found in the coastal waters of Taiwan, there is always a possibility of human cases outbreak in that country where people have recently begun to eat raw hairtail. As it is not possible to distinguish *A. typica* from the other *Anisakis* type I larvae through morphology, molecular methods are indispensable for determining the incidence of *A. typica* infection in humans. This is important not only in Taiwan, but also in other countries where the presence of *A. typica* and other species comprising the *Anisakis* type I has been noted.

Acknowledgements

We thank Dr. Stefano D'Amelio of the University of Rome 'La Sapienza' and Dr. Nelio Barros of the Mote Marine Laboratory for supplying adult specimens of *Anisakis typica*. Dr. Hiroshi Yamasaki, Dr. Yasuyuki Morishima and Dr. Masanori Kawanaka of the National Institute of Infectious Diseases, Dr. Jun Araki of the Meguro Parasitological Museum and Dr. Kazuo Nakamura of Kitasato University provided invaluable suggestions and encouragement throughout this study. This research was supported in part by grants for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labor and Welfare of Japan. This research was also partially supported by the Promotion and Mutual Aid Corporation for Private Schools of Japan, Grant-in-Aid for Matching Fund Subsidy for Private Universities.

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International Journal for Parasitology 40 (2010) 1651–1658

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Paragonimus westermani possesses aerobic and anaerobic mitochondria in different tissues, adapting to fluctuating oxygen tension in microaerobic habitats

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ARTICLE INFO

Article history:
Received 26 April 2010
Received in revised form 1 July 2010
Accepted 2 July 2010

Keywords:
Aerobic and anaerobic mitochondria
Respiratory chain
Paragonimus westermani
NADH-fumarate reductase system
Tegument
Parenchyma

ABSTRACT

We previously showed that adult *Paragonimus westermani*, the causative agent of paragonimiasis and whose habitat is the host lung, possesses both aerobic and anaerobic respiratory chains, i.e., cyanide-sensitive succinate oxidase and NADH-fumarate reductase systems, in isolated mitochondria (Takamiya et al., 1994). This finding raises the intriguing question as to whether adult *Paragonimus* worms possess two different populations of mitochondria, one having an aerobic succinate oxidase system and the other an anaerobic fumarate reductase system, or whether the worms possess a single population of mitochondria possessing both respiratory chains (i.e., mixed-functional mitochondria). Staining of trematode tissues for cytochrome *c* oxidase activity showed three types of mitochondrial populations: small, strongly stained mitochondria with many cristae, localised in the tegument and tegumental cells; and two larger parenchymal cell mitochondria, one with developed cristae and the other with few cristae. The tegumental and parenchymal mitochondria could be separated by isopycnic density-gradient centrifugation and showed different morphological characteristics and respiratory activities, with low-density tegumental mitochondria having cytochrome *c* oxidase activity and high-density parenchymal mitochondria having fumarate reductase activity. These results indicate that *Paragonimus* worms possess three different populations of mitochondria, which are distributed throughout trematode tissues and function facultatively, rather than having mixed-functional mitochondria.

1. Introduction

Parasitic helminths including nematodes, trematodes and cestodes exhibit a variety of metabolic pathways (Tielens and van den Bergh, 1993; Komuniecki and Komuniecki, 1995; Behm, 2002; Kita and Takamiya, 2002). Their life cycle consists of two stages, a free-living larval stage that lives in an aerobic environment and a parasitic stage inside a host, in which oxygen tension depends on the tissues penetrated by migrating larvae or dwelt in by adult parasites. For example, the adult stage of *Ascaris suum*, a nematode that parasitises swine, dwells in the lumen of host small intestine, where oxygen tension is very low, while its fertilised eggs develop aerobically to form infective L3s. Therefore, during development parasitic helminths undergo aerobic–anaerobic transitions in energy metabolism resulting from adaptation to their environments. During its aerobic larval stage, *A. suum* possesses a functional tricarboxylic acid (TCA) cycle and a cyanide-sensitive respiratory chain similar to those in mammals (Takamiya et al., 1993; Amino et al., 2003; Iwata et al., 2008); both of these are localised in mitochondria and produce ATP by oxidative phosphorylation. That is, electrons derived from substrate oxidation are transported via NADH or flavin adenine dinucleotide (FADH) to oxygen by the proton-pumping electron-transfer complexes of the respiratory chain, and a proton gradient formed across the inner membrane drives ATP-synthase, resulting in ATP production with a backflow of protons. The respiratory chain consists of four electron-transfer complexes, i.e., complexes I (NADH-ubiquinone oxidoreductase), II (succinate-ubiquinone oxidoreductase, SQR), III (ubiquinol-cytochrome *c* oxidoreductase), and IV (cytochrome *c* oxidase, CCO), and two low-molecular weight electron carriers, cytochrome *c* and ubiquinone. Complexes I, III and IV have proton-pumping activity. Thus, mitochondria that produce ATP at the expense of oxygen can be defined as aerobic mitochondria, with 38 mol of ATP produced during the complete oxidation of each mol of glucose. Adult *Ascaris* nematodes, however, possess anaerobic mitochondria that yield ATP in the absence of oxygen.

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doi:10.1016/j.ijpara.2010.07.009

Mitochondria from adult *Ascaris* muscle have reduced levels of complex III and cytochrome *c*, and almost completely lack complex IV. These nematode mitochondria catalyze the NADH-dependent reduction of fumarate, which is endogenously derived from malate dismutation and serves as a terminal electron acceptor. NADH-linked fumarate reduction is mediated by complex I and by complex II, an isoform of the larval complex II (Takamiya et al., 1986). The latter, together with rhodoquinone, catalyzes rhodoquinol-fumarate reduction, the reverse of the succinate oxidation mediated by the larval enzyme, a reaction coupled to site 1 phosphorylation of ADP. Using this pathway, approximately 5 mol of ATP are produced per mol of glucose; a value higher than that for lactate fermentation (2 mol ATP per mol glucose) but lower than that observed using the aerobic pathway. Since adult *Ascaris* mitochondria can produce ATP by oxidative phosphorylation in the absence of oxygen, they are termed "anaerobic" mitochondria or more precisely, "anaerobically functioning mitochondria". Similar anaerobic mitochondria are present not only in parasitic helminths but also in other hypoxically functioning eukaryotes that live in microaerobic environments such as marine or freshwater sediments (Van Hellemond et al., 1995; Tielens et al., 2002). Therefore, oxygen tension where anaerobic mitochondria function is quite varied from extremely anoxic to less hypoxic or microaerobic. Although the molecular properties of anaerobic mitochondria, especially of their respiratory chains, have been elucidated in detail, the mechanisms of aerobic–anaerobic transition still remain to be determined with respect to mitochondrial biogenesis.

Paragonimus spp., lung flukes that infect mammals including humans, may provide appropriate material to investigate aerobic–anaerobic transitions in mitochondria. Adult *Paragonimus westermani* and *Paragonimus ohirai* inhabit mammalian lungs by forming cysts, in which oxygen tension fluctuates much more than in the lumen of the small intestine. We previously showed that the mitochondria of adult flukes possess both aerobic and anaerobic respiratory chains, i.e., mammalian-type, cyanide-sensitive respiratory chains and the NADH-fumarate reductase system (Ma et al., 1987; Takamiya et al., 1994). This finding raises the interesting question as to whether the adult *Paragonimus* spp. possess separate populations of aerobic and anaerobic mitochondria or a single mitochondrial population containing both the succinate oxidase and NADH-fumarate reductase systems. Our cytochemical study using *P. ohirai* supported the former view but we did not have sufficient biochemical evidence (Fujino et al., 1996). We have therefore attempted to separate the aerobic and anaerobic mitochondria, and to further characterise each individually.

2. Materials and methods

2.1. Isolation of P. westermani mitochondria

Adult *P. westermani* (3*n* type, 62 or 88 worms) were obtained from worm cysts from the lungs of dogs 8 or 12 months after inoculation with metacercariae. After removal of ovary tissue, the mitochondria were prepared as described (Takamiya et al., 1994). All procedures performed on laboratory animals were approved by the institutional animal care and use committee of the National Defense Medical College, Japan (the approval No. 09097) and all the animal experiments were carried out in compliance with the guidelines for animal experimentation of National Defense Medical College.

2.2. Isopycnic density-gradient centrifugation

Continuous 26 ml-sucrose density gradients, 32–52% (w/w) or 35–55% (w/w) containing 10 mM Tris–HCl, pH 7.5, 1 mM EDTA and 0.1% BSA were prepared using a density gradient fractionator (DGF-U, Hitachi, Japan). A mitochondrial suspension (0.9–1.2 ml) was loaded onto each gradient, which was centrifuged at 108,000*g* for 2 h in a Hitachi RP70T rotor at 4 °C. The contents of each tube were fractionated through the fractionator and CCO (Takamiya et al., 1994), fumarate reductase (FRD) (Kita et al., 1988) and SQR (Takamiya et al., 1994) activities, and the protein contents of these fractions, were measured. Using one drop of each fraction, the buoyant density was measured by measuring the sucrose concentration of each fraction with Abbe's refractometer and comparing each with a curve relating sucrose concentration to density. For protein assays, a 0.1 ml aliquot of each fraction was suspended in 0.9 ml of 0.025 M Tris–HCl buffer, pH 7.5, containing 0.21 M mannitol, 0.07 M sucrose and 0.1 mM EDTA. The suspensions were centrifuged at 14,000*g* for 15 min (Tomy MRX 150) to remove BSA and recover the mitochondria in the pellet. The supernatant was thoroughly removed and the protein content of each pellet was assayed (Markwell et al., 1978). For quinone determination, mitochondrial fractions were diluted with 10 vol. of 10 mM Tris–HCl, pH 7.4, containing 0.2 mM EDTA and the mixtures were centrifuged at 100,000*g* for 1 h to recover the mitochondrial particles. Each pellet was suspended in a small volume of the same buffer and used for quinone determination (Takamiya et al., 1994).

