# ISOLATION OF PARASITES ON FRUITS AND VEGETABLES

Jeffrey W Bier

Division of Microbiology, Food and Drug Administration, Washington, DC 20204, USA.

**Abstract.** The current FDA method to recover parasites from fruits and vegetables is derived from procedures used to isolate parasitic protozoa from water. A 1kg portion of fruit or vegetable is divided into 200 g subportions. The subportions are sequentially processed in a sonic cleaning bath with 1.5 liters of detergent solution (1% sodium dodecyl sulfate, 0.1% Tween 80) and sonicated for 10 minutes. As each subsample is removed, it is thoroughly drained. After this sonic treatment, the wash water is collected in a polypropylene beaker, transferred to 50 ml polypropylene centrifuge tubes and centrifuged for 15 min at 1500 × *g*. The sediment is consolidated into one tube along with two rinsings of each tube. The final sediment is fixed in 4% formaldehyde for 10 minutes before examination for parasites. Indirect fluorescent antibody is applied to stain the parasites (*Giardia* spp. and/or *Cryptosporidium* spp.) by using commercial kits when available. If a large quantity of extraneous matter is contained in the sediment it may be reduced by layering on Sheather's fluid and centrifuging at 1500 × *g* for 15 minutes. The supernatant is collected and washed twice in distilled water. This procedure is adequate for protozoa and nonoperculate helminth eggs; operculate helminth eggs may be cleaned by extraction with ethyl acetate. When cabbage and lettuce were seeded at 1 organism/g, the rate of recovery for *Cryptosporidium parvum* with the FDA method was 1%. When cabbage was seeded at 1 egg/g and 10 eggs/g, the average rate of recovery of decorticated eggs of *Ascaris* sp. or untreated *Trichuris* sp. was 10%. The use of the sonic bath and the mixture of anionic and neutral detergent increased recovery 100–fold over washing in water or saline. Recovery using no detergent did not improve with the use of a cationic detergent.

## INTRODUCTION

Methods for the examination of foods for parasites are primitive, compared to those for bacterial and fungal contaminants. The principal cause is the lack of simple culture methods for growing parasites *in vitro*. A method used by the US Food and Drug Administration is presented here.

## MATERIALS AND METHODS

A test portion is considered to be 1 kg of fresh fruits or vegetables. It is divided into five subportions of about 200 g each. If the vegetable grows in a tight head, eg European cabbage, only the outer two layers of leaves are removed and analyzed. The subportions are sequentially processed in a sonic cleaning bath with 1.5 l detergent solution (1% sodium dodecyl sulfate, 0.1% Tween 80) and sonicated intermittently for 10 minutes. The sonication interval is dependant on the environmental temperature. In the laboratory, the intervals are adjusted so that the bath does not to exceed 37° C. As each subportion is removed, it is allowed to drain thoroughly into the bath. After a test portion has been processed the contents of the bath are transferred to a polypropylene beaker, and then to 50 ml polypropylene centrifuge tubes that are centrifuged for 15 min at 1,500 $\overline{xg}$. The sediment is consolidated into one tube along with two rinsings of each tube. The final sediment is fixed in 4% formaldehyde for 10 minutes prior to examination or staining. Indirect fluorescent antibody is applied to stain the parasites (*Giardia* spp. and or *Cryptosporidium* spp.) by using commercial kits when available. If a large quantity of extraneous material is present in the sediment, it is reduced by suspending the sediment in detergent and layering the suspension on Sheather's fluid then centrifuging at 1,500 xg 15 minutes. The supernatant is collected and washed twice in distilled water. This procedure is adequate for protozoa and nonoperculate helminth eggs; operculate helminth eggs may be cleaned by extraction with ethyl acetate ( Rude *et al*, 1982).

Table 1
Recovery of parasites from seeded vegetable samples.

| *Cryptosporidium* sp. | *Giardia* sp. | *Trichuris* sp. | | *Ascaris* sp. | |
|---|---|---|---|---|---|
| 1/g | 1/g | 1/g | 10/g* | 1/g | 10/g* |
| 14 | 7 | 120 | 1155 | 105 | 934 |
| 8 | 13 | 89 | 1040 | 106 | 1010 |
| 12 | 9 | 95 | 931 | 87 | 1020 |
| 5 | 9 | 98 | | 116 | |
| 9 | 11 | 102 | | 90 | |

* Derived by multiple 3 × samples of 1 ml of 1:100 dilution of final concentrate.

## RESULTS AND DISCUSSION

When cabbage and lettuce samples were seeded at levels of 1 organism/g, the rate of recovery of *Cryptosporidium parvum* with the FDA method averaged 1%. When cabbage was seeded at 1 egg/g and 10 eggs/g the average recovery of decorticated eggs of *Ascaris* sp. or untreated *Trichuris* sp. was 10% (Table 1). Similar recovery rates were obtained with protosa seeded on strawberries. The use of the sonic bath and the mixture of anionic and neutral detergent increased recovery 100–fold over washing in water or saline. Recovery using no detergent was not improved by the use of a cationic detergent (data not shown). Recovery of parasites from fruits and vegetables is improved by the use of anionic detergent coupled with the sonic cleaning bath. Recovery of helminth eggs throughout this study was lower than previously reported (Rude *et al*, 1982). Recovery will not equal that of microbiological methods for bacteria and fungi until improved culture methods become available, or a method is developed to extract the DNA from parasites in fruit and vegetable samples and amplify the DNA signal by the use of the polymerase chain reaction, so that it may be detected by a specific probe.

## CONCLUSION

The use of anionic detergent and sonication in the recovery of parasites from fruits and vegetables improves recovery.

## REFERENCE

Rude, RA, Bier JW, Jackson GJ, McClure FD. Recovery of eggs of two parasitic nematodes, *Ascaris* sp. and *Trichuris* sp.: Interlaboratory Study. *J Assoc Off Anal Chem* 1982; 65: 79-81.