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# CRAB HOSTS FOR *PARAGONIMUS WESTERMANI* (KERBERT, 1878) IN MALAYSIA

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**Abstract:** During July and August 1985, a field survey on the lung fluke was carried out at six localities in the peninsular Malaysia. A total of 1,009 fresh-water crabs belonging to eight species were examined and *Paragonimus westermani* metacercariae were found in four species of the crabs, *Parathelphusa maculata* from Kuala Pilah and Ulu Langat, *Parathelphusa malaysiana, Johora tahanensis* and *Irmengardia pilosimana* from Sungai Wa. The three species of crabs except *Parathelphusa maculata* are recorded as new crustacean hosts of *P. westermani. Parathelphusa maculata* harboured more than 60% of the total metacercariae in the muscle, 20-25% in the gills and about 10% in the liver, but no metacercariae were found in the heart. In contrast, a substantial number of metacercariae parasitized the heart of the crab, *Parathelphusa malaysiana.*

## INTRODUCTION

In Malaysia, three species of fresh-water crabs, *Johora johorensis* (Roux, 1936), *Parathelphusa maculata* de Man, 1879 and *Potamiscus cognatus* (Roux, 1936) have been reported as the second intermediate hosts of *Paragonimus westermani* (Kim, 1967; Lee and Miyazaki, 1965; Miyazaki *et al.*, 1968; Miyazaki and Kwo, 1969).

During July and August 1985, the authors carried out a field survey on the Malaysian lung fluke. In the investigation, three species of the fresh-water crabs were found as new second intermediate hosts of *P. westermani.*

We report here the new crab hosts of *P. westermani* in Malaysia, the prevalence of *Paragonimus* infection in crabs, and the distribution of its metacercariae in the crab tissues.

## MATERIALS AND METHODS

A total of 1,009 fresh-water crabs belonging to eight species were collected from the following six different localities: Kampong Langkap near Kuala Pilah (Negeri Sembilan), several streams in Baling District (Kedah), Sungai Wa and Sungai Kuching at Taman Negara (Pahang), Sungai Lui at Ulu Langat (Selangor) and Sungai Lalang (Selangor) in the peninsular Malaysia (Figure 1 and Table 1). They were brought back to the Institute for Medical Research in Kuala Lumpur and were examined for *Paragonimus* infection after being classified by species, sex and carapace width. The gills of each crab were examined under a stereomicroscope.

Some of crabs were examined in detail for the distribution of metacercariae in the crab tissues. The examination procedure used is as follows. Gills, liver, intestine, heart and gonad were separately pressed between two glass plates and examined under a stereomicroscope. Muscles of the cephalothorax and legs were chopped up into small pieces by scissors and then transferred into a metal sieve of about 5-mesh. The chopped muscle was further ground with a wooden rod in the sieve immersed in a beaker of tap-water. The filtrate was allowed to stand still for a few minutes and then the supernatant was discarded. The sediment was washed several times by repeating this procedure and then strained through a sieve of 30-mesh. The supernatant of the final filtrate was poured off and the sediment was examined for metacercariae under a stereomicroscope. The metacercariae recovered from crabs were measured and used for experimental infection. Identification of the crabs was performed by Dr. M. Takeda of National Science Museum, Tokyo and the results have been published (Ng and Takeda, 1992).

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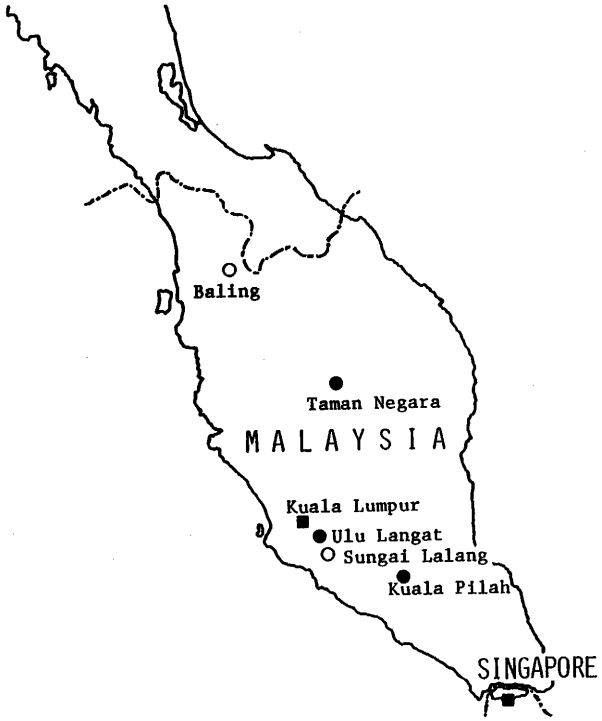


Figure 1 A map showing the localities surveyed in Malaysia ●: positive for metacercariae of *P. westermani* ○: negative

## RESULTS

The results of the crab examination for *Paragonimus* infection are shown in Table 1. *Paragonimus* metacercariae were recovered from *Parathelphusa maculata* collected from Kuala Pilah and Ulu Langat, *Parathelphusa malaysiana* Ng and Takeda, 1992, *Irmengardia pilosimana* (Roux, 1936) and *Johora tahanensis* (Bott, 1966) from Sungai Wa (Photos. 1-4). The table also indicates the prevalence rate and the number of metacercariae in the gills.

Table 2 Relationship between carapace size of crabs, *Parathelphusa maculata,* and number of *Paragonimus westermani* metacercariae in crab gills

| Carapace size (in mm) | No. of crabs | | Ave. No. of metacercariae in a positive crab |
|---|---|---|---|
| | examined | infected | |
| 25.0-29.9 | 17 | 6 (35.3%) | 1.7 |
| 30.0-34.9 | 49 | 12 (24.5%) | 2.3 |
| 35.0-39.9 | 79 | 29 (36.7%) | 1.5 |
| 40.0-44.9 | 57 | 31 (54.4%) | 2.5 |
| 45.0-49.9 | 60 | 45 (75.0%) | 2.9 |
| 50.0-54.9 | 21 | 18 (85.7%) | 2.6 |
| 55.0- | 4 | 4(100.0%) | 5.8 |

The crabs were collected from Kuala Pilah

145 (50.0%) of 287 *Parathelphusa maculata* (carapace width 25-57 mm) from Kuala Pilah, were positive. Although the infection rate was relatively high, the number of metacercariae per positive crab was low. One or two metacercariae per crab were found in many the positive crabs, and eleven was the maximum number in the gills. The prevalence rate and number of metacercariae in gills increased with carapace size, therefore they are proportional to the age of the crab (Table 2).

In Ulu Langat, 6 (24.0%) out of 25 *Parathelphusa maculata* (carapace width 20-36 mm) were positive. The number of metacercariae per positive crab ranged from 1 to 7, and 3.0 on the average.

In Sungai Wa (Taman Negara), 1 (1.0%) of 98 *Johora tahanensis,* 4 (6.2%) of 65 *Irmengardia pilosimana* and 12 (63.2%) of 19 *Parathelphusa malaysiana* were found to be infected with *Paragonimus* metacercariae. The average number of metacercariae

Table 1 Prevalence of *Paragonimus westermani* metacercariae in crab gills in Malaysia

| Locality | Crab host | No. of crabs | | No. of metacercariae /positive crab |
|---|---|---|---|---|
| | | examined | infected | |
| Kuala Pilah | *Parathelphusa maculata* | 287 | 145 (50.0%) | 2.5 |
| Ulu Langat | *Parathelphusa maculata* | 25 | 6 (24.0%) | 3.0 |
| Sungai Lalang | *Parathelphusa maculata* | 14 | 0 | |
| | *Johora intermedia* | 3 | 0 | |
| | *Geosesarma peraccae* | 12 | 0 | |
| Baling | *Somanniathelphusa sexpunctata* | 213 | 0 | |
| | *Stoliczia tweedei* | 52 | 0 | |
| Taman Negara Sungai Kuching | *Johora tahanensis* | 221 | 0 | |
| Sungad Wa | *Parathelphusa malaysiana* | 19 | 12 (63.2%) | 11.2 |
| | *Irmengardia pilosimana* | 65 | 4 ( 6.2%) | 1.0 |
| | *Johora tahanensis* | 98 | 1 ( 1.0%) | 6.0 |

Photo. 1 *Parathelphusa maculata* de Man, 1879

Photo. 2 *Parathelphusa malaysiana* Ng and Takeda, 1992

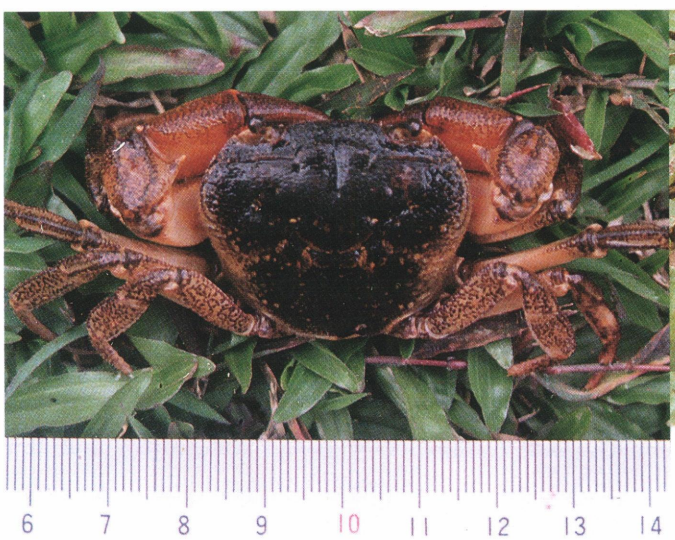

Photo. 3 *Johora tahanensis* (Bott, 1966)

Photo. 4 *Irmengardia pilosimana* (Roux, 1936)

in the gills per positive crab was 6.0, 1.0 and 11.2, respectively (Table 1).