2.3. Electron microscopy

For cytochemical studies of CCO, worm tissues were fixed for 20 min at 4 °C in phosphate-buffered 2% glutaraldehyde, which had been purified overnight using active carbon. Small blocks of tissue were rinsed for several hours in the same buffer with 7% sucrose and sectioned at 40 μm on a Sorvall TC-2 tissue sectioner. The sections were collected in the same buffer and incubated for 2 h in medium (Seligman et al., 1968) at 37 °C. Control sections were incubated with the inhibitor, 10 mM KCN. The tissues were post-fixed for 45 min at 4 °C in 1% osmium tetroxide buffered to pH 7.4 with 0.05 M Veronal buffer and washed for 30 min with 0.1 M sodium acetate. Before dehydration with ethanol, the tissues were stained for 30 min in 0.5% uranyl acetate at 4 °C and examined using a JEOL JEM 100c electron microscope. Electron micrographs at magnifications of 5,000–10,000 were prepared to measure the maximum cross-sectional length and width and areas of mitochondria in trematode cells. The size of mitochondria in the micrographs were recorded using analysis Five software (Soft Imaging System GmbH, Münster, Germany).

Isolated mitochondria were fixed in 4% glutaraldehyde buffered with 10 mM Tris–HCl (pH 7.4), 0.21 M mannitol, 0.07 M sucrose and 0.1 mM EDTA, post-fixed in 1% osmium tetroxide in the same solution, dehydrated in ethanol, embedded in Quetal 812, and cut into sections of thickness 300–500 Å. These sections were stained with 4% uranyl acetate, treated with lead citrate hydroxide and examined under a Hitachi H-7,000 transmission electron microscope.

Electron micrographs at magnification 7,000 were prepared to measure the areas (μm^2) and brightness of cross-sections of individual fractionated mitochondria. The micrographs were examined using a computer-controlled image analyzer (KS400, Zeiss, Germany) equipped with a scanner (Epson GT-9100). Typically, for each fraction, 476–502 mitochondrial cross-sections were analyzed.

2.4. Statistical analyses

The areas and brightness of mitochondrial cross-sections were analyzed by Kaleida Graph ver. 4. For in situ observation, statistical analyses were performed using Prism 4 software (GraphPad Software, Inc., San Diego, CA, USA). Differences between means were

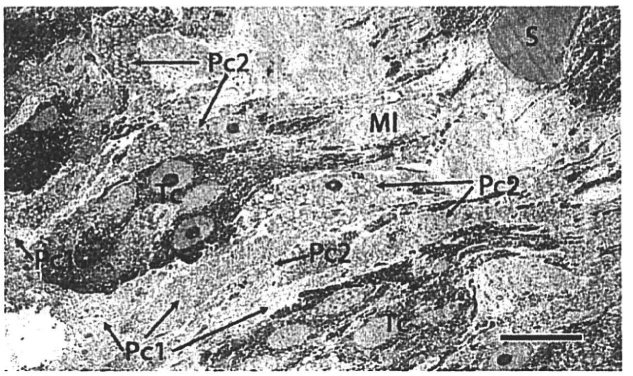

Fig. 1. Low magnification electron micrograph of the *Paragonimus* body wall. Ml, muscle layer; Pc1, parenchymal type-1 cells; Pc2, parenchymal type-2 cells; S, spine; T, tegument; Tc, tegumental cells. The magnification is 1000×. Scale bar = 20 μm.

analyzed using the Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. In situ morphological features

Examination of the body wall sections of *P. westermani* by transmission electron microscopy showed that body wall cells could be categorised into two types, tegumental and parenchymal cells, based on their shape, the density of their cytoplasm and the number and morphology of mitochondria (Fig. 1). The morphological features of the two cell types were similar to those described for *P. ohirai*; the parenchymal cells could be further divided into two types, parenchymal cells types-1 and -2 (Pc1 and Pc2, respectively) (Fujino et al., 1996). Pc1, with cytoplasm less dense than that of Pc2, contain a few round or oval mitochondria with several long cristae in rather dense matrices (Fig. 2A). In contrast, Pc2 possess

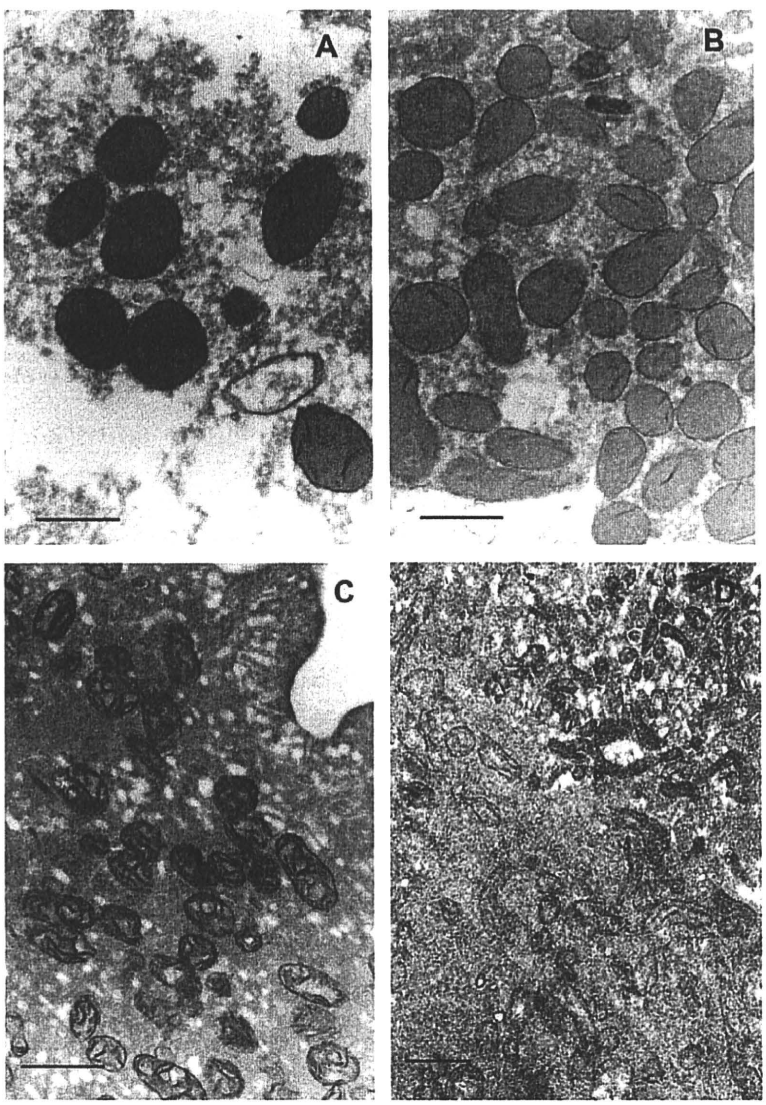

Fig. 2. Cytochrome *c* oxidase (CCO) staining of *Paragonimus* mitochondria in the (A) parenchymal type-1 cells; (B) parenchymal type-2 cells; and (C) tegument. (D) Tegumental cells for control. No significant staining was observed in the presence of KCN, a potent inhibitor of CCO. The magnification for A–C and D is 10,000× and 2,000×, respectively. Inserted bar represents 1 μm.

Table 1
In situ morphology of *Paragonimus westermani* mitochondria.

Sourc	Cross-section area (μm²)	Shape	Crista development	Matrix density	CCO staining
T or Tc	0.130 ± 0.0819	Oval	Well	Dense	Heavy
Pc1	0.330 ± 0.193	Round or oval	Moderate	Dense	Moderate
Pc2	0.350 ± 0.197	Round or oval	Poor	Less dense	Little

Cross-section areas (n = 190) were measured as described in Section 2.
T, tegument; Tc, tegumental cells; Pc1, parenchymal type-1cells; Pc2, parenchymal type-2 cells; CCO, cytochrome *c* oxidase.

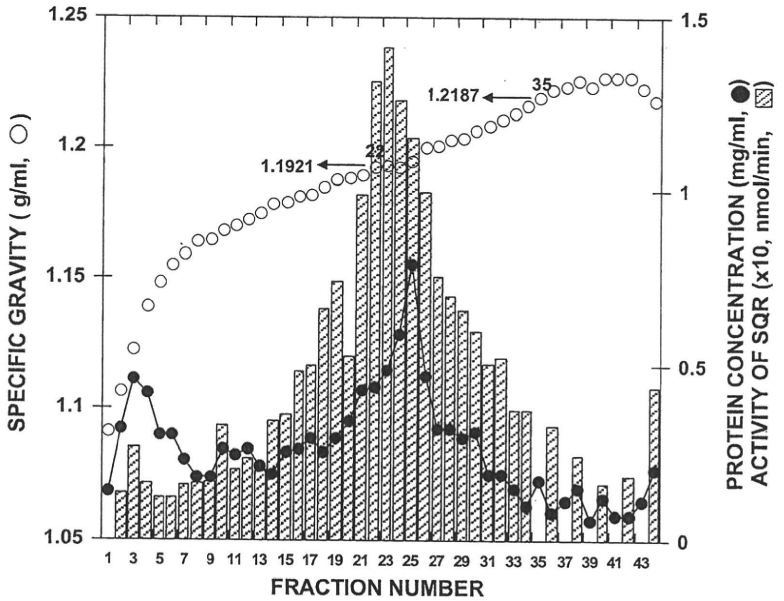

Fig. 3. Distribution of isolated *Paragonimus* mitochondria on isopycnic density gradient (32–52% (w/w) sucrose) centrifugation. Trematode mitochondria (10.0 mg protein) were centrifuged and the specific gravity (open circles), protein concentrations (closed circles), and activity of succinate-ubiquinone oxidoreductase (SQR) (shaded bars) were determined in each fraction. Mitochondria with high fumarate reductase (FRD)/cytochrome *c* oxidase ratios were present in Fractions 22–35. Detailed experimental procedures are described in Section 2.

numerous mitochondria, round or oval in shape, with less dense matrices than Pc1 and poorly developed cristae (Fig. 2B). Since the cristae of Pc1 mitochondria were positive for CCO, the terminal oxidase that reduces molecular oxygen, Pc1 mitochondria appear more aerobic than Pc2 mitochondria. In contrast to the parenchymal cells, the tegumental cells contain many small mitochondria with well-developed cristae and heavily positive for CCO activity (Fig. 2C). Therefore, among the three types of body wall mitochondria, the tegumental mitochondria appear most aerobic. Morphological features of *P. westermani* body wall mitochondria are summarised in Table 1.

