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Recovery of Parasitic Nematodes from Fish by Digestion or Elution

G. J. JACKSON,[1]* J. W. BIER,[1] W. L. PAYNE,[1] AND F. D. McCLURE[2]

Division of Microbiology[1] and Division of Mathematics,[2] Food and Drug Administration, Washington, D.C. 20204

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Two methods, digestion and elution, were used to recover parasitic nematodes from 470 flatfish belonging to species in the family Pleuronectidae. Samples of similar fish were collected from market lots; half of each sample was subjected to digestion, and half was subjected to elution (sedimentation). The edible (flesh) and the inedible (viscera) portions of each fish were analyzed separately. The total number of nematodes recovered by digestion was 1,110, which was not significantly greater than the 922 nematodes recovered by elution. However, digestion recovered 1,062 nematodes of the anisakine genera *Anisakis* and *Phocanema*, which are potentially pathogenic for human consumers of raw or semiraw fish. This number is significantly greater than the 608 pathogenic nematodes recovered by elution. Digestion also recovered 242 more nematodes from the edible flesh than did elution. Conversely, more nonpathogenic nematodes were recovered by elution. Approximately half the fish (240) had been collected in Boston markets, and the other half (230) had been collected in San Francisco markets. Fish from San Francisco each contained an average of eight nematodes, and those from Boston contained an average of less than one nematode per fish.

Human anisakiasis is a parasitic disease that has been recognized with increasing frequency since the 1950s. In the United States, the first well-documented cases were diagnosed in the 1970s (5). This "new" incidence of human infections prompted investigators to undertake surveys of edible marine fish (4, 6, 7), the sources of these parasites. The parasites, larval anisakine nematodes, belong primarily to the genera *Anisakis* and *Phocanema*. If these nematodes are not killed by cooking, freezing, or salting, they may survive long enough after being consumed by humans to cause the varied symptoms of anisakiasis (2). These symptoms range from mild nausea or gastrointestinal distress to severe abdominal pain.

Once the nematodes have been ingested by humans (or by experimental or accidental mammalian hosts), they may molt once. However, they mature and reproduce only in marine mammals, which are their definitive hosts.

Different methods for detecting the nematodes in their fish hosts have been used in various surveys, the most common being dissection, digestion, elution, and candling. These techniques are applied singly or in combination. Procedures for detection by chemical and serological means are still experimental (3).

This study was undertaken to compare two of the methods for detecting nematodes in fish—digestion and elution. Samples from Boston and San Francisco were compared because previous examinations of fish from other markets along the east and west coasts of the United States had indicated that there were more total nematodes as well as pathogenic nematodes in offshore Pacific fish (6, 7).

MATERIALS AND METHODS

Collection of fish. Approximately every other week, 11 fresh, unfrozen flatfish (weighing approximately 1 kg each) were purchased from the same lot in a commercial market in Boston and San Francisco. These collections were continued for a year, beginning in October 1977.

The Atlantic coast flatfish sampled consisted of two species: the American plaice (*Hippoglossoides platessoides*) and the blackback flounder (*Pseudopleuronectes americanus*). The Pacific coast flatfish sampled consisted of seven species: petrale sole (*Eopsetta jordani*), rex sole (*Glyptocephalus zachirus*), rock sole (*Lepidopsetta bilineata*), Dover sole (*Microstomus pacificus*), English sole (*Parophrys vetulus*), starry flounder (*Platichthys stellatus*), and sand sole (*Psettichthys melanostictus*). All nine species of fish are members of the family Pleuronectidae.

Of the 11 fish of each lot, 1 was frozen for subsequent identification. The common and scientific names of the flatfish are the terminology of the American Fisheries Society (1).

Preparation of fish. The viscera were removed, weighed, and stored in a numbered container. The

eviscerated fish were skinned and filleted. Fillets and viscera of five fish in each lot were analyzed by elution, and those of the other five were analyzed by digestion. The fillet sample (200 g) consisted of the anterior 100 g of the dorsal and ventral fillets. In whiting this area (the belly flap) contains more parasites than other areas of the fillets (8). Samples were stored in the refrigerator at 5°C, and tests were performed within 24 h of purchase.

Elution method. The elution technique is sometimes referred to as a "sedimentation" method, although both elution and sedimentation are involved.

The viscera and flesh of each fish were examined separately. Up to 200 g of sample of a fish was distributed evenly on a no. 4 sieve. The sieve was placed in a funnel and stabilized with four "feet," consisting of pieces of 3.2-mm-bore tubing, 20- to 30-mm long, which were slit and placed around the lower lip of the sieve. Tubing at the bottom of each funnel was closed with a clamp, and the funnel was filled with 0.85% NaCl (approximately 4 liters per funnel). To prevent the gonads from breaking and clogging the stem of the funnel, saline was not poured over them.

After standing 16 to 18 h, the clamped tubing at the bottom of the funnel was opened, and 100 ml of sediment was drained into a 250-ml beaker. The upper 50 to 75 ml of fluid was removed after 10 min, and the remaining suspension was examined in 15-ml portions. Each portion was placed in a 15- by 100-mm petri dish and, if the contents were too opaque for examination with a microscope at 10×, the portion was diluted with saline.

