TESTING FOR FOOD-BORNE PARASITES, THEIR METABOLIC PRODUCTS AND SYMBIONTS

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Abstract. Microscopic animals associated with foods include free-living and saprophytic invertebrates, parasites of hosts other than humans, and parasitic animals specifically designated as food-borne that can infect a human host by the gastrointestinal route. The first general method used to screen for food-borne species was digestion with pepsin and hydrochloric acid at 36° C, based on the "artificial stomach juice" technique for recovering larvae of the nematode *Trichinella spiralis* from muscle. This method selects for forms capable of surviving a mammalian digestive enzyme at mammalian temperatures. It has been used successfully to recover a variety of food-borne helminths, not only from mammalian flesh but also from fish, shellfish and molluscs, and can be adapted to greatly reduce the "background of living animals" associated with soils and the crops grown in them. However, not all animal forms that survive digestion are food-borne parasites, and all that succumb are not necessarily noninfectious. Methodology to test for food-borne parasites is, in general, not as efficient as that for food-borne bacteria. Recent developments in food parasitology indicate a need to identify not only the parasite, but also its metabolic products and associated symbionts.

INTRODUCTION

In testing foods for parasites that are of health significance to the consumer, one must also deal with other invertebrates that are associated with edible produce. These microscopic or small macroscopic animals in or on foods include free-living forms and saprophytes as well as organisms, which, although parasitic to other hosts, are incompatible with the human digestive tract. Often these associates are present in sufficient numbers to interfere with the testing for pathogens and prove bothersome to the expert as well as the less experienced analyst.

Some of these invertebrates are unavoidable in foods (one expects to find soil nematodes on crops grown in soil); however, the presence of others (such as maggots in meat and beetles in dried food) are the result of poor practices in food harvesting, processing, storage or distribution. As desirable as a complete tabulation of all animals that have become associated with a food might be for ecological data banks or filth profiles, such a thorough accounting is usually impractical. Of primary concern from the point of view of health are those invertebrates that are potential agents of infections or producers of deleterious substances.

When the US Food and Drug Administration (FDA) established its Laboratory of (Food) Parasitology in 1972, two priorities became apparent immediately. One was to develop and/or standardize techniques for testing food for the presence of parasites because, compared to testing for food-borne bacteria, few parasitological methods were approved for regulatory purposes, ie, few had been studied collaboratively by the Association of Official Analytical Chemists or designated as the method of choice for FDA laboratories on other grounds. The second urgent need was for a way to reduce the background of miniscule live animals ("animalcules") in foods and select for the potential parasites of humans.

To invade the human host by the gastrointestinal route, a small animal must be able to survive along the mammalian digestive path at least through the stomach and, for a number of parasite species, into the small intestines. Certain physical and chemical factors of *in vivo* digestion, therefore, are, in part, reproduced by the pepsin/hydrochloric acid digestion method for recovering parasites. This recovery technique is familiar to those who work with *Trichinella spiralis* and obtain this nematode's larval form from its cysts in mammalian muscle.

MATERIALS AND METHODS

A detailed description of supplies and procedures for pepsin/hydrochloric acid digestion is given by Bier *et al* (1984) in the FDA Bacteriological Analytical Manual. In principle, the methods consists of:

- preparation of the test portion (100 g);
- digestion in 15 g pepsin powder, 750 ml physiological saline (0.85% NaCl) adjusted with a 1:1 solution of water and concentrated hydrochloric acid (37%) to pH 2 and stirred (250 rpm) in a water bath at 36 ± 0.5° C until digestion of the test portion is visually complete (but not exceeding 24 hour);
- sedimentation by sieving the digested mixture into a stoppered funnel or settling cone, adding rinses of the digestion vessel and allowing parasites 1 hour to gravitate to the bottom.

DISCUSSION

Of course, test conditions do not exactly duplicate the digestive factors of the gastrointestinal tract. Only one digestive enzyme is used; acidity is similar to that of the stomach; microaerobic conditions are gradually attained; continuous stirring generates movement forces and a mixing action that only approximates those of biological stomaching; and the temperature is in the mammalian range.

How well does the method work and what are its critical control points? Preparatory conditions differ for different foods, but choosing the right one is critical. Blending, grinding, macerating or even cutting the test portion of a food may kill or destroy some parasites. Papain predigestion may be helpful or even necessary to aid the pepsin/hydrochloric acid digestion of some foods. A certain amount of trial and error must precede the determination of optimal preparatory conditions, but these should be adhered to once they have been established.

According to a comparison by members of the European Economic Community (Thompsen *et al*, 1985), the efficiencies of four digestion method variants for enumerating *T. spiralis* larvae did not differ significantly (93.8–96.9% recovery) but were superior to that of trichinoscopy (88.6% enumerated) when the level of infection exceeded 1 larva/g of test portion. All five methods were poor (results of 22% or less) with low levels of infection (less than 1 larva/g).

A comparison by the FDA (Jackson *et al*, 1981) of digestion and elution for enumerating nematodes in fish, found that the two methods did not differ significantly in the total numbers of worms recovered from split portions of 470 flatfish. However, of the 1,110 nematodes recovered by digestion, 1,062 were potential pathogens for human consumers of raw or semiraw fish, whereas with elution, significantly fewer (608 pathogens from a total of 922 nematodes) were recovered. In other words, digestion served as a screen, selecting more invasive larvae of the anisakid genera *Anisakis* and *Pseudoterranova* (*Phocanema*) and eliminating many nonpathogenic nematodes of anisakid genera and such other genera as *Cuculanus*, *Metabronema*, *Acuaria* and the Spirurinae.

In edible snails, digestion tended to eliminate one nematode (a *Rhabditis* sp. that was a snail saprophyte) but recovered another (a larval protostrongyle) that was infective for rats (Payne and Jackson, 1978). Although this result is desirable in screening for food-borne parasites, the method is far from perfect, as exemplified by the survival of the larvae of a nematode parasitic in insects that also survived more than 24 h in the rat peritoneal cavity (Jackson and Bradbury, 1970).

To date, pepsin/hydrochloric acid digestion has proved useful in screening for pathogenic helminths in mammalian meat, seafood and escargot. Obviously, there is a need to test it with more foods and other invertebrates, particularly the protozoa. Today, however, in judging the safety of foods we should not just identify the known parasitic pathogens but also look for their metabolic products and symbionts, which may play important roles in epidemiology (Jackson 1990a,b). The question of whether the digestion method preserves metabolites in an unaltered state and symbionts intact has yet to be answered.

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