3.2. Buoyant density and SQR activity of mitochondria fractionated by isopycnic density-gradient centrifugation

Paragonimus westermani mitochondria were analyzed by sucrose density-gradient centrifugation between 32% (w/w) and 50% (w/w) sucrose, with two gradient ranges, a low-density gradient range (1.0914–1.1546 g/ml) of Fractions 1–6 and a high density gradient range (1.1546–1.2267 g/ml) of Fractions 6–42. Since the density of 32% (w/w) sucrose solution is 1.1426 g/ml, higher than those for Fractions 1–4 (1.0914–1.1390 g/ml), the steep initial gradient was formed by dilution with sucrose–mannitol medium used to suspend mitochondria. The mitochondria were layered on top of the gradient.

Using SQR activity as the mitochondrial marker enzyme, *P. westermani* mitochondria were distributed at sucrose densities of 1.123–1.2267 g/ml (Fractions 2–42) with a main protein peak at 1.1946 g/ml (Fraction 25) (Fig. 3). The low-density peak at Fractions 2–4 contained small mitochondria with well-developed cristae, although cross-contaminating microsomal vesicles were

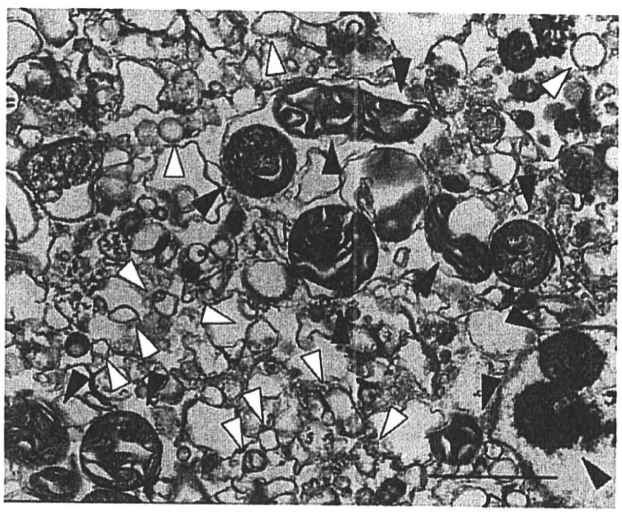

Fig. 4. Electron micrograph of *Paragonimus* mitochondrial preparation with lower densities (Fraction 2, 1.1065 g/ml) fractionated by isopycnic density-gradient (32–52% (w/w) sucrose) centrifugation. Black and white arrowheads indicate mitochondria and microsomal vesicles, respectively. Inserted bar represents 1 μm.

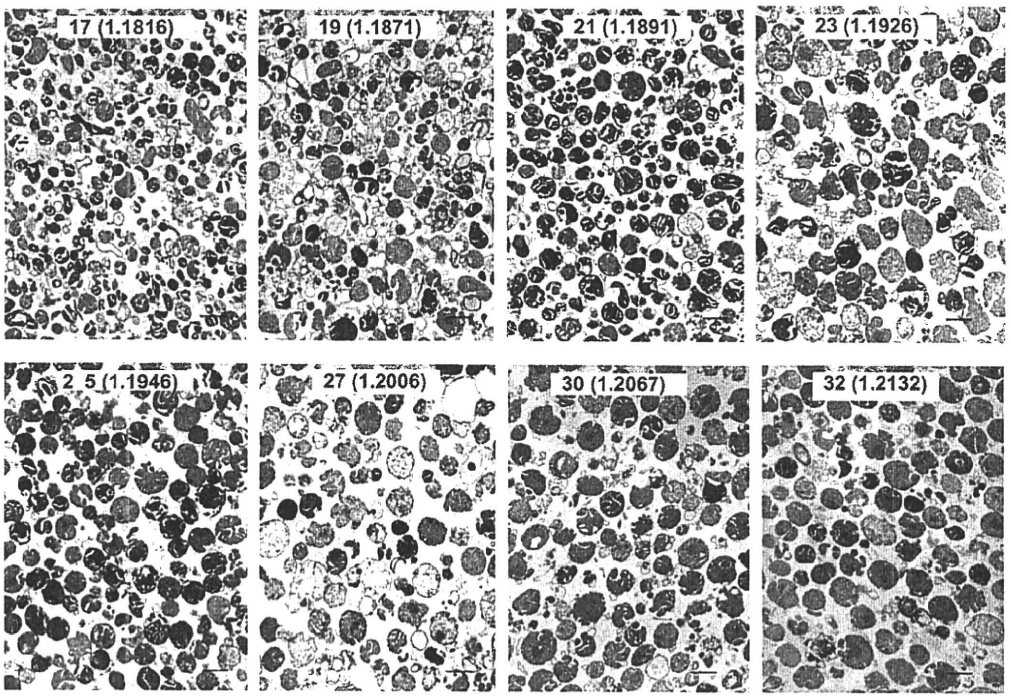

Fig. 5. Electron micrographs of *Paragonimus* mitochondria with higher densities (Fractions 17, 19, 21, 23, 25, 27, 30 and 32) fractionated by isopycnic density-gradient (32–52% (w/w) sucrose) centrifugation. Numbers and numerals in brackets represent fraction numbers and densities, respectively. Inserted bar represents 1 μm.

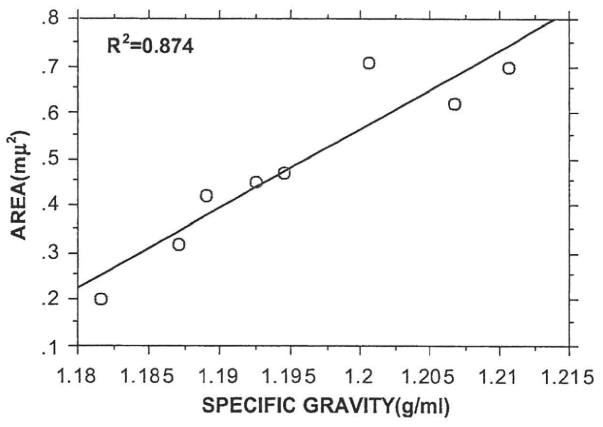

Fig. 6. Relationship between specific gravities and cross-sectional areas of *Paragonimus* mitochondria fractionated by isopycnic density-gradient (32–52% (w/w) sucrose) centrifugation. Medians of mitochondrial cross-sectional areas, as determined by a computer-controlled image analyzer, were plotted against the specific gravities of Fractions 17, 19, 21, 23, 25, 27, 30 and 32. Detailed experimental procedures are described in Section 2.

also detected (Fig. 4). Thus, the specific activity of SQR was lower in Fractions 1–7 than in mitochondria before centrifugation. The specific activity of this mitochondrial marker enzyme in the rest of fractions (Fractions 8–42) ranged from 0.177 (Fraction 8) to 0.595 μmol/min/mg protein (Fraction 22). This variation also suggested heterologous mitochondrial populations that differed in energy metabolism. The average specific activity was calculated to be 0.405 μmol/min/mg, indicating a 2.7-fold purification after centrifugation. Intact rat liver mitochondria have been fractionated by sucrose density gradient ultracentrifugation into two bands, with mean densities of 1.184 and 1.216 g/ml (Pollak and Munn, 1970) and human liver mitochondria exhibited an equilibrium density of 1.20 g/ml (Peters and Seymour, 1978). Thus, the buoyant densities of most fluke mitochondria were similar to those of mammalian aerobic mitochondria.

3.3. Morphological features of fractionated P. westermani mitochondria

Since we found that trematode mitochondria were heterologous in situ, we analyzed the morphology of mitochondria fractionated by isopycnic-density-gradient centrifugation. Electron micrographs showed mitochondria that differed in size and staining density; the small mitochondria appeared to be fractionated at lower densities and the large mitochondria at higher densities (Fig. 5). The size (area, μm^2) and staining density (brightness) of individual mitochondrial cross-sections in each fraction were determined one by one using a computer-controlled image analyzer. When we plotted the sizes and brightness of mitochondria versus their densities, we observed a linear relationship (Fig. 6), with small mitochondria present at low sucrose densities and large mitochondria at higher sucrose densities. However, we observed no relationship between the brightness of mitochondria and their density (data not shown). Rather, there were two populations of mitochondria, bright and dark stained, irrespective of their sizes (Figs. 7 and 8). The relative fraction of bright mitochondria appeared to be higher in the high sucrose density fractions, indicating morphological heterogeneity of the fractionated mitochondria.

