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Bacteriological Analytical Manual

Chapter 19: Parasitic Animals in Foods

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Introduction

Humans unknowingly consume microscopic and small macroscopic animals with their food. The intestinal tract is inhospitable to most of these organisms, which are either digested or evacuated in the feces. However, some obligate or facultative parasites may become established in the human body. Although a number of parasites produce no symptoms and are not associated with disease, others may cause mild, moderate, or severely acute illness and even permanent damage. The following methods are used to examine foods and food-contact materials for the presence of parasites. For the most part, these techniques are labor-intensive and tedious; work continues to refine them and to develop additional techniques and rapid methods. Several alternative ways to examine fish and shellfish are presented. However, candling is the only method currently used for regulatory purposes with finfish.

I. Digestion to Select Mammalian Parasites in Edible Flesh

By mimicking the chemical and temperature conditions of the mammalian stomach, this method frees parasites from the surrounding flesh and reduces the background of nonparasitic organisms.

A. **Equipment and materials**

1. Balance, at least 250 g capacity
2. Stirrer or rotating incubator shaker
3. Water bath, 37 ± 0.5°C
4. Beakers, 100 and 1500 ml
5. Sedimentation cone and support, 1 L, plastic with removable plug, e.g., Imhoff cone

6. Tubing, amber gum, 2.4 and 9.5 mm diameter
7. Tubing clamp
8. Microscopes, dissecting and inverted
9. Culture dishes, plastic, various sizes
10. Sieve No. 18 (U.S. standard sieve series), 1 mm mesh, 204 mm diameter, 51 mm high; other sizes optional
11. Tray, rectangular, polypropylene, about 325 × 260 × 75 mm
12. Cylinder, graduated, 1 L
13. pH meter
14. Pasteur pipets, or polypropylene eyedroppers
15. Rubber bulb, about 2 ml capacity
16. Spoon
17. Spatula
18. Optional materials: blender, meat grinder, food processor, negative pressure hood, foil, plastic wrap, tweezers, and dissecting needles

B. **Reagents**

1. Physiological saline (R63)
2. Pepsin, laboratory grade
3. pH reference solutions
4. HCl, concentrated
5. Optional reagents: papain, ethanol, glacial acetic acid, glycerine, lactophenol, phenol, formalin, Lugol's iodine (R40), ether

C. **Sampling and sample preparation**

From a sample weighing 1 kg, take a subsample (100 g) of beef, pork, or poultry, or 250 g of fish. Subsamples of most mammalian meat, poultry, or fish require no further preparation. They may be torn or separated into 5 or more pieces to increase the surface area. Samples with relatively large amounts of connective tissue are not readily digested; snail meat, for example, is digested very poorly. The following methods improve digestion. A 100 g sample is blended in 750 ml saline. Ten intermittent, instantaneous bursts in a blender will destroy some macroscopic organisms but usually will not affect microscopic organisms. A meat grinder is less destructive, although not suitable for some foods such as snails. Least destructive is initial digestion with papain followed by pepsin digestion.

CAUTION: Pathogens that are easily disseminated may be contained in samples and will be liberated by digestion. Of special concern are macroscopic tapeworm cysts and microscopic cysts of protozoa. When the presence of such pathogens is suspected, carry out the digestion and subsequent sample handling in a negative pressure hood until the suspect digest is placed in a safely closed dish. Handle all utensils as if contaminated, and autoclave or incinerate after use.

D. **Digestion, sedimentation, and examination**

Adjust incubator-shaker or water bath to 37 ± 0.5°C. Prepare digestion fluid in 1500 ml beaker by dissolving 15 g pepsin in 750 ml saline, add sample, and adjust to pH 2 with concentrated HCl (about 3 ml). Place in incubator or water bath and stir (about 100 rpm) after equilibration for about 15 min; check and adjust pH again. Cover beaker with aluminum foil (if using stirrer, pierce hole for stirring rod) and continue incubating until digestion is complete. The time required for digestion will vary but should not exceed 24 h.

