **i** show combined IFA and DIC images. All images were observed by laser scanning confocal microscopy

conjunction with IFA or PCR methodology can recover and detect the presence of protist parasites contaminating various fresh produce and environmental surfaces. In this study, as few as 10 *Cryptosporidium* oocysts were applied as a contaminant on the surfaces of four types of produce, and 250 were applied on a laminate countertop typical of many used in kitchens for food preparation. Oocysts were detected on each piece of produce and each contaminated area of countertop. Only one earlier attempt to use adhesive tape to detect the protist parasites (*Eimeria* oocysts) has been reported and none were detected (Krecek et al. 2010).

At concentrations of 100 and 50 oocysts within a circle 5 mm in diameter (19.6 mm<sup>2</sup>), the level of detection using double-sided transparent adhesive tape examined either by

PCR or by IFA was 100 % for 16 pieces of produce. This was the same level of detection achieved by PCR on excised contaminated skin from the same pieces of produce. There was one exception in which a tomato contaminated with 50 oocysts per circle was negative by PCR but positive by IFA which probably was the result of experimental error because tomatoes contaminated with only 10 oocysts were found positive. At the contamination level of only ten oocysts per 5-mm circle, the most sensitive method of detection was PCR of excised contaminated skin. By this method, every piece of produce tested positive at one or more of the contaminated circles: six of six apples and peaches, and four of four cucumbers and tomatoes. However, for all produce contaminated with ten oocysts per circle, adhesive tape

examined by PCR was generally less sensitive than either PCR of contaminated skin without prior tape application or IFA examination of adhesive tape. Except for one tomato and the peaches, the IFA examination of adhesive tape detected oocysts on all 12 other produce items. Peaches were especially problematic because the skin (exocarp) of the peach contains hair-like projections (trichomes) that project from the surface (contributing to the fuzzy feeling and appearance) while providing a physical barrier preventing tape from reaching all oocysts that could be seen adhering to the exocarp at the base of the trichomes (Fig. 2). When adhesive tape used to recover oocysts from the surface of peaches was observed by SEM or fluorescence microscopy (Figs. 2 and 3), the number of trichomes observed sticking to the tape was far greater than the number of oocysts observed.

Successful use of adhesive tape to detect low level contamination of produce was reflected in differences in the surface structures among fruits and vegetables, as found with peaches versus those produce items with relatively smooth surfaces that were more amenable to this method of detection, and with the smooth surface of the laminate used for food preparation. These findings demonstrated that to determine the benefits and difficulties of using adhesive tape for detection, each surface should be tested individually.

Application of the adhesive tape method for recovering *Cryptosporidium* oocysts or other encysted protists from contaminated food surfaces may prove useful because current microscopic methods for investigating *Cryptosporidium* oocyst contamination of food either underestimate contamination or misidentify organisms that have no public health significance (Smith et al. 2007). The annual number of cases of cryptosporidiosis is highly underreported and as a result underestimated (Kortbeek 2009). The true disease burden for cryptosporidiosis in the USA is estimated to be 165,380–826,900 cases, compared to 8,269 cases reported annually to the CDC (CDC 2007). Likewise, in Scandinavia, the vast majority of cryptosporidiosis infections are also greatly underestimated in national registers of infectious diseases, with a single reported case estimated to represent 4,072 to 15,181 undetected cases (Hörman et al. 2004). Also underreported, and usually unidentified, is the etiological agent responsible for over half of all reported foodborne outbreaks (DeRoeve 1998). Even when a pathogen associated with a foodborne outbreak is identified, finding the food product harboring the pathogen is difficult. Detection of protist parasites in or on fresh produce is especially difficult because these parasites adhere tenaciously to plant surfaces and cannot be enriched in culture medium like bacteria to produce large numbers that facilitate detection. Current detection methods for *Cryptosporidium* on fresh produce use PCR to detect parasite DNA in washings from produce, direct microscopic observation of concentrated produce eluates, or similar examinations of chopped or ground food matrices, which is problematic

because of the large ratio of matrix volume to parasite number (Robertson and Gjerde 2001; Robertson et al. 2002). The present study has demonstrated the concept that adhesive tape can detect the presence of a protist such as *Cryptosporidium* on some fresh produce and on a laminate work surface with little other matrix volume. Other applications for this method may be found to expand its usefulness. Ultimately, its usefulness lies in further evaluation of the specimens to be examined versus the levels of detection desired.