3.4. Distribution of CCO and FRD activities on separated mitochondria

To further analyze *P. westermani* mitochondria, they were centrifuged on a higher density gradient, i.e., from 35% (w/w) to 55% (w/w) sucrose (Fig. 9). Two linear density gradients were observed, one from Fractions 1 to 6 (1.0865–1.163 g/ml) and the other from Fractions 6 to 37 (1.163–1.253 g/ml). Activities of CCO and FRD, the marker enzymes of the aerobic and anaerobic respiratory chains, were determined in each fraction, and relative ratios of their specific activities were calculated. Mitochondria with high CCO/FRD

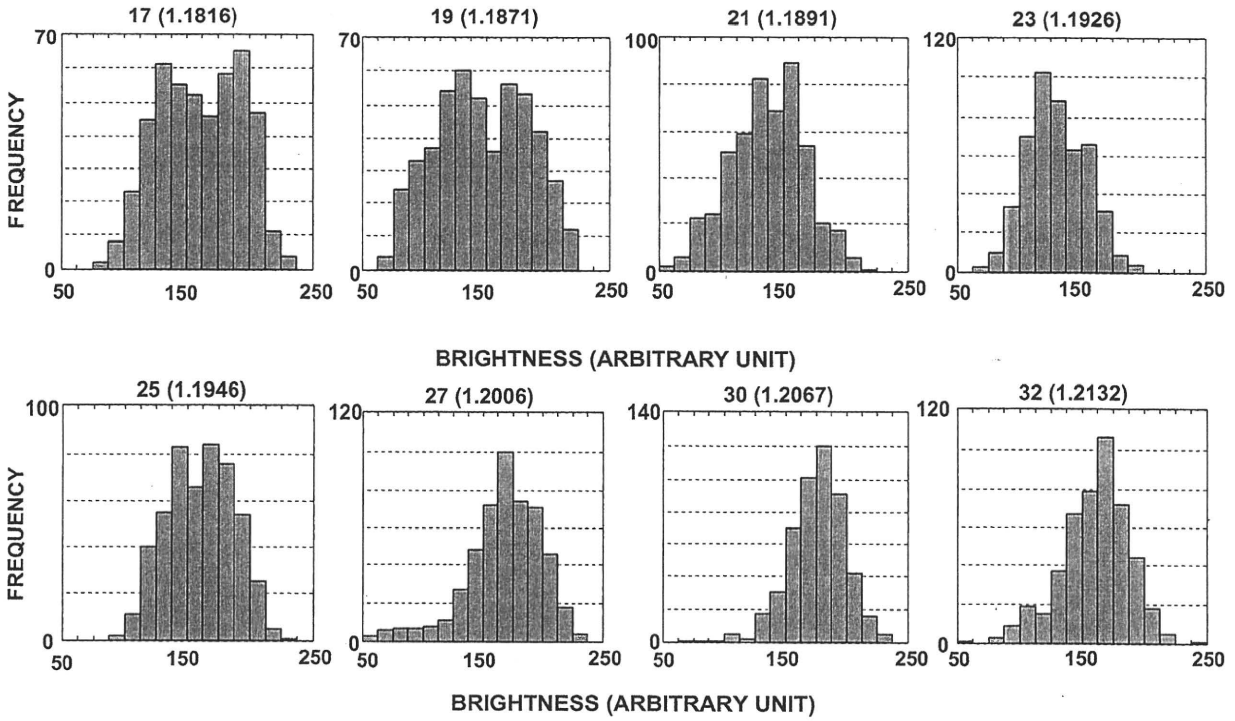

Fig. 7. Histograms of the brightness of *Paragonimus* mitochondria fractionated by isopycnic density-gradient (32–52% (w/w) sucrose) centrifugation. Numbers and numerals in brackets represent fraction numbers and densities, respectively. Brightness of individual mitochondria was determined by a computer-controlled image analyzer. Sample numbers for Fractions 17, 19, 21, 23, 25, 27, 30 and 32 were 477, 491, 502, 479, 502, 502, 502 and 476, respectively.

ratios were mainly recovered at low densities (Fractions 1–7, 1.0865–1.168 g/ml), and those with high FRD/CCO ratios at high densities (Fractions 13–23, 1.192–1.1285 g/ml), indicating that aerobic mitochondria are lighter than anaerobic mitochondria. We also determined the ubiquinone-10 and rhodoquinone-10 contents of fractionated mitochondria to compare the ubiquinone/rhodoquinone ratio in low- and high-density mitochondria. The ubiquinone/rhodoquinone molar ratio was 8.13 and 3.29 in low- and high-density mitochondria, respectively, indicating that the low-density mitochondria are also aerobic in terms of quinone contents.

4. Discussion

Although there have been several studies on the morphological and functional heterogeneity of *Paragonimus* mitochondria (Hamajima et al., 1982; Yamakami et al., 1984; Fujino et al., 1995, 1996), substantial evidence for the occurrence of aerobic and anaerobic mitochondria has been limited. We previously reported that there were three types of mitochondria, which differed in morphology and cytochemistry, in *P. ohirai* tissues (Fujino et al., 1996), but the relationships between morphology and function could not be determined due to limited amounts of material. No direct evidence was presented for Pc2 mitochondria with high FRD activity. By using *P. westermani*, which are larger, for mitochondrial preparation, we were able to further characterise trematode mitochondria. We found that adult *P. westermani* possess three types of mitochondria, small aerobic mitochondria localised in the tegument and tegumental cells and two types of large mitochondria, one localised in Pc1 and highly stained for CCO activity, and the other derived from Pc2 and poorly stained for CCO activity. Fractionation of these trematode mitochondria by isopycnic density-gradient centrifugation based on their buoyant densities, which

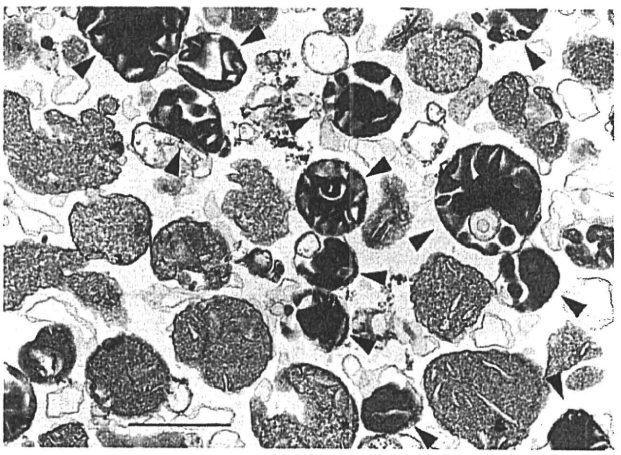

Fig. 8. Electron micrograph of *Paragonimus* mitochondria (Fraction 21) exhibiting different staining densities. Arrowheads show heavily stained mitochondria, with low brightness scores, with the remainder being lightly stained mitochondria, with high brightness scores. Inserted bar represents 1 μm.

are directly proportional to their cross-section areas or sizes, showed that the small mitochondria localised in the tegumental cells were present in the low-density fractions. These mitochondria, of average size $0.180 \pm 0.102\ \mu m^2$, comparable to their in situ average size of $0.130 \pm 0.0819\ \mu m^2$ (Table 1), had high CCO/FRD ratios and well-developed cristae. The parenchymal mitochondria were larger, with in situ sizes of $0.33 \pm 0.193\ \mu m^2$ for Pc1 mitochondria and $0.35 \pm 0.197\ \mu m^2$ for Pc2 mitochondria (Table 1), and were of average size $0.619 \pm 0.314\ \mu m^2$ after gradient fractionation. The larger cross-sectional areas of isolated compared with in situ mitochondria may have been due to unavoidable swelling during isolation.

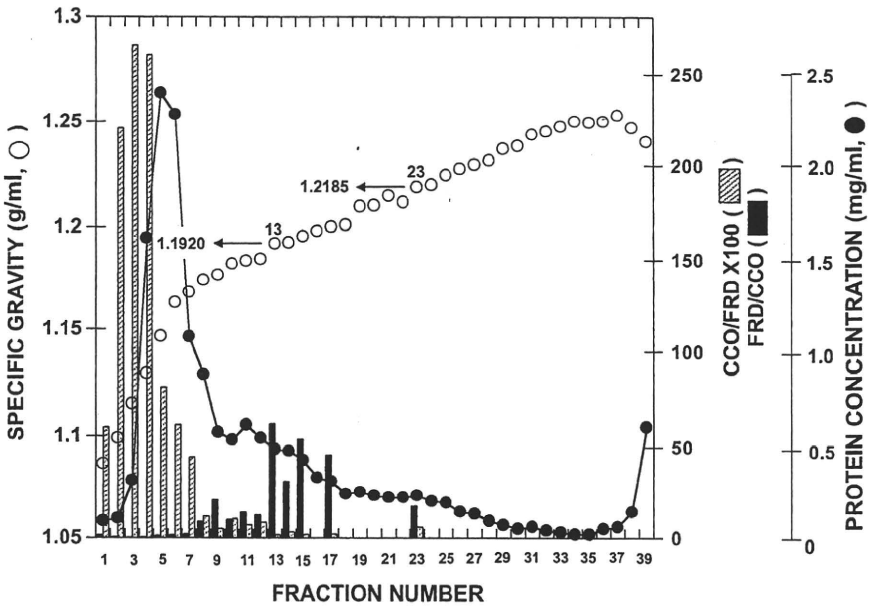

Fig. 9. Distribution of isolated *Paragonimus* mitochondria on isopycnic density gradient (35–55% (w/w) sucrose) centrifugation. Trematode mitochondria (24.9 mg protein) were centrifuged and the specific gravity (open circles), protein concentration (closed circles), and ratios of specific activity of cytochrome *c* oxidase (CCO) to fumarate reductase (FRD) (shaded bars) and of FRD to CCO (closed bars) were determined for each fraction. Specific activity of CCO and FRD for Fraction 3, giving the highest CCO/FRD × 100 value, is 38.7 s^{-1}/ml/mg and 14.6 μmol/min/mg, respectively. Specific activity of CCO and FRD for Fraction 13, giving the highest FRD/CCO value is 0.225 s^{-1}/ml/mg and 14.1 μmol/min/mg, respectively. Mitochondria with high FRD/CCO ratios were distributed between Fractions 13 and 23, with densities of 1.1920 and 1.2185 g/ml, respectively. Detailed experimental procedures are described in Section 2.

