

# DIPHYLLOBOTHRIUM, ANISAKIS AND OTHER FISH-BORNE PARASITIC ZOONOSES

Terry A Dick<sup>1</sup>, Brent R Dixon<sup>2</sup> and Anindo Choudhury<sup>1</sup>

<sup>1</sup> Department of Zoology, University of Manitoba, Winnipeg Manitoba, Canada, R3T 2N2; <sup>2</sup> Bureau of Microbial Hazards, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0L2.

**Abstract.** Fish-borne parasitic zoonoses such as anisakiasis and diphyllbothriasis occur infrequently in Canada and more work needs to be done on the interactions and transmission dynamics of marine and freshwater anisakids in North America. The diphyllbothriid tapeworms are primarily restricted to the northern Canada. Problems with the specific identification of these parasites from their fish hosts prompted the development of a series of nucleic acid probes. Use of the polymerase chain reaction proved to be quick, accurate and requires little skill, once developed.

## INTRODUCTION

There is an increasing awareness of the importance of fish-borne parasites as human pathogens by consumers in North America. Such parasites include *Diphyllbothrium*, the anisakids and two fresh water trematodes. New sources of fresh fish supply from cultured fish, semi-intensive (ponds) and intensive (marine and freshwater cage culture), have added to the logistic problems of inspection. Superimposed on these are the potential effects of warming of the temperate region and the desire of humans to preserve remaining natural resources such as fish eating birds and mammals which serve as primary hosts of these parasites.

This paper will emphasize the Canadian situation with respect to fish-borne zoonoses, particularly anisakiasis and diphyllbothriasis, and will discuss some of the unresolved and potential new problems and methods of identification.

## DISCUSSION

### Anisakid roundworms

A large volume of literature exists on anisakids in fish and their pathogenicity to humans (Desowitz, 1986; Hafsteinsson and Rizvi, 1987), and a recent case of human infection from Alberta, Canada (Kowalewska-Grochowska *et al*, 1989) reinforces the health

problems caused by these parasites. There is little difficulty in distinguishing between larval worms of the genera *Pseudoterranova*, *Anisakis*, and *Contracaecum/Phocascaris*, from fish, provided the worms are complete. However L<sub>3</sub> larvae of *Contracaecum osculatum*, *C. spiculigerum* and *Phocascaris* spp. are almost impossible to differentiate and aspects of their transmission dynamics and potential pathogenicity to humans remain unclear. *Anisakis simplex* and *A. physeteris* L<sub>3</sub> larvae are also difficult to separate. There is considerable overlap in the distribution of the anisakids in Canadian waters (Margolis and Arai, 1990) which adds to the problem. Little is known about *Contracaecum* spp. in Canadian freshwater systems. Recently, semi-intensive stocking programs with rainbow trout, for example, resulted in very heavy infections in the viscera and flesh and a 10-fold increase over 3 years, thus rapidly altering the parasitofauna of a pristine system by attracting piscivorous birds (Dick *et al*, 1987). There are at least three species of *Contracaecum* reported from pelicans form birds in North America (Deardorff and Overstreet, 1980) and their impact on inshore fisheries is unclear.

### Diphyllbothriid tapeworms

Of the 12 species of *Diphyllbothrium* reported in North America, three species are known to infect humans in Canada; *D. latum*

and *D. ursi* utilize mammals as the primary host while *D. dendriticum* utilizes a bird host (Anderson *et al.*, 1987). It is generally believed that larval plerocercoids can be differentiated on the basis of their morphology but considerable morphological variability among individual plerocercoids in fish makes species identification tentative, at best. The location of plerocercoids in the viscera (*D. dendriticum*) and in the musculature (*D. latum*) generally holds as a distinguishing feature but from our experience, *D. dendriticum* often occurs in the flesh in both salmonid and coregonid fishes. Positive identification of plerocercoids is only possible by obtaining adult worms from experimentally infected hosts. Gulls are likely the primary hosts of *D. dendriticum* while the natural primary host of *D. latum* in Canada is less well understood but generally thought to be aquatic mammals or bears. It is also not clear how the North American *D. latum* compares with the Eurasian species which infect humans.