Table 3 shows the distribution of metacercariae in the crabs hosts. In 21 (13 males and 8 females) *Parathelphusa maculata* from Kuala Pilah, out of 250 metacercariae counted, 156 (62.4%) were found in the muscles, 58 (23.2%) in the gills, 18 (7.2%) in the liver, 4 (1.6%) in the genital organs and 14 (5.6%) in other parts of the body (mostly in the inner membrane of the carapace). The infection rate of each organ or tissue was follows: 20 (95.2%) out of 21 crabs harboured 1-31 metacercariae (7.8 on the average) in the muscles; 14 (66.7%), 1-10 in the gills; 7 (33.3%), 1-5 in the liver and 4 (19.0%), a single metacercariae each in genital organs. No metacercariae were found in the heart region of the crabs. There was no significant difference between both sexes of crabs in the infection rate of metacercariae in each organ or tissue.

An infected crab, *Parathelphusa maculata* from Ulu Langat, was examined for the distribution of the metacercariae in the host. The crab (carapace width 35.8 mm) harboured 21 metacercariae in the muscles, 7 in the gills and 2 in the liver.

A total number of 437 metacercariae were recovered from 4 *Parathelphusa malaysiana* from Sungai Wa. More than half, 252 (57.7%), were obtained from mus-

Table 3 Distribution of *Paragonimus westermani* metacercariae in the crab hosts

| Crab host | No. of crabs examined | No. of Meta-cercariae obtained | No. of metacercariae recovered from | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | gills | liver | heart | genital organs | muscle | other tissues |
| *Parathelphusa maculata* | 21* | 250 | 58 (23.2%) | 18 (7.2%) | 0 | 4 (1.6%) | 156 (62.3%) | 14 (5.6%) |
| *Parathelphusa malaysiana* | 4† | 437 | 52 (11.9%) | 38 (15.6%) | 68 (8.7%) | 8 (1.8%) | 252 (57.7%) | 19 (4.4%) |
| *Irmengardia pilosimana* | 4† | 18 | 4 (22.2%) | 0 | 0 | 0 | 14 (77.8%) | 0 |
| *Johora tahanensis* | 1† | 18 | 6 (33.3%) | 2 (11.1%) | 0 | 0 | 9 (50.0%) | 1 (5.6%) |

* Collected from Kuala Pilah. † Collected from Sungai wa, Taman Negara
All crabs harboured metacercariae somewhere in their bodies.

Table 4 Measurements of the inner cyst of metacercariae of Malaysian *Paragonimus westermani* (in $\mu$m)

| Crab host | Locality | No. cysts measured | Cyst size | |
|---|---|---|---|---|
| | | | maximam | minimam |
| *Parathelphusa maculata* | Kuala Pilah | 56 | 334.9±17.0 | 312.2±17.0 |
| *Parathelphusa maculata* | Ulu Langat | 14 | 352.4±20.8 | 320.6±20.8 |
| *Parathelphusa malaysiana* | Sungai Wa | 52 | 342.9±15.1 | 314.7±15.1 |
| *Johora tahanensis* | Sungai Wa | 13 | 345.5±23.6 | 327.0±16.1 |
| *Irmengardia pilosimana* | Sungai Wa | 18 | 341.0±15.1 | 321.8±13.2 |

cles and 68 (15.6%) from heart or pericardium. Only 52 (11.9%) of them were distributed in the gills and the rest in the liver, genital organs, and other part of the body.

Another infected crab, *Johora tahanensis,* from Sungai Wa, harboured 18 metacarcariae, 9 of them were found in the muscles, 6 in the gills, 2 in the liver and 1 elsewhere.

In the examination of 4 infected individuals of *Irmengardia pilosimana* from Sungai Wa, only one crab harboured a single metacercaria in the gills but all four

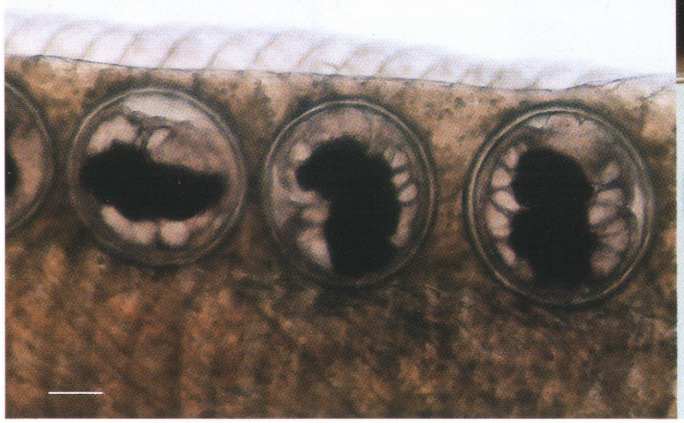

Photo. 5 Metacercariae in the gill of a host crab, *Parathelphusa malaysiana.* (Scale=100 $\mu$m)

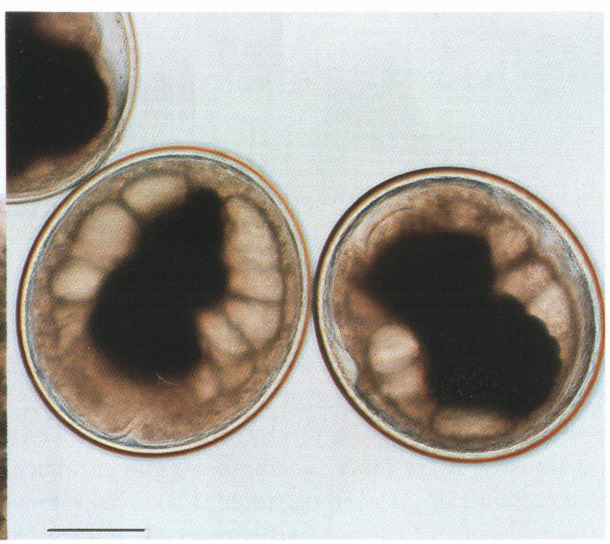

Photo. 6 Metacercariae removed from a host crab, *Parathelphusa malaysiana.* (Scale=100 $\mu$m)

had 3-4 metacercariae in the muscles.

*P. westermani* metacercariae obtained from different crab hosts in Malaysia were oval in shape and had two cyst walls, i.e., outer and inner layers. Thickness of outer layer was 2-3 $\mu$m and that of inner layer varied from 5 to 25 $\mu$m. The measurements of the encysted metacercariae from different localities are shown in Table 4. Pinkish granules in the metacercariae were rarely seen (Photos. 5 and 6).

On the basis of the morphological features of the metacercariae together with those of the adults obtained from experimentally infected animals and the cytogenetical studies of the flukes, these flukes were comfirmed as *P. westermani* (diploid type).

## DISCUSSION

Up to the present, the following 3 species of the fresh-water crabs have been reported as the second intermediate host of *P. westermani* in Malaysia: *Potamon johorense* from Ulu Langat (Lee and Miyazaki, 1965), *Parathelphusa maculata* from Ulu Langat (Miyazaki *et al.*, 1968) and *Potamiscus cognatus* from Baling (Miyazaki and Kwo, 1969). Kin (1967) also reported two species of crabs, *Potamon johorense* and *Parathelphusa maculata* as second intermediate host. The genus *Johora* (type species: *Potamon* (*Potamon*) *johorense* Roux, 1936) was first established by Bott (1966) as a subgenus of *Stoliczia* Bott, 1966 and Ng (1987) elevated it to a full genus. Nowadays, *Potamon johorense* comes under the genus *Johora*. In the present investigation, 4 species of the fresh water crabs, *Parathelphusa maculata* from Kuala Pilah and Ulu Langat, *Parathelphusa malaysiana, Johora tahanensis* and *Irmengardia pilosimana* from Sungai Wa, were found to be infected with *P. westermani*. These crab hosts except *Parathelphusa maculata* are recorded as new crustacean hosts of *P. westermani*.