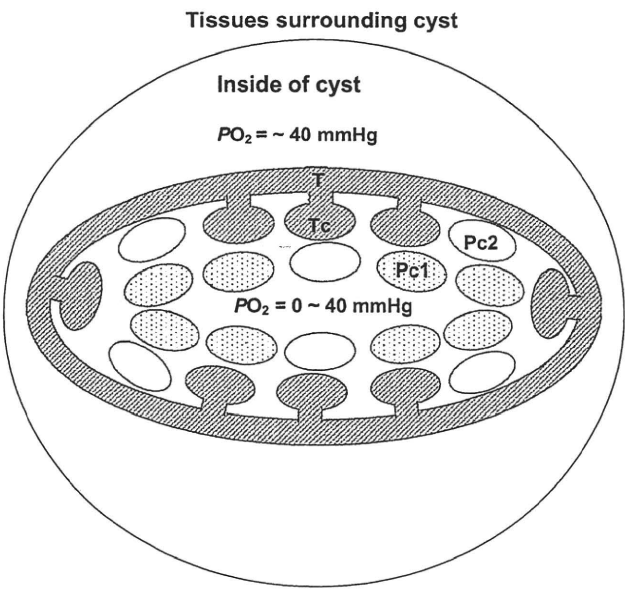

Fig. 10. Schematic cross-section of a cyst inhabited by adult *Paragonimus*. T and Tc represent the tegument and tegumental cells with aerobic mitochondria, and Pc1 and Pc2 represent types-1 and -2 parenchymal cells, respectively, the latter of which contain anaerobic mitochondria. PO_2 represents oxygen tension.

At present, it is difficult to separate Pc1 and Pc2 mitochondria because they do not differ significantly in size. Mitochondria present in Fractions 8–12 (1.174–1.1845 g/ml), with low CCO/FRD and FRD/CCO ratios, may be intermediate between aerobic and anaerobic mitochondria. Alternatively, their higher CCO activity and larger size might suggest that they consist primarily of Pc1 mitochondria. Although we previously reported that Pc1 mitochondria in *P. ohirai* are aerobic (Fujino et al., 1996), the staining intensity for CCO activity of *P. westermani* Pc1 mitochondria was significantly lower than that of tegument/tegumental cell mitochondria. Interestingly, we observed two types of mitochondria, one heavily stained with clear cristae and the other lightly stained with few cristae, which appear to correspond to our in situ observations, although artifacts may have been introduced during staining. The Pc2 mitochondria can be safely categorised as anaerobic with high FRD/CCO ratios. To summarise the results, the distribution of the three types of mitochondria is schematized in Fig. 10.

The presence in adult *P. westermani* of mosaic cells containing different types of respiratory chains, with varied energy efficiency, is intriguing. This situation may be due to the environmental oxygen tension surrounding the trematodes. In contrast to adult *A. suum*, which dwells in the host small intestinal lumen with low oxygen tension (PO_2 = 0–10 mm Hg; Minning et al., 1999), oxygen tension inside the cysts in which adult *P. westermani* live is likely higher than in the intestinal lumen, although this has not been demonstrated to date. Since the PO_2 of mammalian lung tissues through which blood circulates is approximately 40 mm Hg (Guyton, 1986), the PO_2 inside cysts may be similarly high enough to drive the aerobic respiratory chain of mitochondria localised in the outermost tegument and tegumental cells. In contrast, the parenchymal cells, which are located under the tegument and in the innermost part of the body, are in an environment of much lower PO_2. Therefore, the PO_2 of some parenchymal cells may be close to zero. Under these anaerobic conditions, as for adult *A. suum* (Kita and Takamiya, 2002; Yokota et al., 2006; Hashimoto et al., 2008; Takamiya et al., 2009), the parenchymal cells must depend on anaerobic respiration, in which fumarate functions as the electron acceptor instead of oxygen and oxygen homeostasis is maintained using haemoglobin and myoglobin. Thus, adult *P. westermani* adapt to their environmental conditions by optimising the mosaicism of their own tissues. Further studies are required to elucidate the mechanisms underlying aerobic–anaerobic transitions and the mosaic formation of tissues in these parasitic trematodes.

Acknowledgements

This work was supported in part by Grants-in-Aids for Scientific Research (C) (Nos. 18590406 and 22590383) from the Ministry of Education, Science, Sports and Culture of Japan, a research grant from the Institute of Health and Medical Sports Science, Graduate School of Health and Sports Science, Juntendo University, Japan (HRC-H17) to S.T., and a research grant from the Institute for Environmental and Gender Specific Medicine, Juntendo University to S.T. We are also indebted to the staff of the Division of Ultrastructural Research, Biomedical Research Center, Graduate School of Medicine, Juntendo University, for their helpful advice and guidance in performing the electron microscopic analyses and to Dr. Shinji Nakamura, Division of Biomedical Imaging Research, Biomedical Research Center, Graduate School of Medicine, Juntendo University, for statistical analyses.

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EXPERIMENTAL INFECTION WITH *PARAGONIMUS HETEROTREMUS* METACERCARIAE IN LABORATORY ANIMALS IN MANIPUR, INDIA

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Abstract. This study was aimed to find out the host-parasite relationship between *Paragonimus heterotremus* isolated as metacercariae from mountain crabs, *Indochinamon manipurensis*, in Manipur, India and laboratory animals such as puppies, albino rats, Swiss mice, guinea pigs, and rabbits, as experimental animals. The animals were fed with the metacercariae. Infected animals were sacrificed 35 to 430 days after feeding to recover worms, which were used to determine the developmental stages. Adult worms (n = 14) were recovered from 3 puppies ≥70 days after feeding and immature worms (n = 25) were recovered from 2 other puppies 35 or 43 days after infection. The infection rate in puppies was 100%. Juvenile worms were recovered from 3 of 13 rats: 1 of 11 rats whose viscera and cavities were examined and both of two rats whose muscles were examined. Rats were not a suitable animal model for pulmonary infection with *P. heterotremus*. Mice, guinea pigs, and rabbits were also found to be insusceptible to pulmonary infection with *P. heterotremus*.

Keywords: *Paragonimus heterotremus*, puppy, metacercariae, host-parasite relationship, infectivity, India

INTRODUCTION

In India, *Paragonimus westermani* isolation from tigers, bear cats, mongooses, civet cats and dogs has been reported (Rao, 1935; Dutt and Gupta, 1978; Singh and Somvanshi, 1978; Ravikumar *et al*, 1979; Parihar and Shirivastava, 1988). *Paragonimus* eggs were also detected in fecal samples of civet cats and toddy cats in Manipur, India (Singh *et al*, 1998). A suitable animal model for *Paragonimus* infection is required to study the host-parasite relationship, pathogenesis, host immune response, and for the therapeutic evaluation of drugs. Adult worms are required for morphological and molecular characterization, as well as antigen preparations for the immunodiagnosis of paragonimiasis. Studies have shown that dogs and cats, especially puppies and

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kittens, are susceptible to most *Paragonimus* species prevalent in Asia, while rats and mice served as paratenic hosts when infected naturally or experimentally with some *Paragonimus* species (Shibahara, 1981; Sugiyama *et al*, 1990).

Little is known about experimental animal infection by *Paragonimus* species in India. Therefore, this study was conducted to develop a suitable laboratory animal model for *Paragonimus heterotremus* in a laboratory in Manipur, India. Human infection with this lung fluke species has been confirmed in Manipur (Singh *et al*, 2007) and Nagaland (Singh *et al*, 2009), a state neighboring Manipur.

MATERIALS AND METHODS

Paragonimus heterotremus metacercariae were isolated from freshwater crabs, *Indochinamon manipurensis* (Alcock, 1909), which were formerly referred to as *Potamiscus manipurensis*, at Churachandpur, Pashong Luwangsangbam Matai, and Motbung in the Manipur State of India (Singh *et al*, 2007). The isolation procedure of metacercariae from crabs was described previously (Rangsiruji *et al*, 2006). Using Pasteur pipettes, puppies (n = 5, 8 to 50 metacercariae each), albino rats (Wistar strain, n = 11, 20 metacercariae each), rabbits (n = 4, 20 metacercariae each), Swiss mice (n = 4, 20 metacercariae each), and guinea pigs (n = 4, 18 metacercariae each) were inoculated orally with isolated metacercariae. Pipettes were all heated over a flame to smooth their edges before using for oral inoculation. The metacercariae, 20 each, were also inoculated to the peritoneal cavity of 2 other albino rats under ether anesthesia using the method described previously (Sugiyama *et al*, 1990). The animals were confirmed negative for *Paragonimus* infection by fecal examinations by the formalin-ether sedimentation technique prior to experimental infection. They were autopsied 35 to 430 days after infection and the lungs, liver, and pleural and peritoneal cavities were examined. The skeletal muscles were also examined in some rats (Sugiyama *et al*, 1990). Recovered worms were examined under a microscope before and/or after Borax carmine staining and mounting to determine the developmental stage as adult, pre-adult, immature, and juvenile according to the criteria of Shibahara (1984).