Carefully pour beaker contents through sieve into tray. Rinse remains with 250 ml saline and add to digest. Examine rinsed contents of sieve and record results. Larger parasites will remain on sieve. Replace plug of sedimentation tube with rubber tubing and clamp folded tubing. Carefully transfer contents of tray to sedimentation cone. Transfer undigested sample or parasites to a petri dish, using spoon, tweezers, or dissecting needle.

After 1 h of sedimentation, remove bottom 50 ml of sediment by releasing clamp and collecting in 100 ml beaker. Transfer fluid to petri dish(es) with eyedropper. (Digests vary in their clarity; if digest is dense, dilute with saline until it is translucent.) Cover dish and examine macroscopically for parasites; then examine with dissecting microscope and finally with inverted microscope (a contrasting background can be helpful). Count, tentatively identify, and record observations. Count total number of organisms and differentiate those that are living (motile) and dead (nonmotile), if possible. Examine complete contents of beaker. Light infections may require repeated sampling to detect parasites.

Interpretation and further identification

Further information about recovered organisms is usually required, both to classify them and to decide whether the criterion of movement is valid for determining viability. For example, the eggs of *Ascaris* must be "embryonated," i.e., allowed to incubate so that moving embryos develop inside. Cysts of some protozoa must be excysted to detect motion; those of *Toxoplasma gondii* can be judged viable only by the outcome of experimental inoculation into the peritoneal cavity of mice. Brief summaries of fixation and staining methods for frequently recovered parasites are given below and in the following references: invertebrates in general parasitology (2); animals' parasites (11); medical aspects of parasitology (10); food parasitology: methods, references, expert consultants (4,7); immunology and serology of parasitic diseases (8); protozoa (9); nematodes (3,16); trematodes (1,14); cestodes (15); arthropods (5).

E. **Fixation and staining**

Protozoan cysts and helminth eggs. Fix and stain fresh material with Lugol's iodine solution (R40) or use fluorescent antibody stain (if available) on formalin-fixed material.

Nematodes. Fix in glacial acetic acid overnight and store in 70% ethanol with 10% glycerin. Study nematode morphology in temporary mounts by removing from alcohol and clearing in glycerin, lactophenol, or phenol ethanol. Before returning to storage, rinse away clearing fluid(s) with 70% ethanol. Sectioning and staining may be necessary for detailed identification of nematodes.

Trematodes and cestodes. Before fixation, relax both trematode and cestode flatworms in cold distilled water for 10 min. Fix flukes (trematodes) in hot (60°C) 10% formalin. Fix tapeworms (cestodes) by adding 10× volume of 70°C fixative to the relaxing fluid, or dip them in 70°C water repeatedly; then fix in a mixture of ethanol, glacial acetic acid, and formalin (85:10:5) overnight. Store in 70% ethanol. Flatworms are usually stained and mounted as permanent slides, but some may require sectioning and staining for detailed identification.

Acanthocephala. Place in water to evert the proboscis. (Some proboscises may evert almost immediately; others require several hours. Do not extend over 8 h.) Fix in steaming 70% ethanol with a few drops of glacial acetic acid. Store in fixative or 70% ethanol. Acanthocephala may be stained and mounted as permanent slides or, like the nematodes, cleared in phenol or glycerol.

Arthropoda. Fix fleas, lice, mites, copepods, fly larvae, and other parasitic or food-inhabiting arthropods in hot water. Store in 70% ethanol.

For additional information concerning the preparation of specimens, contact Clarke Beaudry.

F. **Viability determination**
The major criterion for the viability of helminths is spontaneous movement. Observe organisms for 10 min to see if spontaneous movement occurs. If autonomous movement is not observed, touch with a dissecting needle and observe to see if movement has been stimulated. Allow specimens from salted products to equilibrate in at least 20× volumes of physiological saline for 3 h before viability determination; osmotic pressure may cause apparent movement. Culturable protozoa should be cultured in vitro to determine viability. If culture is not feasible, dye exclusion is the method of choice for viability determination.