*P. westermani* is widely distributed in Asia, and at each locality the fluke may have many crustaceans as the second intermediate host and some differences have been observed in the distribution of the metacercariae in the crab hosts. Observations on the crab, *Geothelphusa dehaani* White, in Japan showed that 86.5% of *P. westermani* (diploid type) metacercariae were found in the muscles, 7.8% in the liver, and 4.0% in the gills (Habe and Miyazaki, 1982). In the case of the Japanese *P. westermani* (triploid type) in *Eriocheir japonicus* (de Haan), 76% of metacercariae were found in the muscles, 16% in the gills, and 5% in the liver, while in *Geothelphusa dehaani*, 82.9% were obtained from the muscles and the remaining from liver or gills (Habe, 1979., Habe and Terasaki, 1982). As for Philippine *P. westermani*, namely *P. w. filipinus* Miyazaki 1978, in the crab, *Sundathelphusa philippina* Martens, 70.0% were found in the muscle, 22.3% in the heart and 4.9% in the gills (Miyazaki and Habe, 1979). The above mentioned results were obtained by the same methods as used in the present study.

In the present investigation on the distribution of *P. westermani* metacercariae in the crab, *Parathelphusa maculata* harboured more than 60% of the total metacercariae in the muscles, 20-25% in the gills and about 10% in the liver. No metacercariae were found in the heart region. The distribution of metacercariae showed no found to be significant difference between the Japanese *P. westermani* in *Eriocheir japonicus* and the Malaysian *P. westermani* in *Parathelphusa maculata*. The distribution of metacercariae in *Johora tahanensis* and *Irmengardia pilosimana* was similar to that in *Parathelphusa maculata*. Lee and Miyazaki (1965) also obtained 23 *Paragonimus* metacercariae from 4 infected crabs, *Johora johorensis*, 14 were in the muscle, 6 in the gills and 3 in the liver. On the other hand, *Parathelphusa malaysiana* showed a high percentage of the metacercariae in the heart region. This is similar to the Philippine *P. westermani* in *Sundathelphusa philippina*.

In the present study, it became clear that the Malaysian *P. westermani* shows different metacercarial distribution in accordance with the host species. The distribution pattern of metacercariae in the crab hosts is important to know accurate prevalence of *Paragonimus*. For example, Table 1 shows the prevalence rate and the number of metacercariae per crab host by examining only the gill. This value is quite a low rate of infection as compared with the whole body examination.

## ACKNOWLEDGMENTS

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# ETIOLOGIC AGENTS OF DIARRHEAL DISEASES IN SURABAYA, INDONESIA

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**Abstract:** Isolation frequency of enteropathogens from diarrheal stools was examined in Surabaya, Indonesia. A total of 385 patients with diarrhea including 264 children under 2 years of age and 121 adults were enrolled. Bacterial enteropathogens other than *Campylobacter jejuni* were positive in 164 cases out of 385 (44%), while *C. jejuni* was isolated from 13 cases out of 173 examined (7.5%). Rotavirus was detected in 91 cases of 184 examined (49%), and 34 cases of these 91 were co-infected with bacterial pathogens. Diarrheagenic *Escherichia coli*, which were isolated from about 30% of all 385 patients, were highly resistant to ampicillin and tetracycline. *Vibrio cholerae* O1 was isolated in 17 of 121 adult diarrhea cases.

## INTRODUCTION

Diarrheal disease was the first leading cause of death among young children in developing countries until around 1980. It was estimated in 1976 that 5 to 18 milion people die each year as a direct result of this disease (7). Although the mortality of diarrheal disease in the past decade appears to be decreasing because of intensive world wide promotion of Primary Health Care (PHC) activities including the enhancement of oral rehydration therapy (ORT), it still remains as a major cause of death among young children in these areas. Moreover, about 50% of tourists to the Third World develop traveler's diarrhea (3). From these viewpoints, diarrheal disease should be regarded as a global health problem.

Although there have been many reports on etiologic agents of diarrheal diseases in various countries and districts, repeated studies are needed since the isolation frequency of the enteropathogens differs from place to place, and the drug sensitivity pattern can change. In this communication, the isolation frequency of enteropathogens from diarrheal patients in Surabaya, Indonesia, and the drug sensitivity of *E. coli* isolated from them are described.

## PATIENTS, MATERIALS AND METHODS

Patients: A total of 385 patients with diarrhea at Dr. Soetomo Hospital (Surabaya, Indonesia) during the period from August to December in 1992 were examined. Of 385 patients, 264 were children under 2 years of age and 121 were adult cases. Of 264 pediatric patients, 137 were admitted to the hospital and 127 went back to their home after initial diagnosis and treatment were conducted at the outpatient department.

Specimens: Stool samples were taken before giving antibiotics. Self medication prior to visiting the hospital could not be completely excluded. An apropriate amount of stool was collected in a plastic container, but rectal swab was used for 70 children out of 127 who returned home directly from the outpatient department.

Microbiological examinations: The target bacteria for isolation from all patients were Escherichia, Salmonella, Shigella, Vibrio, Aeromonas, and Plesiomonas. Serial 10-fold dilutions of stool samples were made in normal saline solution, and 50 $\mu$l of the dilution at $10^5$ was inoculated on to agar plate of modified Drigalsky medium (Eiken). The inoculum was spread over half of the agar plate by a glass bar spreader and then streaked them to the other side with a wire inoculation loop. In the same manner, the dilution at $10^4$ was inoculated on to an agar plate of SS medium (Eiken), and the dilution

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at $10^3$ on to TCBS agar (Eiken). The colonies grown on agar plates were identified by routine laboratory tests. *Campylobacter jejuni* was isolated on "Campylobacter blood free selective agar medium (Oxoid), and was identified by confirming the morphology with Gram staining and degradation activities for hippuric acid. For the detection of rotavirus, the stools were centrifuged and a drop of the supernatant was mixed with a drop of anti-rotavirus antibody sensitized latex (Rota virus detection kit; Denka Seiken Co., Tokyo, Japan) on a slide glass to detect rotavirus. Enterotoxins were detected using Biken method (5) for LT, and using ST detection kit for ST (COLIST EIA; Denka Seiken Co. Tokyo, Japan). Verotoxin was examined by the cytopathic effect of *E. coli* culture supernatant on verocyte monolayer.

Drug sensitivity test: Two hundreds and seventy-three strains of *E. coli* isolated from 273 cases were examined for susceptibility against ampicillin (ABPC), tetracycline (TC), minocycline (MINO), cefdinir (CFDN), and ofloxacine (OFLX); preparations for oral administration are available for all 5 drugs. The *E. coli* examined contained 113 diarrheagenic strains and 160 non-diarrheagenic strains. Heart infusion agar plates containing serial 2-fold increasing concentrations of each drug ranging from 0.0125 to 100 $\mu$g/ml were prepared. The organisms were cultured in heart infusion broth at 37°C overnight. The culture was diluted 1 to 10 with saline solution and inoculated on the drug containing plates using a microplanter (Sakuma-MITP, Tokyo, Japan). The growth of organisms was observed after 24-hour incubation at 37°C, and minimum inhibitory concentrations of each drug against each organism were recorded.

## RESULTS

Isolation frequency: Facultative anaerobic enteropathogenic bacteria were isolated from 164 cases out of 385 examined (44%). More than half of the isolates were diarrheagenic *E. coli* (DEC). The isolation rate of

Table 1 Isolation Frequency of Enteropathogens

| | Total | In(Ped) | Out(Ped) | Adult(In/Out) |
|---|---|---|---|---|
| Number of patients | 385 | 137 | 127 | 121 |
| DEC | 113 | 44 | 39 | 29 |
| *Shigella* | 8 | 1 | 3 | 5 |
| *Salmonella* | 8 | 4 | 0 | 4 |
| *V. cholerae* O1 | 22 | 5 | 0 | 17 |
| non-O1 | 3 | 2 | 1 | 0 |
| *V. parahemolyt.* | 0 | 0 | 0 | 0 |
| Other vibrios | 2 | 2 | 0 | 0 |
| *Aeromonas* | 7 | 3 | 4 | 0 |
| *Plesiomonas* | 1 | 0 | 1 | 0 |
| Total | 164(44%) | 61(47%) | 48(38%) | 55(45%) |

In(Ped): pediatric inpatient, Out(Ped): pediatric outpatient
DEC: Diarrheagenic E. coli

Table 2 Details of the Isolated Diarrheagenic *E. coli* (DEC)

| | Total | In(Ped) | Out(Ped) | Adult(In/Out) |
|---|---|---|---|---|
| Cases with DEC | 113 | 44 | 39 | 29 |
| EPEC | 70 | 30 | 23 | 16 |
| EIEC | 7 | 2 | 2 | 3 |
| ETEC toxin(+) | 15 | 8 | 7 | 1 |
| serovar | 21 | 5 | 7 | 9 |
| VTEC | 0 | 0 | 0 | 0 |
| (E. coli-O157 | 4 | 1 | 2 | 1) |

EPEC: enteropathogenic *E. coli*, EIEC: enteroinvasive *E. coli*, ETEC: enterotoxigenic *E. coli*, VTEC: verotoxin-producing *E. coli*. ETEC serovar: characteristic serovar for ETEC such as 06, 015, 078, etc., but the toxin was not detected in a single examination.