RESULTS

The 5 puppies used in this study were all positive for *P. heterotremus* infection, and 39 flukes were recovered (Table 1). Fourteen flukes recovered 70 or more days after infection were adult (Fig 1), and those (n = 25) recovered 35 and 43 days after infection were at the immature stage. Twenty-eight worms were recovered from 3 of the 13 rats. The flukes from rats were all determined to be at the juvenile stage (Table 2, Fig 2). No worms were recovered and no cysts were identified from the infected rabbits, mice, and guinea pigs.

DISCUSSION

The infection rate of puppies was 100%, and the worms recovered 70 or more days after infection with *P. heterotremus* metacercariae were all identified as adults. Employing puppies as an experimental host animal seems to yield sufficient numbers of worms required for morphological identification, antigen preparations, and molecular studies. The study of host immune responses and therapeutic evaluation of antihelminthic drugs can also be carried out in puppies.

The recovery of juvenile worms alone from only 3 of the 13 rats fed with *P. het-*

Table 1
Results of experimental infection of puppies with *P. heterotremus* metacercariae.

Puppy no.	Sex	Route of infection	No. of MC[a]	Autopsy at days after infection	No. (%) of worms recovered	No. of worms recovered from				
						Cyst in lungs	Lungs	Pleural cavity	Liver	Peritoneal cavity
1	F	Oral	20	35	5 (25)	0	1	2	0	2
2	M	Oral	50	43	20 (40)	0	0	20	0	0
3	F	Oral	20	70	5 (25)	0	0	5	0	0
4	F	Oral	25	330	5 (20)	4	1	0	0	0
5	F	Oral	8	430	4 (50)	4	0	0	0	0

[a] MC, metacercariae

Table 2
Results of experimental infection of albino rats with *P. heterotremus* metacercariae.

Rat no.	Sex	Route of infection[a]	Autopsy at days after infection	No. of worms recovered (%)	No. of worms recovered from					
					Cyst in lungs	Lungs	Pleural cavity	Liver	Peritoneal cavity	Muscle[b]
1	F	Oral	50	0	0	0	0	0	0	NE
2	F	Oral	50	1 (5)	0	0	1	0	0	NE
3	F	Oral	90	0	0	0	0	0	0	NE
4	F	Oral	90	0	0	0	0	0	0	NE
5	M	Oral	90	0	0	0	0	0	0	NE
6	M	Oral	112	0	0	0	0	0	0	NE
7	M	Oral	114	0	0	0	0	0	0	NE
8	M	Oral	120	0	0	0	0	0	0	NE
9	F	Oral	142	0	0	0	0	0	0	NE
10	M	Oral	152	0	0	0	0	0	0	NE
11	M	Oral	159	0	0	0	0	0	0	NE
12	M	IP	95	14 (70)	0	0	0	0	0	14
13	M	IP	95	13 (65)	0	0	0	0	0	13

[a] 20 metacercariae were given orally or intraperitoneally (IP); [b] NE, not examined

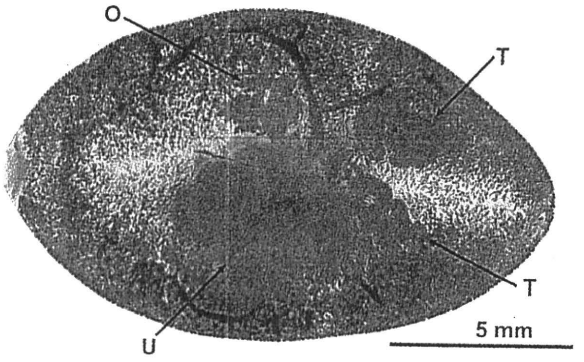

Fig 1–An adult fluke recovered from the lung cyst of tested puppy No. 3, 330 days after oral infection with *P. heterotremus* metacercariae. T, testes; O, ovary; U, uterus.

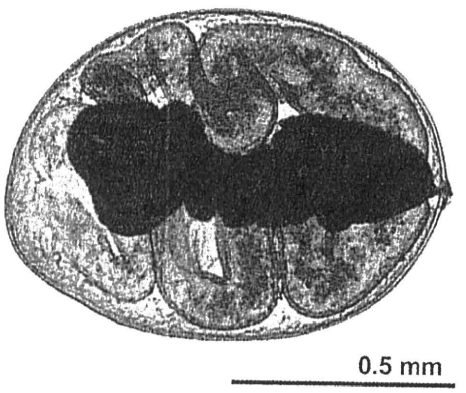

Fig 2–A juvenile fluke recovered from the muscle tissue of tested rat No. 12, 95 days after intraperitoneal infection with *P. heterotremus* metacercariae.

erotremus metacercariae showed that rats are not a suitable final host for this isolate. The rats served as paratenic hosts may be important for natural sources of infection of wild and domestic carnivorous animals.

Adult worms were recovered from rats experimentally infected with *P. heterotremus* experimenting in Thailand (Sugiyama *et al*, 1990) and in Arunachal Pradesh, India (Narain *et al*, 2003). From our observations, together with the previous findings, the host-parasite relationship between *Paragonimus* species and laboratory mammalian hosts might be related to strain variation of the species and the selection of mammalian hosts in different geographical areas (Fan and Chiang, 1970; Habe, 1978; Habe *et al*, 1996). Dogs and cats, especially puppies and kittens, are susceptible to all *Paragonimus* species in Asia; these animals can serve as suitable models for experimental studies which require a greater number of adult worms and a longer host life span.

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Case Report

Cerebral paragonimiasis mimicking tuberculoma: First case report in India

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KEY WORDS

Cerebral paragonimiasis, Brain CT Scan, Tuberculoma, India

ABSTRACT

An 8-year-old male child of Tuensang District, Nagaland, India, attended Civil Hospital, Tuensang, complaining of cough, fever, headache, and inability to move right arm since one month. On clinical suspicion of tubercular meningitis, anti-tubercular therapy was initiated and the patient was referred to the Naga Hospital Authority. A brain computed tomography scan revealed an isodense area with surrounding edema on the left parietal lobe, which was diagnosed as tuberculoma and the anti-tubercular therapy was continued. As there was no sign of clinical improvement on completion of the three-month-ATD regimen, the patient was investigated for paragonimiasis. Laboratory investigations revealed peripheral blood eosinophilia, raised ESR, *Paragonimus* egg-positive sputum, and positive Enzyme-linked immunosorbent assay (ELISA) and ID tests for paragonimiasis. The Bacillus Calmette-Guérin (BCG) test and Acid Fast Bacilli (AFB) sputum smears were negative. Chest roentgenogram showed no abnormal findings. A final diagnosis of pulmonary paragonimiasis associated with cerebral paragonimiasis was made. The patient responded to praziquantel therapy. Cerebral paragonimiasis is a serious extrapulmonary form of paragonimiasis, sometimes life-threatening, but curable with praziquantel. It should be included in the differential diagnosis of cerebral granulomatous and other space-occupying lesions.

How to cite this article
Singh TS, Khamo V, Sugiyama H. Cerebral paragonimiasis mimicking tuberculoma: First case report in India. Trop Parasitol 2010;1:39 - 41.

DOI: ***

INTRODUCTION

Paragonimiasis is commonly manifested as pulmonary paragonimiasis, but extrapulmonary forms are not infrequent in humans. Singh *et al.*, in a review of 45 cases of paragonimiasis in children, found 20 (44.4%) extrapulmonary cases.[1] In another review of 247 cases in adults, the extrapulmonary forms were found in 2% of all the cases.[2] Extrapulmonary forms resulted from the erratic migrations of juvenile worms, which deviated from the normal route of migration to the lungs through the intestinal wall, peritoneal cavity, and diaphragm. The cerebral form is one of the typical examples of extrapulmonary forms due to erratic migrations. In this form, the worms enter the cranial cavity through the jugular or carotid foramen and commonly invade the temporal and occipital lobes.[3] Cerebral paragonimiasis is a serious and sometimes fatal disease, although the symptoms are solely dependent on the site of localization and number of parasites.[4] Cerebral paragonimiasis have been reported mainly from China;[5] Korea;[6] Formosa;[7-8] and Japan.[9] We report the first case of cerebral paragonimiasis in India, which was combined with the pulmonary form.