II. Candling to Detect Parasites in Finfish

The following procedures are used to determine parasites in finfish. The candling procedure is applicable to fresh or frozen fish with white flesh processed as fillets, loins, steaks, chunks, or minced fish. The ultraviolet (UV) light procedure is for fish with dark flesh and for breading removed from fish portions.

NOTE: This method is not applicable to dried fish or fish in the round.

A. **Equipment and materials**

1. **Sharp knife**

2. **Candling table**. Rigid framework to hold light source below rigid working surface of white, translucent acrylic plastic or other suitable material with 45-60% translucency. Length and width of working surface should be large enough to permit examination of entire fillet, e.g., 30 × 60 cm sheet, 5-7 mm thick.

3. **Light source**. "Cool white" with color temperature of 4200°K. At least two 20-watt fluorescent tubes are recommended. Tubes and their electrical connections should be constructed to prevent overheating of light source. Average light intensity above working surface should be 1500-1800 lux, as measured 30 cm above center of acrylic sheet. Distribution of illumination should be in ratio of 3:1:0.1, i.e., brightness directly above light source should be 3 times greater than that of outer field, and brightness of outer limit of visual field should be not more than 0.1 that of inner field. Illumination in examining room should be low enough not to interfere with detection of parasites, but not so dim as to cause excessive eye fatigue.

B. **Reagents** (for preservation reagents, **see** section I. B-5, above)

C. **Sample preparation**
Weigh entire sample and record weight on analytical reporting form.

Fillets. If fillets are large (200 g or larger), use one fillet for each of the 15 subsamples. If fillets are small (less than 200 g), randomly select fillets to prepare 15 subsamples of approximately 200 g each. Record actual weight analyzed for each subsample. If fillets are more than 30 mm thick, cut with a sharp knife into 2 pieces of approximately equal thickness (not to exceed 30 mm per fillet). Examine both pieces as described below. If fillets have a thickness of 20 mm or less, examine whole.

Fish blocks. Analyze 15 subsamples randomly selected from 2 thawed and drained blocks. Prepare the subsamples as described for fillets, above. Note separately any parasites observed in minced fish added to block around subsamples.

Steaks, loins, chunks. Prepare as for fillets.

Minced fish. If frozen in blocks, analyze 15 subsamples randomly selected from 2 thawed and drained blocks. Prepare subsamples as described for fillets, above. Select portions from different parts of block. If not in blocks, analyze 15-200 g portions. Do not further shred or chop minced fish.

Breaded fish portions. Thaw frozen products at room temperature in a beaker of appropriate size. After thawing, pour hot (50°C) solution of 2% sodium lauryl sulfate in water over fish in increments of 100 ml per 300 g of product. Stir with glass rod for 1 min. Let stand for at least 10 min or until breading separates from flesh. Transfer individual portions to No. 10 sieve nested over No 40 sieve. Wash breading through No. 10 sieve with gentle stream of warm tap water. Periodically examine No. 40 sieve containing the breading, using UV light. Parasites will appear fluorescent under this light. Note any parasites detected and record on the analytical reporting form. Discard breading by backflushing the No. 40 sieve with tap water. Examine fish portions by candling, using white light. If the flesh is pigmented, use UV light.

D. **Examination**
Parasites near the surface will appear red, tan, cream-colored, or chalky white. Parasites deeper in the flesh will appear as shadows. Remove representative types of parasites or other defects found. Record general location, size, identification, and other observations as outlined below. For minced fish, spread portion on light table to depth of 20-30 mm for examination. Select representative parasites for descriptive analysis.