Table 3 Detection Rate of *Campylobacter jejuni* and Rotavirus (No. of positive cases/No. of Patients examined, %)

| | Total | In(Ped) | Out(Ped) |
|---|---|---|---|
| *C. jejuni* | 13/173, 7.5% | 2/46, 4.3% | 11/127, 8.7% |
| Rotavirus | 91/184, 49% | 71/137, 52% | 20/47, 43% |
| Rotavirus + DEC | 26/184, 14% | 21/137, 15% | 5/47, 11% |
| Rotavirus + other | 8/184, 4.4% | 6/137, 4.4% | 2/47, 4.3% |

DEC: Diarrheagenic E. coli

DEC among pathogens isolated was 72% in children and 52% in adults (Table 1). Most of the DEC belonged to so called enteropathogenic *E. coli* (EPEC). Enterotoxins were detected from 15 strains by singe examination (14 strains with ST, one strain with ST/LT, and no strain with LT only). There were 4 strains with serovar O157, but their H antigens were not type 7 (Table 2). These 4 strains did not produce verotoxin, and verotoxin-coded gene was not detected from them as examined by polymerase chain reaction. *Campylobacter jejuni* was isolated from 13 cases out of 173 children (7.5%), but was not isolated from 58 adult cases examined. Rotavirus was detected in 91 pediatric diarrhea cases out of 184 examined (49%). However, bacterial pathogens were also isolated from 34 cases of the 91 with rotavirus-positive diarrhea (Table 3). The serovar of EPEC showed 15 types, of which serovar O127a was dominant (Table 4).

Table 4 Serovar Distribution of EPEC, 70 strains

| Serovar | No. of strain |
|---|---|
| O18 | 8 |
| O20 | 1 |
| O26 | 2 |
| O44 | 7 |
| O55 | 0 |
| O86a | 1 |
| O111 | 2 |
| O114 | 3 |
| O119 | 3 |
| O125 | 1 |
| O126 | 8 |
| O127a | 23 |
| O128ab | 4 |
| O142 | 0 |
| O146 | 1 |
| O151 | 0 |
| O158 | 1 |
| O166 | 5 |
| Total | 70 |

Drug sensitivity of *E. coli* revealed that more than 50% of the isolates were highly resistant to ampicillin and tetracycline (MIC=100 $\mu$g/ml or higher). Minocycline was better than tetracycline, but the MIC was 6.25 $\mu$g/ml or higher against 50% of the isolates. Cefdinir inhibited 95% of the isolates at the concentration of 6.25 $\mu$g/ml. Ofloxacin revealed excellent activity against the isolates. It inhibited the growth of all isolates at the concentration of 0.78 $\mu$g/ml except one which was inhibited at 3.13 $\mu$g/ml (Table 5). Drug sensitivity patterns of diarrheagenic *E. coli* and non-diarrheagenic *E. coli* were essentially the same, as shown in Table 6.

## DISCUSSION

The isolation rate of enteropathogens from diarrheal stools is largely dependent on the effort of the person conducting the test, and this should be considered in evaluating reports of isolation frequency. Although more than 10 genuses and many species of enteropathogens are known, it is usually difficult to focus attention on all these pathogens. In well conducted studies, the isolation rates of bacterial enteropathogens from diarrheal stools in developing countries have been reported as about 40 to 60% (1, 6, 9).

The present study also revealed on isolation rate of about 50% including *Campylobacter*. However, it may have been possible to increase the isolation rate by using enrichment media such as Selenite-F broth for Salmonella, alkaline peptone water for Vibrios, and ampicillin-blood agar for *Aeromonas*. Adkins et al reported that approximately 60% of *Salmonella* isolates were obtained only after enrichment (1), and we also have the same experience. Four colonies of *E. coli*

Table 5 Antimicrobial Susceptibility of *E. coli*

| Drug concentration (μg/ml) | Antimicrobials | | | | |
|---|---|---|---|---|---|
| | ABPC | TC | MINO | CFDN | OFLX |
| 0.0125 | 0 | 0 | 0 | 0 | 0 |
| 0.025 | 0 | 0 | 0 | 0 | 4 |
| 0.05 | 0 | 0 | 0 | 2 | 72 |
| 0.1 | 0 | 0 | 0 | 12 | 125 |
| 0.2 | 0 | 0 | 1 | 98 | 38 |
| 0.39 | 0 | 1 | 7 | 57 | 17 |
| 0.78 | 0 | 4 | 49 | 16 | 16 |
| 1.56 | 4 | 56 | 39 | 15 | 0 |
| 3.13 | 42 | 11 | 43 | 16 | 1 |
| 6.25 | 37 | 2 | 58 | 44 | 0 |
| 12.5 | 19 | 3 | 43 | 12 | 0 |
| 25 | 3 | 2 | 24 | 1 | 0 |
| 50 | 0 | 30 | 7 | 0 | 0 |
| 100 | 5 | 62 | 1 | 0 | 0 |
| 200≦ | 163 | 102 | 1 | 0 | 0 |

E. coli: 273 strains isolated from diarrheal patients, included 113 diarrheagenic *E. coli*.
Numerals indicate number of strains inhibited by the drug concentration.

Table 6 Drug Susceptibility of Diarrheagenic *E. coli*=DEC (113) and Non-diarrheagenic *E. coli*=NDEC (160)

| Drug conc. (μg/ml) | ABPC | | TC | | MINO | | CFDN | | OFLX | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DEC | NDEC | DEC | NDEC | DEC | NDEC | DEC | NDEC | DEC | NDEC |
| 0.0125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.025 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| 0.05 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 24 | 28 |
| 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 6 | 49 | 43 |
| 0.2 | 0 | 0 | 0 | 0 | 1 | 0 | 39 | 33 | 11 | 16 |
| 0.39 | 0 | 0 | 1 | 0 | 4 | 1 | 21 | 20 | 6 | 7 |
| 0.78 | 0 | 0 | 3 | 1 | 17 | 19 | 1 | 10 | 7 | 5 |
| 1.56 | 2 | 1 | 24 | 17 | 16 | 14 | 6 | 5 | 0 | 0 |
| 3.13 | 14 | 16 | 3 | 5 | 14 | 17 | 3 | 7 | 1 | 0 |
| 6.25 | 16 | 12 | 0 | 1 | 16 | 24 | 24 | 11 | 0 | 0 |
| 12.5 | 6 | 8 | 0 | 2 | 19 | 14 | 1 | 7 | 0 | 0 |
| 25 | 1 | 1 | 1 | 1 | 9 | 8 | 0 | 1 | 0 | 0 |
| 50 | 0 | 0 | 10 | 11 | 3 | 2 | 0 | 0 | 0 | 0 |
| 100 | 3 | 1 | 28 | 19 | 0 | 1 | 0 | 0 | 0 | 0 |
| 200≦ | 58 | 61 | 30 | 43 | 1 | 0 | 0 | 0 | 0 | 0 |

Numerals indicate percent of strains inhibited at the drug concentration.

isolated from 1 sample were examined for their enteropathogenicities in the present study, however, enterotoxin production is a delicate phenomenon and the repeated examination may result in higher detection rate.

Rotavirus was detected in almost 50% of infantile diarrhea with no definite seasonal variation. This epidemiological feature is in contrast to that in Japan where the epidemic of rotavirus infection is concentrated in the first 3 months of the year (4). The reason for this epidemiological contrast is of great interest in the field of tropical medicine. More than one third of the cases with rotavirus infection were also infected with another enteropathogen. The pathogenic role of micro-

organisms in the dual infection is obscure.

Drug sensitivities of *Shigella* were not examined since the number of isolates was very low. Nevertheless, antibiotic therapy is most important for shigellosis. However, the drug sensitivity pattern of *Shigella* is considered to be simillar to that of *E. coli* because of the presence of transmisible plasmid. The drug sensitivity pattern of diarrheagenic *E. coli* and non-diarrheagenic *E. coli* are quite similar as shown in the present study (Table 6).