CASE HISTORY

An 8-year-old boy of Tuensang district, Nagaland, India was admitted to the district hospital of Tuensang on 15 October, 2007, with chief complaints of cough, fever, headache, and inability to move the right arm since one month prior. The patient was provisionally diagnosed as a case of tubercular meningitis. Without any laboratory investigations, he was discharged on 22 October, 2007 to be admitted in a Civil Hospital, Tuensang, on 25 October,

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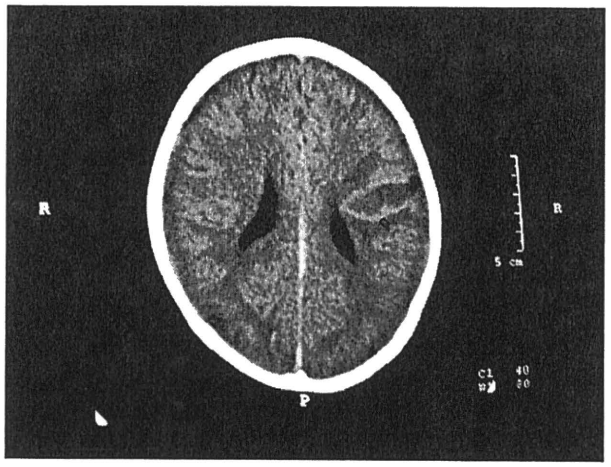

Figure 1: CT scan of the brain showing an isodense area surrounded by edema (arrow) in the left parietal lobe

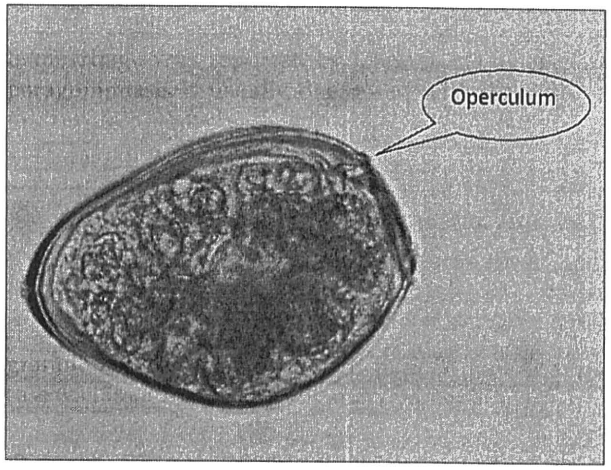

Figure 2: *Paragonimus* egg seen in the sputum smear (40×) not to be scaled

where he was given antitubercular therapy (Cat-II). In the absence of any appreciable improvement, the patient was transferred on 6 November, 2007 to the Naga Hospital Authority, where specialists and facilities for laboratory investigations, including computed tomography (CT) / X-ray / Ultrasonography were available. On 13 November, 2007, few laboratory investigations and CT scan of the brain were performed. The routine hematological test revealed Hb 11gm%, total leucoctye count 8800 per cumm, neutrophils-58%, lymphocytes-25%, eosinophil-17%, and ESR (Westergren) 60 mm, at the end of the first hour. The BCG test was negative. A CT scan of the brain showed an isodense area with surrounding edema in the left parietal lobe [Figure 1]. A diagnosis of tuberculoma in the left parietal lobe was made and antitubercular therapy was continued. Sputum samples were negative for AFB, but positive for *Paragonimus* eggs [Figure 2]. Micro-ELISA and double immunodiffusion tests of the serum were positive for paragonimiasis. *Paragonimus heterotremus* adult extract was used for these tests, as an antigen. The patient gave a history of consumption of mountainous crabs. Based on these findings, the case was finally diagnosed as pulmonary paragonimiasis with cerebral involvement. The morphological and molecular characterizations of eggs from the sputum sample revealed features of *P. heterotremus*. Hence, the causative agent was identified as *P. heterotremus*. A high incidence of *P. heterotremus* metacercariae in mountainous crabs (Singh TS *et al.*, manuscript in preparation) supports this conclusion. A course of praziquantel was given in doses of 25 mg per kg body weight, thrice a day, for five days. Following the praziquantel therapy, the patient showed dramatic improvement clinically, during the follow-up period of three months post therapy.

DISCUSSION

Cerebral paragonimiasis is one of the important extrapulmonary forms with serious consequences and high mortality compared to the pulmonary form, which is usually benign. Most cases of cerebral disease are associated with chronic morbidity due to epilepsy, dementia, and various neurological sequelae. Rarely, the patient may die of cerebral hemorrhage. A rare case of cerebral paragonimiasis combined with cerebral hemorrhage was reported by Choo *et al.*[3] Cerebral paragonimiasis is usually diagnosed with the help of imaging techniques, such as, X-ray, Computerized Axial Tomography (CAT) scan, and magnetic resonance (MRI), and serodiagnosis (ELISA and others) is complemented. The CT and MRI of cerebral paragonimiasis generally show as conglomerates of multiple ring-shaped shadows or enhancements, called the 'grape cluster' or 'soap bubble' forms, in one hemisphere.[10] The present case is an exception of cerebral paragonimiasis with a granulomatous lesion in the left parietal lobe. As the case was associated with pulmonary paragonimiasis, the causative agent was identified as *P. heterotremus*, based on the morphological and molecular characterization of the eggs from the sputum sample. This case emphasizes the need for differential diagnosis between tuberculoma and cerebral paragonimiasis by careful history taking and laboratory investigations. If the clinicians were aware of the prevalence of paragonimiasis and its various clinical presentations, the delay in the correct diagnosis and therapy for cerebral paragonimiasis could have been avoided.

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Source of Support: Nil, **Conflict of Interest:** None declared.
DOA: ***, **DOP:** ***

日本食品微生物学会雑誌 Jpn. J. Food Microbiol., 27(1), 1–7, 2010

総 説

食品媒介寄生虫による食中毒

Food-borne Parasitic Infection as Food Poisoning

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はじめに

食の安全・安心は，我々にとって大きな関心事となっている．この食の安全を担保する法律として，我が国では「食品衛生法」が重要な役割を果たしてきた．本法は「食品の安全性の確保のために公衆衛生の見地から必要な規制・措置を講じることにより，飲食に起因する衛生上の危害の発生を防止し，もって国民の健康の保護を図ることを目的とする（第1条）」として，1947年に制定された法律である．この条文に書かれている「飲食に起因する衛生上の危害」を，「飲食に起因する健康被害」と読み替え，さらにこれを「食中毒」としてとらえるのが，最近の行政上の，すなわち厚生労働省の方針となっている[16,20] (http://www1.mhlw.go.jp/topics/syokueihou/tp1228-1_13.html).

食中毒の原因として対策を取る寄生虫

寄生虫も「飲食に起因する衛生上の危害」を引き起こすことから，「食中毒」の原因物質としてとらえる必要がある．我が国では，生鮮魚介類などの生食が嗜好され，食文化としても定着していることから，食品に媒介される寄生虫症の発生を認めることは，経験的にも知られている．この食品媒介寄生虫による疾患への対策を検討するため，1997年9月に当時の厚生省において，食品衛生調査会食中毒部会食中毒サーベイランス分科会が開催された．そのときにまず，我が国において特に対策が必要な寄生虫が指定された．検討に際しては，

イ）全国的に発生が多いもの，あるいは近年増加傾向にあるもの．

ロ）海外では発生が多く日本でも増加が懸念されるもの．

ハ）発生は多くなくとも重篤な被害が出る恐れのあるもの．

という三つの条件が考慮され，以下の14種類の寄生虫が対象として指定されることになった．

1) 原虫類（4種類）：単細胞の寄生虫（著者注）

クリプトスポリジウム，サイクロスポーラ，ジアルジア，赤痢アメーバ

2) 蠕虫類（計10種類）：多細胞の寄生虫，「ぜんちゅう」（著者注）

(1) 生鮮魚介類により感染するもの（6種類）

アニサキス，旋尾線虫，裂頭条虫，大複殖門条虫，横川吸虫，顎口虫

(2) その他の食品（獣生肉等）により感染するもの（4種類）

肺吸虫，マンソン孤虫，有鉤嚢虫，旋毛虫

これら寄生虫の感染を予防するために当面取るべき対策として，まず「国民及び関係者への安全な摂食方法等についての普及啓発」が挙げられた．また「国内外での食品の寄生虫汚染の実態及び当該疾患の発生状況についての情報把握」などの事項も取るべき対策とされた[17] (http://www1.mhlw.go.jp/houdou/0909/h0917-1.html).

食品衛生法に則した食中毒の届出

寄生虫疾患の発生状況についての情報を把握するには，法に則した届出を確実とすることが有効な手段となる．寄生虫も「食中毒」の原因物質としてとらえることから，飲食に起因した寄生虫症が発生すれば，これを食中毒として届け出ることになる．

「食中毒」が発生した場合の届出に関しては，食品衛生法の第58条に規定がある．その第1項には「食品，添加物，器具もしくは容器包装に起因して中毒した患者もしくはその疑いのある者を診断し，またはその死体を検案した医師は，直ちに最寄りの保健所長にその旨を届け出なければならない」と記載されている（医師の届出は文書，電話または口頭により24時間以内に行わなければ

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ならない：食品衛生法施行規則第72条）．実際には，「食中毒患者等届出票」の当該箇所（原因）に，食中毒事件票の「食中毒病因物質の分類」（表1）にリストアップされている病因物質を記載して，医師から保健所に，食中毒事案としての届出が行われることになる．医師以外の者からの報告・苦情等は，食中毒の疑いのある事案として保健所が受け付ける[20]．

食品衛生法に則した寄生虫症の届出

「食中毒病因物質の分類」を見ると，寄生虫が食中毒の病因物質として，「22. その他」という範疇で取り上げられていることがわかる（表1）．しかも具体的な例として，「クリプトスポリジウム，サイクロスポラ，アニサキス」の3種類の寄生虫が「等」という文字を付して掲げられている．この3種の寄生虫は，上述の食中毒部会で検討・指定された14種類の中に含まれる．

このように寄生虫名が「食中毒病因物質の分類」の中に例示された直接の契機は，1999年の食品衛生法施行規則の一部改正にある．改正に当たり当時の厚生省から都道府県知事等に発出された通知を見ると，「原虫及び寄生虫による飲食に起因する健康被害についても食中毒としての取扱いを明確にするために，食中毒病因物質の分類のその他にクリプトスポリジウム等の例示を掲げた」と明記されている[19]（http://www1.mhlw.go.jp/topics/syokueihou/tp1228-1_13.html）．寄生虫を原因とするものであっても，「飲食に起因する衛生上の危害」は「食中毒」として取扱い，食品衛生法に基づいて事案発生を届け出る．その結果として，事故が調査され，被害の拡大が防がれ，再発が防止される．このような寄生虫症への対応に関する厚生労働省の見解には，現時点でも変更はない．