## References

- Aidar Sobrinho T, Coelho LM, de Oliveira SM, Martins JT, Rabello Júnior JA, de Oliveira CR, de Paula MA, Perroud Júnior MW, Miyazaki SM (1995) Frequency of intestinal helminth eggs in public restrooms in Sorocaba, SP. *Rev Soc Bras Med Trop* 28:33–37, Portuguese
- Beaver P (1949) Methods of pinworm diagnosis. *Am J Trop Med Hyg* 29:577–587
- Bisha B, Brehm-Stecher BF (2009) Simple adhesive-tape-based sampling of tomato surfaces combined with rapid fluorescence in situ hybridization for *Salmonella* detection. *Appl Environ Microbiol* 75:1450–1455
- Borges CA, Costa-Cruz JM, Paula FM (2009) Intestinal parasites inside public restrooms and buses from the city of Uberlândia, Minas Gerais, Brazil. *Rev Inst Med Trop Sao Paulo* 51:223–225
- Centers for Disease Control and Prevention (2007) *Cryptosporidiosis* surveillance—United States, 2003–2005. *MMWR* 56:1–10
- Cordray JC, Huffman DL (1985) Comparison of three methods for estimating surface bacteria on pork carcasses. *J Food Prot* 48:582–584
- DeRoeve C (1998) Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9:321–347
- Fayer R, Santin M, Dargatz DA (2010) Species of *Cryptosporidium* detected in weaned beef calves on cow-calf operations in the United States. *Vet Parasitol* 170:187–192
- Fung DY, Lee CY, Kastner CL (1980) Adhesive tape method for estimating microbial load on meat surfaces. *J Food Prot* 43:295–297
- Hörman A, Korpela H, Sutinen J, Wedel H, Hänninen M (2004) Meta-analysis in assessment of the prevalence and annual incidence of *Giardia* spp. and *Cryptosporidium* spp. infections in humans in the Nordic countries. *Int J Parasitol* 34:1337–1346
- Kortbeek M (2009) Clinical presentation in *Cryptosporidium*-infected patients. In: Ortega-Pierres G, Caccio S, Fayer R, Mank TG, Smith HV (eds) *Giardia and Cryptosporidium* from molecules to disease. CAB International, Cambridge, pp 131–137
- Krecek RC, Moura L, Lucas H, Kelly P (2010) Parasites of stray cats (*Felis domesticus* L., 1758) on St. Kitts, West Indies. *Vet Parasitol* 172:147–149
- Langvad F (1980) A simple and rapid method for qualitative and quantitative study of the fungal flora of leaves. *Can J Microbiol* 26:666–670
- Macarasin D, Bauchan G, Fayer R (2010a) *Spinacia oleracea* L. leaf stomata harbouring *Cryptosporidium parvum* oocysts: a potential threat to food safety. *Appl Environ Microbiol* 76:555–559
- Macarasin D, Santin M, Bauchan G, Fayer R (2010b) Infectivity of *Cryptosporidium parvum* oocysts after storage of experimentally contaminated apples. *J Food Protect* 73:1824–1829
- McEwan NA (2001) *Malassezia* and *Candida* infections in bull terriers with lethal acrodermatitis. *J Small Anim Pract* 42:291–297
- Miranda MF, Silva AJ (2005) Vinyl adhesive tape also effective for direct microscopy diagnosis of chromomycosis, lobomycosis, and paracoccidioidomycosis. *Diagn Microbiol Infect Dis* 52:39–43

- Nichols G (2008) Epidemiology. In: Fayer R, Xiao L (eds) *Cryptosporidium* and cryptosporidiosis, 2nd edn. CRC Press, Boca Raton, pp 80–118
- Pinter L (1999) *Leporacarus gibbus* and *Spilopsyllus cuniculi* infestation in a pet rabbit. *J Small Anim Pract* 40:220–221
- Piperaki ET, Spanakos G, Patsantara G, Vassalou E, Vakalis N, Tsakris A (2011) Characterization of *Enterobius vermicularis* in a human population, employing a molecular-based method from adhesive tape samples. *Mol Cell Probes* 25:121–125
- Robertson LJ, Fayer R (2012) *Cryptosporidium*. In: Robertson LJ, Smith HV (eds) Foodborne protozoan parasites. Nova Science Publishers Inc., Hauppauge, pp 33–64
- Robertson LJ, Gjerde B (2001) Occurrence of parasites on fruits and vegetables in Norway. *J Food Protect* 64:1793–1798
- Robertson LJ, Johannessen GS, Gjerde BK, Loncarevic S (2002) Microbiological analysis of seed sprouts in Norway. *Int J Food Microbiol* 75:119–126
- Sberna P (1957) New method for microscopic examination of dermatophyte colonies; use of cellophane adhesive tape in skin mycology. *Rass Dermatol Sifilogr* 10:137–140
- Smith HV, Cacciò SM, Cook NC, Nichols RA, Tait A (2007) *Cryptosporidium* and *Giardia* as foodborne zoonoses. *Vet Parasitol* 149:29–40
- Stinson CG, Tiwari NP (1978) Evaluation of quick bacterial count methods for assessment of food plant sanitation. *J Food Prot* 41:269–271
- Tang N, Luo NJ (2003) A cross-sectional study of intestinal parasitic infections in a rural district of west China. *Can J Infect Dis* 14:159–162
- Teplitsky V, Mumcuoglu KY, Babai I, Dalal I, Cohen R, Tanay A (2008) House dust mites on skin, clothes, and bedding of atopic dermatitis patients. *Int J Dermatol* 47:790–795
- Xiao L, Escalante L, Yang C, Sulaiman I, Escalante AA, Montali RJ, Fayer R, Lal AA (1999) Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. *Appl Environ Microbiol* 65:1578–1583