Prevailing etiologic agents of diarrheal diseases and their drug sensitivities vary from place to place, and from year to year. The recent emergence of a new serovar of non-O1 *Vibrio cholerae* and the disappearance of *V. cholerae* O1 in India and Bangladesh are good examples (2, 8). Continuous monitoring of etiologic agents of diarrheal diseases is required.

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# IMMUNOBLOT ANALYSIS OF ANTIBODY RESPONSE BEFORE AND AFTER TREATMENT OF HUMAN STRONGYLOIDIASIS

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**Abstract:** Qualitative analysis of antibody response before and 12 months after treatment of human strongyloidiasis was performed to identify the antigenic component which may be useful for serological evaluation of the effectiveness of postchemotherapy. The immunoblotting patterns changed significantly after successful treatment in almost all patients; some responses decreased in their intensity and some disappeared. Whereas, the patterns of reactivity after treatment could not be distinguished from those before treatment in patients who were interpreted to be equivocal for complete cure because their antibody levels did not show a significant decrease after treatment. The immunoblotting patterns, however, varied considerably for each patient and it was difficult to identify the antigenic component which may be effective to detect reduced antibody responses early after treatment.

## INTRODUCTION

Strongyloidiasis, which is relatively common in Okinawa, Japan, is a parasitic disease resulting from an infection with a nematode *Strongyloides stercoralis*. One of the unique properties of the parasite is its ability to propagate in a host by internal autoinfection. The parasite is usually nonpathogenic in immunocompetent hosts, but due to the autoinfection, the asymptomatic infection often progresses to a severe or fatal infection under immunosuppressed conditions.

The chemotherapy of such severe cases is known to be difficult and it is essential to treat the patients during their chronic infection to prevent a severe infection. On the other hand, assessment of therapeutic efficacy by coprological examination is difficult because strongyloidiasis patients frequently fail to respond to anthelminthic treatment and also because the stool examination is not sensitive enough to detect continuous chronic infection after unsuccessful treatment. In the previous study, the authors have demonstrated a significant decrease in ELISA antibody levels a year after treatment, indicating that the serologic testing is useful for postchemotherapy evaluation (Kobayashi *et al.*, 1993).

In the present study, the antibody responses specific to *Strongyloides* were qualitatively compared before and after treatment by the immunoblotting method to identify the antigenic component which may be useful for serological evaluation of successful treatment.

## MATERIALS AND METHODS

### Patients

Thirty-three individuals were found to be harboring the parasite at a mass screening by stool examination in Sashiki Town, Okinawa Prefecture, Japan. They were 12 males and 21 females ages 49 to 76 years (mean=68.3 years). Two months after the initial diagnosis, they were treated with pyrvinium pamoate at a dosage of 5 mg/kg daily for 3 days and subsequently received follow-up faecal examination 12 months after the treatment to determine the effectiveness of the therapy.

### Experimental group

Out of the 33 individuals treated, stool examination revealed that 10 had not been cured. The remaining 23 were negative in the follow-up examination, but 6 of them were considered to be equivocal for complete cure because their antibody titers did not significantly decrease after treatment. The immunoblotting patterns before and after treatment were analyzed on the 3 groups of therapeutic efficacy.

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### Stool examination

Stool examination after treatment was performed daily for 3 consecutive days by three different methods; direct smear, formalin-ether concentration method and faecal culture with an agar-plate (Arakaki *et al.*, 1988). The details of the follow-up faecal examination were the same as in the previous report (Kobayashi *et al.*, 1993).

### Anti-*Strongyloides* antibody

Serum antibodies to *S. stercoralis* were compared before and after treatment to assess serologically the efficacy of treatment. Three serum samples were collected from each patient on the same times of their diagnosis, treatment and follow-up examination. The antigen used was prepared from *S. stercoralis* filariform larvae collected from faeces of strongyloidiasis patients and the antibodies were measured by an enzyme-linked immunosorbent assay (ELISA) (Sato *et al.*, 1985). The sera were tested at a single dilution of 1 : 50 and the intensity of antibody response was measured as the absorbancy (OD) at 500 nm. The change in antibody titer after treatment was expressed as an antibody ratio against antibody value before treatment, calculating by division of the antibody values at the follow-up examination by those at the diagnosis. On the basis of a previous study (Kobayashi *et al.*, 1993), the individuals with an antibody ratio of over 0.6 were interpreted to be equivocal for effective treatment, regardless of negative results in the follow-up faecal examination.

### Immunoblot analysis

The polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) was carried out with 10% polyacrylamide slab gels (Laemmli, 1970). Samples for electrophoresis were applied at a protein concentration of 1.0 mg/ml in the presence of 5% 2-mercaptoethanol (2-ME) and electrophoresis was performed at a constant voltage of 120 V for 4 hr.

After electrophoresis, the proteins separated were transferred electrophoretically to a nitrocellulose membrane (Towbin *et al.*, 1979). The blotted membrane was

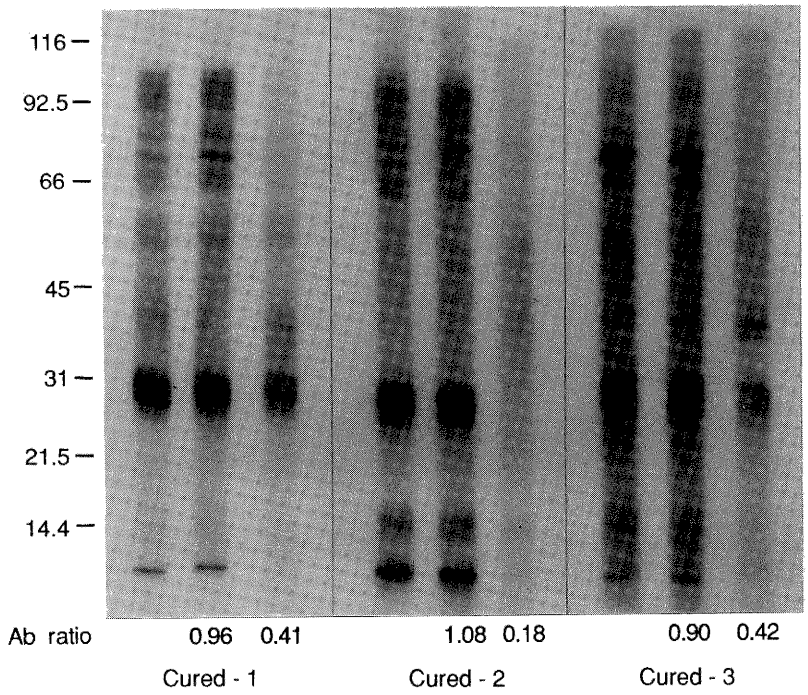


Figure 1 Immunoblotting patterns in three patients who were coprologically and serologically considered to be successfully treated. Three serum samples were collected before and after treatment and the immunoblotting patterns of the sera were compared. For each patient, the left-hand lane shows the pattern of serum samples collected when first diagnosed, the middle lane shows the pattern during treatment and the right-hand lane shows the pattern at the follow-up examination. The numbers at the bottom of each lane show antibody ratio (Ab ratio) against antibody value at the initial diagnosis. The position and molecular weight (KDa) are represented at the far left.

then treated with a 1/10 dilution of patients' sera for 2 hr at 37°C and subsequently overnight at 4°C. The IgG antibodies which reacted with antigens on the membrane were detected by autoradiography using $^{125}I$ -labelled protein A. The above method was described in detail in the previous report (Sato *et al.*, 1990). Autoradiograms were scanned with a Dual-Wavelength Flying-Spot Scanner (Shimazu CS-9000; Shimazu Co. Ltd., Tokyo, Japan). The area values for each peak were calculated by a personal computer (IBM PC-AT) linked to the densitometer.

## RESULTS

Immunoblotting patterns before and after successful treatment in three patients are illustrated in Fig. 1. The patterns of antibody response in serum samples collected at their initial diagnosis could not be distinguished from those at the time of treatment. The reactivities, however, decreased significantly after treatment in all of the patients. Especially, the positive bands before treatment disappeared almost completely after treatment in a case (Cured-2) in which antibody ratio was only 0.18 after treatment. In the remaining two cases, positive reactivities remained to the bands in the region of relatively low molecular masses from 20 to 30 KDa where strong reactions were detected before treatment. On the other hand, no significant difference of pattern was observed in 6 patients who were interpreted to be equivocal for complete cure. The difference in reactivity could also not be recognized in 10 patients who were unsuccessfully treated. The representative patterns of reactivity before and after treatment in an equivocal and an unsuccessful cases are shown in Fig. 2.