Digestion method. Pepsin powder (15 g), saline (750 ml of 0.85% NaCl), and the sample (about 200 g) were added sequentially to a 1.5-liter beaker. The beaker was placed in a water bath of 36 ± 0.5°C so that the level of the water was within 1 cm of the fluid level in the beaker. Contents of the beakers were stirred mechanically for 15 min at 250 rpm, and the solutions were adjusted to pH 2 with 6 N HCl. Beakers were covered with metal foil (with as small an aperture as possible for the stirring shaft) and the contents were stirred mechanically for 24 h. After digestion, the content was poured through a no. 18 sieve into a pan. Digestion of both flesh and viscera was usually 90% efficient, except that the flesh of some petrale sole did not digest over 50%. The sieves were rinsed with 250 ml of saline, and the fluid was collected in the pan. The washed remains were placed in a culture dish and wetted with saline for microscopic examination.

The contents of each pan were transferred into a 1-liter sedimentation cone or funnel, and after 1 h the bottom 50 ml was drained into a 100-ml beaker. Portions of the liquid were placed in a culture dish and examined for nematodes microscopically at 10× magnification.

Preservation and identification of nematodes. Only active nematodes were counted as live; nematode fragments were not counted as whole parasites. All nematodes and fragments were preserved in glacial acetic acid contained in vials. Identification of nematodes was performed by previously stated criteria (6).

RESULTS

Most nematodes recovered by both methods were alive. The 1,110 nematodes recovered by the digestion method did not differ significantly ($P > 0.10$) from the 922 that were recovered by the elution method (Table 1). However, the number of pathogenic nematodes (*Anisakis* and *Phocanema* spp.) recovered by the digestion method was 175% higher than the number recovered by the elution procedure. Nonpathogenic nematodes were species of the anisakine genera, *Contracaecum* and *Thynnascaris*, as well as nonanisakine species of the genera *Cuculanus*, *Metabronema*, the subfamily Spirurinae, and (only from Pacific Ocean fish) the genus *Acuaria*. Digestion also increased the number of nematodes recovered from the edible flesh by 3.6× the number recovered by elution.

Fish from San Francisco markets (i.e., from nearby Pacific Ocean fishing waters) each contained an average of eight nematodes. Fish from Boston markets (i.e., from nearby Atlantic Ocean fishing waters) each contained an average of less than one nematode (Table 2). The number of pathogens in San Francisco fish was significantly higher than that for Boston fish, both for total fish ($P < 0.01$) and for fish flesh ($P < 0.01$).

DISCUSSION

The comparative data on recovery of parasitic nematodes from flatfish by the digestion or elution methods were considered to be reliable,

TABLE 1. *Recovery of parasitic nematodes from 235 flatfish by two methods*

Recovery method	No. of nematodes			
	In viscera		In flesh	
	Pathogens[a]	Nonpathogens[b]	Pathogens[a]	Nonpathogens[b]
Digestion	727	47	335	1
Elution	522	306	86	8

[a] *Anisakis* sp., *Phocanema* sp.
[b] *Contracaecum* sp., *Thynnascaris* spp., *Cuculanus* sp., *Metabronema* sp., *Acuaria* sp., Spirurinae.

TABLE 2. *Recovery of parasitic nematodes from flatfish from west and east coast waters*

Location	No. of fish examined	No. of nematodes			
		In viscera		In flesh	
		Pathogens[a]	Nonpathogens[b]	Pathogens[a]	Nonpathogens[b]
San Francisco	230	1,245	212	390	5
Boston	240	4	141	31	4

[a] *Anisakis* sp., *Phocanema* sp.
[b] *Contracaecum* sp., *Thynnascaris* spp., *Cuculanus* sp., *Metabronema* sp., *Acuaria* sp., Spirurinae.

since each method recovered enough nematodes to make comparison possible. The sensitivities in detecting nematodes by the two methods differed. Digestion destroyed some nonpathogenic nematodes; living nematodes were not digested by artificial gastric juice, whereas those that did not survive the digestion fluid at 35°C were digested. Elution failed to recover some of the pathogens; those that had penetrated deeply into the flesh were probably not stimulated to migrate by the saline alone. For the best estimate of the total number of nematodes in a fish, a combination of methods, therefore, seems best: careful gross dissection followed by candling, then elution, and finally digestion. This, however, is extremely time consuming and, thus, impractical when many fish must be examined. On the average, fish analyzed by digestion contained 4.7 nematodes (range: 0 to 70 in viscera, 0 to 39 in flesh). By adding the pathogens recovered by digestion to the nonpathogens recovered by elution, one can estimate that each fish contains an average of 5.9 nematodes and that approximately 1 nematode per fish is not being recovered by digestion alone.

The finding that fish from San Francisco markets contained more nematodes, including pathogenic *Anisakis* sp. and *Phocanema* sp., in the edible flesh than did fish from Boston markets (Table 2) agrees with findings from other Pacific coast and Atlantic coast fishing areas (4, 6, 7). The higher incidence of pathogenic nematodes in U.S. Pacific coast fish is attributed to the greater population in those waters of marine mammals, the definitive hosts in which these parasites mature and reproduce.

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