#### Larval trematodes

*Clinostomum complanatum* and *Metorchis conjunctus* are considered potential human pathogens in Canada. *Clinostomum*, usually considered a yellow perch parasite, has recently caused heavy infections in fillets of pond (semi-intensive) cultured rainbow trout (Szalai and Dick, 1988). Human infections of *M. conjunctus*, are from wild freshwater fish and occur infrequently. There has been one case only in the last 10 years (Naiman *et al.*, 1980). This parasite is restricted to northern Ontario, Manitoba and Saskatchewan in central Canada.

#### DIAGNOSIS

As stated earlier, problems in identification arise at the species level or when only pieces of worms are available. Methods other than morphological, such as isozyme and molecular biology techniques have been developed to overcome such problems in identification of larval anisakids and diphylobothriids (Orecchia *et al.*, 1986; deVos *et al.*, 1990). Isozymes require considerable care in the handling of specimens, often not the case for the material one receives for diagnosis. Molecular techniques show pro-

mise but the use of restriction digest profiles of genomic DNA require extensive procedures that are time consuming. Use of specific DNA probes are good but require the extraction of DNA prior to hybridization with a radiolabelled probe. Amplification of a specific sequence of DNA has considerable promise as small amounts of DNA can be used and the time to diagnose is about 4-5 hours. We have developed ribosomal DNA probes from the intergenic spacer regions, sequenced this DNA, synthesized specific primers which were used in the polymerase chain reaction (PCR) to accurately identify *D. latum* and *D. dendriticum* plerocercoids. We are able to obtain sufficient DNA, by amplification from pieces of parasite tissue, to serve as a template for the PCR. Future identification methods will incorporate this technology as it is quick, accurate and requires little specialized skill once the primers and methods have been developed.

#### REFERENCES

- Andersen K, Cheng HL, Vik R. A review of freshwater species of *Diphylobothrium* with redescrptions and the distribution of *D. dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825) from North America. *Can J Zool* 1987; 65:2216-28.
- Deardorff TL, Overstreet RM. *Contracaecum multipapillatum* (= *C. robustum*) from fishes and birds in the Northern Gulf of Mexico. *J Parasitol* 1980; 66:853-6.
- Desowitz RS. Human and experimental anisakiasis in the United States. *Hokkaido Igaku Zasshi* 1986; 61:358-71.
- deVos T, Szalai AJ, Dick TA. Genetic and morphological variability in a population of *Diphylobothrium dendriticum* (Nitzsch, 1924). *Syst Parasitol* 1990; 16:99-105.
- Dick TA, Papst MH, Paul HC. Rainbow trout (*Salmo gairdneri*) stocking and *Contracaecum* spp. *J Wild Dis* 1987; 23:242-7.
- Hafsteinsson H, Rizvi SSH. A Review of the sealworm problem: biology, implications and solutions. *J Food Protect* 1987; 50:70-84.
- Kowalewska-Grochowska K, Quinn J, Perry I, Sherbaniuk R. A case of anisakiasis - Alberta. *Can Dis Wkly Rep* 1989; 15:221-3.

FOOD - BORNE PARASITIC ZONOSIS

- Margolis L, Arai HP. Synopsis of Vertebrates of Canada, Parasites of Marine Mammals. Alberta Agriculture, Animal Health Division. 1990: 26pp.
- Naiman HL, Sekla L, Albritton WL. Giardiasis and other intestinal parasitic infections in a Manitoba residential school for the mentally retarded. *Can Med Assoc J* 1980; 122:185-8.
- Orecchia P, Paggi L, Mattiucci S, Smith JW, Nascetti G, Bullini L. Electrophoretic identification of larvae and adults of *Anisakis* (Ascaridida: Anisakidae). *J Helmithol* 1986; 60:331-9.
- Szalai AJ, Dick, TA. Helminths of stocked rainbow trout (*Salmo gairdneri*) with special reference to *Clinostomum complanatum*. *J Wild Dis* 1988; 24:456-60.
-