Densitometric trace of autoradiograms in Figs. 1 and 2 are shown in Fig. 3 and 4. As seen in Fig. 3, some peaks in the relatively high molecular weight region were found to decrease significantly or to disappear after treatment. Total area values of peaks detected decreased 66% after treatment in the Cured-1 patient, as much as 85% decrease in the Cured-2 patient and 48% decrease in the Cured-3 patient, as compared to the area values before treatment. These results were well consistent with those of the ELISA assay, in which the ELISA values are 59% decrease in the Cured-1, 82% decrease in the Cured-2 and 58% decrease in the Cured -3. On the other hand, the densitometric patterns before and after treatment did not change so much in an equivocally cured patient and in a patient of unsuccessful treatment (Fig. 4). The total area values of these cases were only 25% decrease after treatment in the

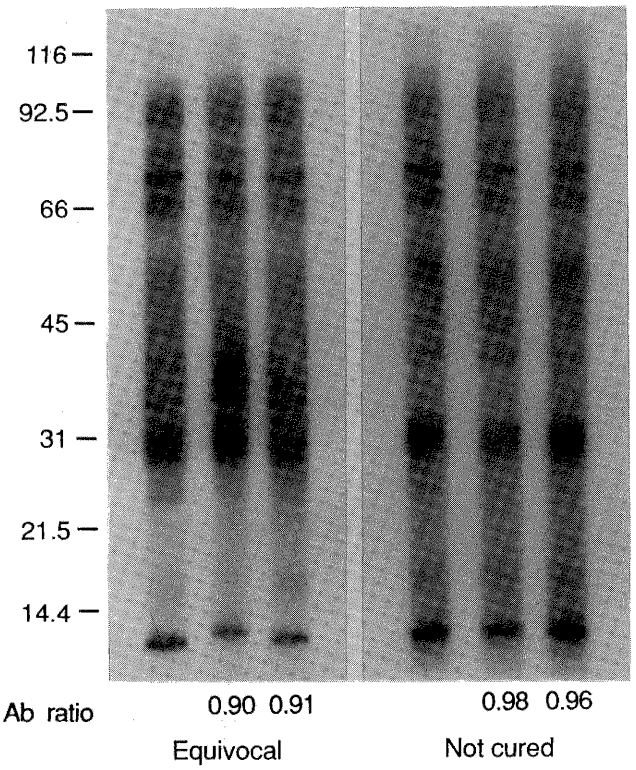


Figure 2 Immunoblotting patterns of patterns of two patients. Three patterns with serum samples collected at the initial diagnosis, treatment and follow-up examination are compared for a patient who was negative in the follow-up faecal examination but serologically equivocal for complete cure (Equivocal), and for a patient who was unsuccessfully treated (Not cured). The other remarks are the same as in Figure 1.

equivocal case and contrarily 8% increase in the case of unsuccessful treatment.

The immunoblotting patterns before and after successful treatment in the remaining 14 cases are represented in Fig. 5. The patterns of reactivity varied considerably for each patient; some bands differed in their intensity and some were absent. The reactivities decreased noticeably after treatment in all but two patients (Cases 5 and 12) whose antibody ratios were 0.58 and 0.56, respectively. The patterns shown after treatment also displayed significant diversity among the patients and it was difficult to determine which bands generally became negative in response with antibodies after successful treatment.

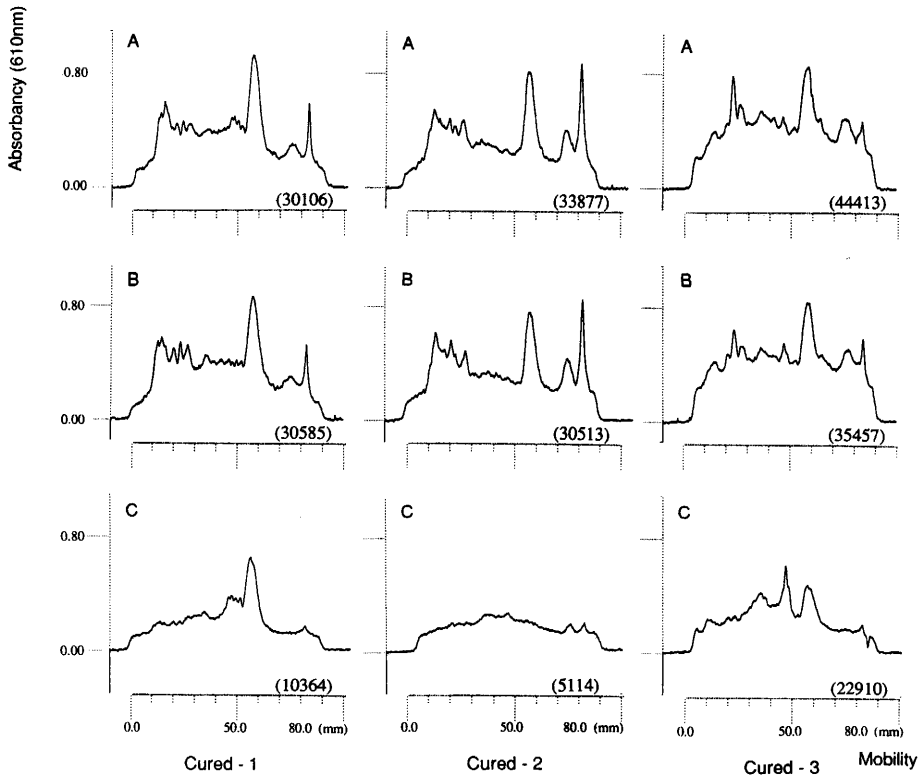


Figure 3 Densitometric patterns of autoradiograms in Figure 1. For each patient, the patterns at their diagnosis(A), treatment (B) and follow-up examination (C) are shown. The number in parentheses are a total area value of peaks detected.

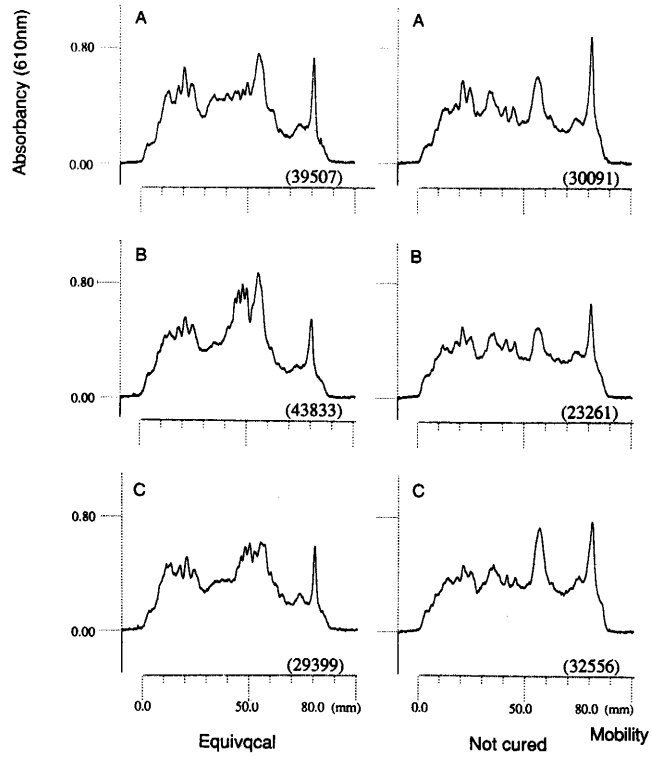


Figure 4 Densitometric patterns of autoradiograms in Figure 2. The remarks are the same as in Figure 3.