E. **Ultraviolet examination of dark-fleshed fish**
Visually examine each portion (de-breaded or de-skinned, as necessary) on both sides under a desk lamp or similar light source. A magnifying desk lamp may be used. Report findings as described below. Conduct UV examination in darkened room. Examine each portion on both sides with reflected longwave UV light (366 nm wavelength). Parasites should fluoresce blue or green under light of this wavelength. Fish bones and connective tissues, which also fluoresce blue, may be differentiated by their regular distribution and shape. Bone fragments will be rigid when probed (6).

CAUTION: Never expose unprotected eyes to UV light from any source either direct or reflected. Always wear appropriate eye protection such as goggles with uranium oxide lenses, welder's goggle, etc., when such radiations are present and unshielded. Keep skin exposure to UV radiations to a minimum.

F. **Parasite identification.** Fix parasites as described in section I.F, above.

III. Compression Candling: Detection of Parasites in Molluscs and other Translucent Foods

I. Parasites may be detected visually in such translucent foods as white-fleshed fish and shellfish by observing the outline of the organism or its capsule in transmitted light. The method described was developed to examine the viscera and muscle of the surf clam *Spisula solidissima* for the presence of *Sulcascaris* sp. nematodes, but is also applicable to other foods and parasites. However, not all parasites are detected (12), probably because they are obscured by the shadows produced by connective tissue. The method was compared with two other visual methods for detecting nematodes in the calico scallop, *Argopectin gibbus*. Compression candling detected more nematodes and yielded fewer false positives than the other two methods.

A. **Equipment and materials**

1. Hinged Plexiglas plates 305 × 305 mm. To construct plates, attach two 305 mm (about 12 inch) squares of 3/8 inch Plexiglas stacked plates to a piano hinge so that they are separated by 3 mm with six 32-5/8 inch machine screws. If proper size piano hinge is not available, a nominal 1-inch hinge can be retapped to give proper spacing. Attach a 3-mm spacer

to each end of the surface of one plate at the edge opposite the piano hinge.

2. Light box
3. Knife
4. Specimen vials or jars
5. Dissecting needles
6. Petri dish

B. **Reagents**

1. Physiological saline solution, 0.85% (R63)
2. Glacial acetic acid
3. 70% Ethanol

C. **Method**

1. Distribute portion of sample on inside of plastic plate. Quantity to be examined at one time depends on size and thickness of sample. Samples over 100 g cannot be compressed. Cut cylindrical samples (e.g., scallops) in half longitudinally to facilitate compression.
2. Close plate and squeeze outer edges firmly.
3. Examine each side of plate for parasites by placing on light table. Parasites in flesh appear as shadows.
4. Record number of parasites. To confirm that parasites are present, mark plate with wax pencil, open, and check by dissection.
5. Fix representative sample to confirm identity (**see** section I.F, above).

IV. Mechanical Disruption and Sedimentation for Detection of Larval Parasites in Fish Flesh

This method detects larval anisakids in the flesh of fillets. It is not applicable to fish that have been treated with salt without being de-boned, such as pickled herring, and, in general, may not be applicable to species such as herring. Subsamples should not exceed 200 g in the food processor, but may be pooled for final analysis.

A. **Materials**

1. Food processor; Cuisinart Model DLC 10, Moulinex Model 663, or equivalent.
2. Glass tray, 350 × 25 × 60 mm
3. Beaker, 1000 ml
4. White fluorescent lamp
5. UV lamp, <365 nm,="" or="" similar,="" light="">
6. Appropriate eye protection
7. Glass rod
8. Forceps
9. Vials or jars
10. Fixative

B. **Method**

Fillet and skin fish before weighing; then place in food processor with plastic dough hook in place. Add 35°C water equal to twice the weight of the fish. Activate food processor intermittently until flesh is dissociated (1-2 min). Pour into beaker and wait 30-60 s before decanting all but 100 ml of the supernatant fluid. Add water and stir; then wait 30-60 s, and decant to 100 ml again (2×).