なお上述の通知では，寄生虫を「原虫及び寄生虫」という形で記述している．「原虫」を寄生虫から分離し，また蠕虫を単に「寄生虫」として表現したものと考えられる．

寄生虫症の届出の実態

上述の食品衛生法施行規則の一部改正以降，食品衛生法に則して届け出る寄生虫症に変化があったか，食中毒統計に見てみたい（表2）．すでに述べたように寄生虫は，食中毒の病因物質「その他」に分類されており，具体的な寄生虫として「クリプトスポリジウム，サイクロスポラ，アニサキス」という3種類が例示されている．しかしながら実際には，蠕虫である「アニサキス，肺吸虫，旋尾線虫」の3種類を病因物質とする事案が，食中毒として届け出られたに過ぎない．

我が国では，生鮮魚介類の生食が嗜好され，これを原因とした寄生虫症の発生があることは経験的にも知られている．なかでもアニサキスは病気の原因として知名度が高い．このアニサキス症の発生状況が，日本内視鏡学会会員や同学会指導施設等を対象としたアンケートにより調べられ，2001年から2005年の5年間に2,511例の症例（年平均：502例）が集計されている[9]．このような成績も背景に，最近でも年間に2,000例以上のアニサキス症が，我が国で発生しているとの推定がある[14]．しかしながら食中毒統計では，例えば2001年から2008年までの8年間の届出は，事件数（患者数）がわずか38件（38名）にとどまる（年平均の患者数は約4.8名）．発

表1．食中毒病因物質の分類

1．サルモネラ属菌，2．ぶどう球菌，3．ボツリヌス菌，4．腸炎ビブリオ，5．腸管出血性大腸菌，
6．その他の病原大腸菌，7．ウエルシュ菌，8．セレウス菌，9．エルシニア・エンテロコリチカ，
10．カンピロバクター・ジェジュニ/コリ，11．ナグビブリオ，12．コレラ菌，13．赤痢菌，14．チフス菌，
15．パラチフスA菌，16．その他の細菌（エロモナス・ヒドロフィラ等），17．小型球形ウイルス，
18．その他のウイルス（A型肝炎ウイルス等），19．化学物質（メタノール，ヒスタミン，ヒ素等），20．植物性自然毒，
21．動物性自然毒，22．その他（クリプトスポリジウム，サイクロスポラ，アニサキス等），23．不明

表2．食中毒の届け出（食中毒統計）

年	食中毒届出総数 事件数（患者数）	病因物質［その他］ 事件数（患者数）	アニサキス 事件数（患者数）	肺吸虫 事件数（患者数）	旋尾線虫 事件数（患者数）
1999	2,697 (35,214)	1 (1)	1 (1)	0 (0)	0 (0)
2000	2,247 (43,307)	5 (53)	4 (4)	0 (0)	0 (0)
2001	1,928 (25,862)	1 (1)	1 (1)	0 (0)	0 (0)
2002	1,850 (27,629)	2 (25)	0 (0)	0 (0)	0 (0)
2003	1,585 (29,355)	1 (1)	1 (1)	0 (0)	0 (0)
2004	1,666 (28,175)	5 (8)	4 (4)	1 (4)	0 (0)
2005	1,545 (27,019)	8 (8)	7 (7)	0 (0)	0 (0)
2006	1,491 (39,026)	7 (23)	5 (5)	0 (0)	0 (0)
2007	1,289 (33,477)	8 (20)	6 (6)	1 (2)	0 (0)
2008	1,369 (24,303)	17 (47)	14 (14)	0 (0)	0 (0)
2009*	580 (12,111)	10 (10)	9 (9)	0 (0)	1 (1)

* 2009年は2009年12月18日までの速報値で，同年11月中旬までの事件数（患者数）を示す．

生の実態と食中毒統計との間に乖離があることは，疑う余地もない．さらに，「クリプトスポリジウム，サイクロスポラ」などを含めた原虫を原因物質とする事案は，食中毒としての届出が全くない（海外では原虫を原因とした食品媒介の症例報告がある[6]）．届出を確実とするような対策の検討が必要である．

ここで本稿では，食中毒の原因として届出がある3種類の寄生蠕虫，すなわちアニサキス，肺吸虫及び旋尾線虫について以下に概説し，各寄生蠕虫に関する最近の話題を提出したいと思う．これら寄生蠕虫症に対する理解を深めて，法に則した届出をさらに促進していただきたい．

アニサキスとアニサキス症

アニサキス症は古くからあった病気と考えられるが，その原因がAnisakis亜科線虫（の幼虫）であることは，1960年にオランダから報告された事例をもって初めて確定された[36]．我が国では，1964年発生の2例が本症としての最初の報告となる[2]．当初は診断の方法がなく，急激な腹部症状から開腹して患部が切除され，病理学的に初めてアニサキス症であると診断された事例がほとんどであった．しかし1970年代になると，内視鏡での検査と生検用鉗子での虫体摘出が普及し，予想以上に多くの症例が発生していることが明らかとなった．

アニサキス症の原因となる虫種としては，クジラやイルカを終宿主とする*Anisakis simplex*（成虫が寄生する宿主を「終宿主」と呼ぶ），マッコウクジラなどを終宿主とする*A. physeteris*，そしてアザラシやトドを終宿主とする*Pseudoterranova decipiens*の3種が重要である．これらの幼虫が魚介類に寄生しており，生きたままヒトに経口摂取されて消化管壁などに侵入し，アニサキス症を引き起こす．

アニサキス症はその発症部位によって，胃アニサキス症，腸アニサキス症及び消化管外アニサキス症に大別される．我が国で発生するアニサキス症の大部分は，激烈な胃痛（心窩部痛）と悪心・嘔吐を主な症状とする胃アニサキス症と考えられる．原因となる主な虫種は，患者に由来する虫体の形態観察から，*Anisakis simplex*であると報告されてきた．

アニサキス同胞種の分類学的解析

*A. simplex*の分類に関しては，アイソザイム解析や塩基配列解読などの先行研究の結果から，これを3種類の同胞種，すなわち*A. simplex* sensu stricto（狭義の*A. simplex*），*A. pegreffii*，*A. simplex* Cに分けるという考えが提出され，国際的にも受け入れらるようになってきた[22]．同胞種とは，形態学的鑑別が困難であるが，自然下では（生殖隔離などで）互いに独立した関係にある種の集団を意味する．

この新しい分類基準に基づく同胞種の解析が我が国でも試みられた．その結果，日本近海の魚に寄生するアニサキスは，北日本（北海道に水揚げされた太平洋産のサバ等）ではいずれも*A. simplex* sensu stricto，また南日本（九州に水揚げされた日本海産・東シナ海産のサバ）ではおおむね*A. pegreffii*であることが示された[32, 35]．一方で，北海道及び九州の人体症例に由来する虫体は，ほとんどすべてが*A. simplex* sensu stictoと同定された[33]．ヒトへの主たるアニサキスの感染源は，例えば九州ではサバと言われていたが[8]，その九州においては，魚（サバ）寄生の優占種と患者由来の優占種とが異なるとの結果が，アニサキスの同胞種解析で得られたことになる[34]．

本州で水揚げされたサバ（日本海産・太平洋産）に由来するアニサキスに関しても，同胞種レベルでの解析が行われ，その結果，*A. simplex* sensu stricto と *A. pegreffii* とが混在して寄生していることが明らかとなった[35]．したがって，サバを原因とする九州のアニサキス症は，地元産ではなく他の地域から搬入された*A. simplex* sensu stricto陽性のサバを原因とする可能性が高い．また，*A. simplex* sensu stictoが人体症例の主たる原因である理由については，*A. pegreffii*に比べてサバの筋肉から検出される虫体数がはるかに多いという検索結果をもって，説明されている[31]．このように同胞種レベルでの解析は，感染源の特定にも有用で，それを適切に応用すれば，アニサキス症の発生予防に有効な啓発活動が展開できると考えられた．

アニサキスによる食物アレルギー

食事をすることでアレルギー症状が起きる「食物アレルギー」に関して，魚介類の消費量が多い我が国では，魚介類も重要な原因食物となってきた．しかしながら，この魚介類アレルギーは，魚介類そのものがアレルゲンではなく，魚介類に寄生するアニサキスが原因であるとの興味深い知見が報告された[10]．すなわち，サバの摂食後に蕁麻疹を呈した複数の症例や，さらに呼吸困難・心悸亢進などのアナフィラキシー症状を呈した症例について，アニサキス抗原を用いたスクラッチテストや血中抗体価(IgE)の測定が行われた．その結果，サバに対しては陰性，アニサキスに対しては陽性となることが示された[11, 12]．アニサキスに起因するアナフィラキシー症状（血圧降下・呼吸不全・意識喪失）は，散発事例だけでなく，集団発生事例でも観察されている（カタクチイワシが原因と推定された事例[1]）．

一方で，魚介類の生食習慣が我が国ほど一般的ではないはずのスペインにおいても，1995年以降に，アニサキスに対するアレルギーの症例が150例以上も報告された[3]．しかも，皮膚炎，喘息発作，関節炎，結膜炎など多彩な病態を示す症例が検出され，さらに虫体を殺滅するような加熱・冷凍などの処理を魚に施しても，アニサキスによるアレルギー反応の発現は必ずしも抑制されな