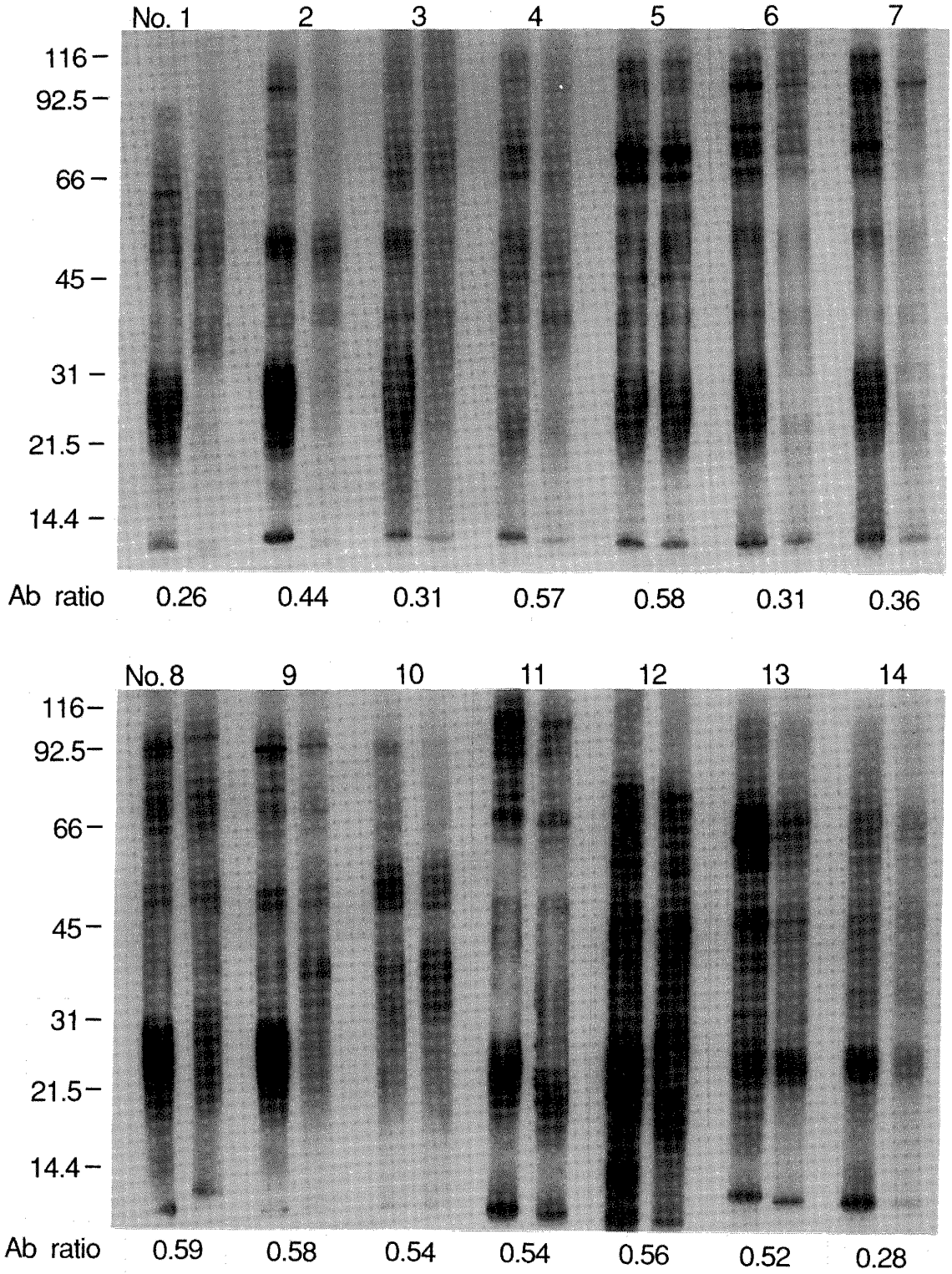


Figure 5 Immunoblotting patterns of 14 patients who were successfully treated. For each patient, the patterns of response at the initial diagnosis (before treatment) are shown on the left lane and those at the follow-up examination (after treatment) are on the right lane. The numbers at the bottom of each lane represents the antibody ratio after treatment. The numbers at the far left represent the position and moleculam weight (KDa) of marker proteins.

## DISCUSSION

Postchemotherapy evaluation of strongyloidiasis is an important clinical problem because strongyloidiasis patients frequently fail to respond to chemotherapy and especially because it is difficult to confirm successful treatment by coprological examination. In a previous study in which 123 persons with chronic *Strongyloides* infection were followed for several months without any treatment, faecal larvae were reconfirmed in only 15% of them by the direct smear, 24.4% by faecal concentration method and 16.7% by faecal culture (Harada-Mori faecal culture), if the faecal examinations were performed only once (Sato *et al.*, 1993). At the present time, several samples collected on different days ought to be examined repeatedly to obtain a correct diagnosis and even after repeated examinations, failure to demonstrate larvae cannot be unequivocally interpreted as absence of the infection (Jones and Abadie, 1954; Grove, 1980). Thus, the postchemotherapy evaluation may be more difficult, if complete cure is not achieved and the infection progressed to a latent infection after treatment.

In Okinawa, where there are many patients with concurrent HTLV-I infection, an unnegligible number of the patients progress to the severe, often fatal, hyperinfection state due to the depressed immune competence condition caused by the viral infection (Nakada *et al.*, 1984, 1987; Takara *et al.*, 1987; Sato & Shiroma, 1989). Most recently, patients with concurrent viral infections were found to be intractable to chemotherapy for strongyloidiasis (Takara *et al.*, 1992; Sato *et al.*, 1992). Therefore, an effective therapy and exact evaluation following the therapy are necessary to prevent such a severe infection.

In the previous study, the authors monitored the changes in the ELISA antibody levels against *Strongyloides* before and 12 months after pyrvinium pamoate treatment and demonstrated that the antibody levels significantly decreased after treatment in patients who become nagative for faecal larvae by the treatment. Among the patients, however, the antibody response did not significantly decrease in about 40% of them after treatment, suggesting that they might be equivocal for complete cure. When further faecal examination was performed on the equivocal cases, about 20% of them were additionally found to be unsuccessfully treated (Kobayashi *et al.*, 1993). In the previous study, Grove (1982) also followed 43 patients for 6 months after thiabendazole treatment and detected a significant decrease in anti-*Strongyloides* antibody levels in many patients who were negative in the follow-up faecal examination. However, on the basis of clinical and serological observations he considered that perhaps one -third of the patients were still infected with the parasite. These results indicate that the serological testing may effectively complement faecal examination in the postchemotherapy evaluation.

In the present study, the authors further compared qualitatively the antibody responses before and after treatment by the immunoblotting method to determine the antigenic component to which positive response predominantly diminish after treatment. The reactivities to the antigenic bands significantly decreased after treatment in almost all of the patients successfully treated. Whereas, the reactivities after treatment did not differ from those before treatment in the patients who were equivocal for complete cure, as well as in patients who were unsuccessfully treated. The authors have shown in a previous study in which antibody responses in human strongyloidiasis were analyzed by immunoblotting method, that the patterns of antigenic recognition by patients' antibodies varied significantly for each patient (Sato *et al.*, 1990). In the present study, the immunoblotting patterns also varied considerably for each patient and due to the diversity in the reactivity, it was difficult to identify the antigenic component which will be useful for serological assessment of successful treatment. At the present time, it should be better to assess the responses to whole extract before and after treatment for the effective evaluation of chemotherapy.

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## 症例報告

# 大分県で発症を見た人体肺犬糸状虫症の一例，特に虫体の形態学的特徴について

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平成5年6月22日受付/平成5年7月15日受理

## はじめに

本来犬の寄生線虫である犬糸状虫 *Dirofilaria immitis* による人体寄生例はこれまで米国南部をはじめ世界各地から報告されている。我国からは，Nishimura *et al.* (1964) により本寄生虫の第一例が発見されているが，真喜屋 (1990) の集計によると1990年5月の段階で85例に達する。これらの多くは近年になって報告された症例である。犬糸状虫の人体症例では無症状の場合が多く，大半は肺寄生であるが，肺以外に寄生する例（主に皮下に寄生し腫瘤を形成する）も知られている（吉村，1988)。肺寄生の場合は，胸部X線撮影によりいわゆる銭型陰影 coin lesion を呈することが多く，開胸手術によって摘出された病理組織から寄生虫の横断像が認められることから，本症と診断されることが少なくない。肺組織中に見いだされる犬糸状虫は，成虫またはそれに近い段階まで発育している場合もあるが，多くは未成熟虫である。また，しばしば変性過程にあって正常の虫体と形態が異なることがある。虫体の組織や形態の保存性が良ければ，角皮の形状などの特徴によって同定が可能であるが，虫体の切断面や変性の度合などによっては形態学的に同定することが困難な場合もみられている（例えば，吉田ら，1984)。

本症例は，これまでの多くの症例と同様に，胸部X線写真の coin lesion に端を発し，術後肺組織中に見いだされた虫体によって診断された典型的な肺犬糸状虫症である。本例では，病変部の肺組織から約25mm の長さに切断された虫体自体が得られた他，肺病変の連続切片標本に虫体の食道部位を含む前方部分と腸部位を含む中間の部分の横断像が得られたので参考に供したい。

## 症　例

患者：39歳。日本人女性。大分県臼杵市在住。主婦。家族歴および既往歴には特記すべきことなし。海外渡航歴なし。犬猫などのペットは飼っていない。

現病歴

1992年8月頃，右前胸下部に軽い鈍痛を覚えたが，2～3日で軽快したため放置。同年9月下旬に行われた住民検診において胸部X線撮影に異常陰影を指摘される。検査のため，同年11月18日国立大分病院外来を受診。初診時，自覚症状なし。胸部理学所見も異常なし。

胸部X線撮影で，右肺中葉に比較的輪郭の明瞭な浸潤陰影（Photos. 1 & 2）が存在した。経気管支肺生検では，慢性気管支炎の所見はあるが，悪性腫瘍の所見は認められなかった。