*Place about 25 ml of sediment in glass tray; dilute until quite translucent or until depth of 10 mm is reached (about 375 ml). Examine, collect, or count parasites. Agitation of sediment with forceps may aid in recovery, and forceps will be useful in collecting parasites. Record parasite movement. Collect and fix a representative portion of the parasites present for identification (**see** section I.F, above). Examine under high intensity (>500 μW/cm^2) shortwave (about 365 μm) light. Parasites will fluoresce blue or yellow-green. Count and record. Repeat from *, above, until sample is complete.

V. Concentration of Helminths and Protozoa from Vegetables

Vegetables may become contaminated with parasitic organisms through contact with animal or human fecal material or through application of sewage-derived fertilizer to croplands (13). The method outlined below can be used to examine fresh vegetables for parasites. A similar method recovered *Cryptosporidium* sp. from 1% of water samples examined. Recovery from vegetables would be estimated at 1% or less. (A sample consists of five 1-kg subsamples.)

A. **Equipment and materials**

1. Balance
2. Polypropylene beakers, 1 L
3. Sonic bath, about 2 L capacity
4. Centrifuge, large capacity, low speed with swinging bucket
5. Polypropylene centrifuge tubes, 50 ml
6. Eye dropper, polypropylene
7. Culture dish with 2 mm grid
8. Microscope slides

B. **Reagents**

1. Lugol's iodine (R40)
2. Sheather's fluid (500 g sucrose, 320 ml deionized water, 6.5 g phenol)
3. Detergent solutions Nos. 1, 2, and 3
   No. 1 - 2.5% formaldehyde, 0.1% sodium dodecyl sulfate (SDS), 0.1% Tween 80
   No. 2 - 1% Tween 80, 1% SDS
   No. 3 - 1% Tween 80
4. Fluorescent antibody kit

C. **Procedure**

Store vegetables in refrigerator before analysis. Separate vegetables into units: **tight head type** (cabbage), remove outer 3 layers of leaves; **loose head type** (leaf lettuce), separate individual leaves; **root type** (carrot), no preparation; **floret type** (cauliflower), separate into florets of about 50 g. *Pour 1-1.5 liters of detergent solution No. 1 into sonic bath and add vegetables loosely to about 250 g; operate bath for 10 min. Remove vegetables individually and drain well. Repeat from *, above, until subsample is completely sonicated.

Transfer detergent to beaker; then dispense all of the material into 50 ml centrifuge tubes. Centrifuge at 1200 × *g* for 10 min. Remove supernatant to 1.5-2 ml and consolidate sediment into one tube with eyedropper or plastic pipet. Rinse each tube twice with 1.5 ml detergent No. 2, and add to consolidation tube. Rinse and centrifuge sediment twice with detergent No. 2. Dilute to 10 ml with detergent No. 3 and sonicate for 10 min. Add 25 ml Sheather's fluid to clean centrifuge tube and layer on detergent suspension from sonic bath. Centrifuge at 1200 × *g* for 30 min. Remove 7 ml of fluid from interface and transfer to centrifuge tube; fill tube with detergent; then centrifuge at 1200 × *g* for 10 min. *Remove supernatant and dilute with detergent No. 3. Then centrifuge for 10 min at 1200 × *g*. Repeat from *, above, 2 times.

For helminth eggs, transfer sediment to gridded petri plate and add 1 ml Lugol's iodine.

Dilute and examine entire plate with inverted microscope. **For protozoa**, dilute sediment sufficiently with detergent No. 3 to make translucent 100 µl thin smears on cleaned polylysine-coated microscope slide cleaned with acid alcohol.

Let slides air-dry. Add positive and negative control samples to separate well or slide and let air-dry. Follow manufacturer's instructions for fluorescent antibody staining. Examine each slide at 200-300× with fluorescent microscope. Record results. If sample is positive, calculate number of cysts present per kg of food specimen by measuring remaining suspension and estimating number. If sample is negative, stain and examine remaining sediment.

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