精密検査の目的で同年12月17日同病院に入院，入院時，身体所見異常なし。血液，尿の生化学検査異常なし。WBC 7180。EOS 4.1%。IgG 1480。IgM 231。IgE 検査せず。ツ反17X16mm。CRP 0.36mg/dl。治療のため抗生剤｛セフォペラゾン・スルバクタム（1：1）2g+クリンダマイシン1200mg／日，7日間｝およびステロイド剤（コハク酸ヒドロコルチゾンナトリウム400mg／日，4日間）投与するも変化なし。

1993年1月18日，同病院にて右肺中・下葉部分切除手術。手術所見：右肺中葉末梢側に約2 cm 大の肉芽腫様病変を認めた。病変の内部は乾酪壊死様に変性。肺胸膜は一部壊れ，中・下葉間に限局した腔洞が形成され，隣接する下葉の一部にも肉芽腫様病変が認められた。中・下葉の病変部を一塊として切除。

同年1月31日退院。

同年1月29日および3月5日に採血した血清は免疫電気泳動法により犬糸状虫抗原に陽性を示した。

病理学的所見

病変部より得られた切片を H・E 染色および EVG 染色により観察した。主病変は壊死組織と虫体の混在であり，その周囲には，線維組織の増生，炎症細胞の浸潤を認める。EVG 染色では，この病変の周囲には弾性線維が輪状に配列する (Photo. 3)。すなわち，上記病変は，虫体による動脈

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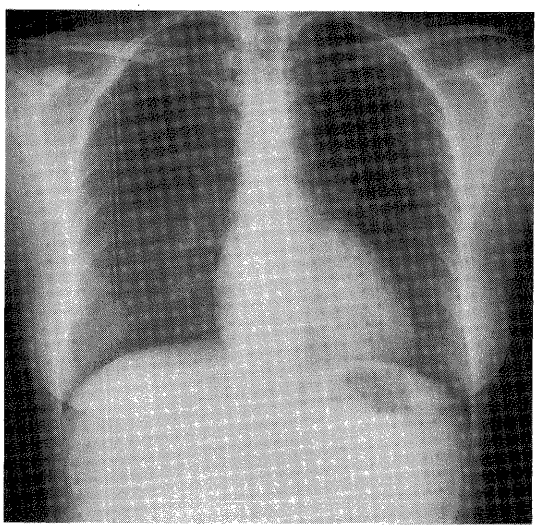
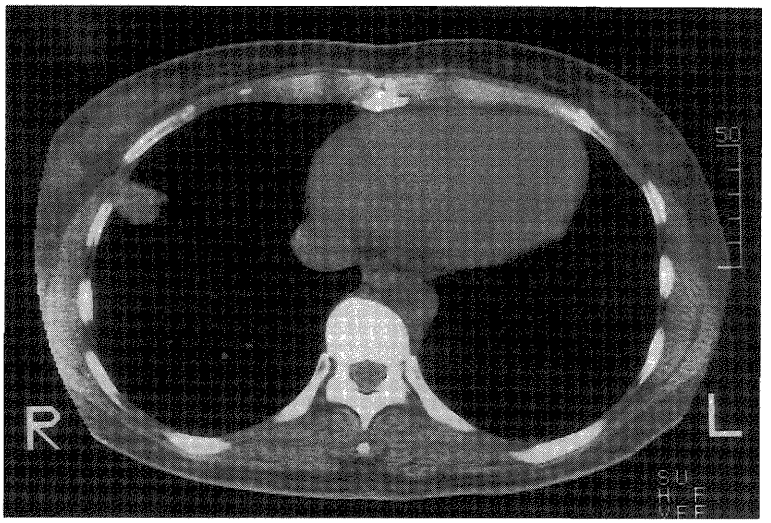
3　国立大分病院呼吸器科（〒870-02 大分県大分市大字横田1000番地1）






Photos. 1 and 2. Preoperative posteroanterior rentgenogram (1) and computed tomogram (2) showing a coin lesion in middle robe of the right lung.

塞栓である。一つの動脈あたり1～3個の虫体断面が認められた。

寄生虫学的検索

病変部から取り出された虫体は切断された4つの部分からなり，総計約25mmの長さで，直径約0.670mmの細い紐状を呈した（Photo. 4）。実体顕微鏡下では虫体は乳白色で，ほぼ全長を通じて内部に黄褐色の縦縞が透けて見えた。ラクトフェノール液で透徹し普通顕微鏡で観察した結果，体壁は厚い角皮（27～47$\mu$m）からなり，外側の表面は平滑で，5～12$\mu$mの間隔で平行する横紋線 transverse striation が認められた（Photo. 5）。また，角皮には互いに直角に交わる2方向に斜走する線維状構造が見られた（Photo. 5）。切口の横断面の観察では，角皮の内側に部分的に剝離した筋肉層と偽体腔中に腸管（直径約90$\mu$m）と2個の生殖管（直径約0.320mmおよび0.250mm）が存在した。側索は変性し，輪郭が不明であったが，側索部の角皮の内層に，縦走する隆起 internal longitudinal ridge（以下，i. l. r. と

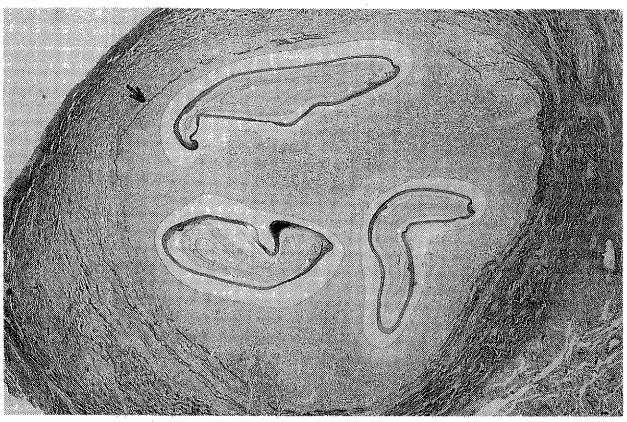


Photo. 3. Obstructed pulmonary artery showing three sections of worm, surrounded by proliferated fibrous tissue. Arrow indicates elastic fibers of the the pulmonary artery (EVG-staining). Scale, 500$\mu$m.

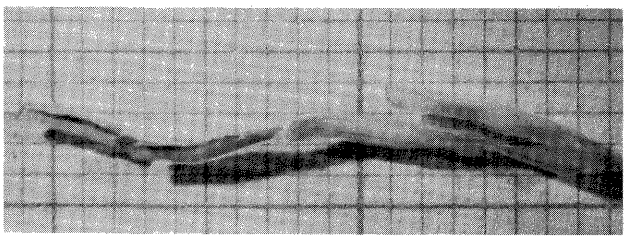


Photo. 4. *Dirofilaria immitis,* portions of midbody of an adult female worm taken from the vessels of the resected pulmonary tissue. Scale, 1mm.

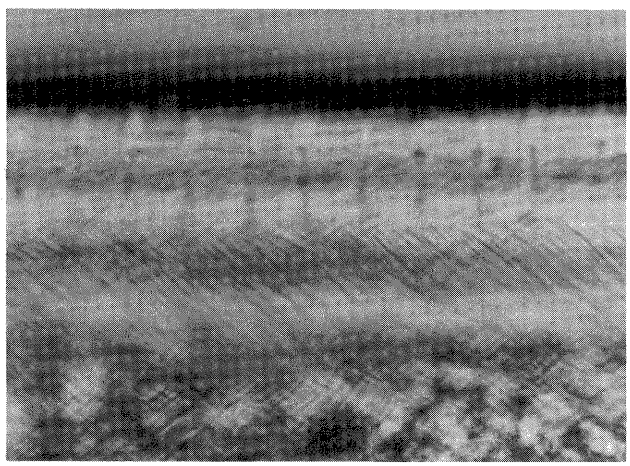
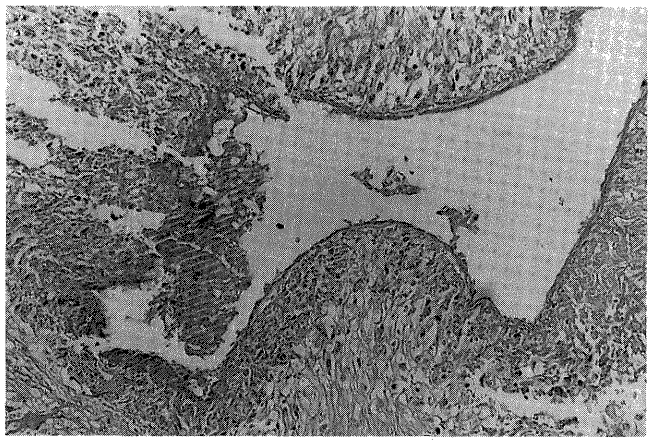


Photo. 5. Enlargement of the body wall of the adult female worm (Photo. 4) showing transverse striations on the smooth outer surface of the cuticle and fibrous layers of cuticle crossing at right angles. Scale, 20$\mu$m.



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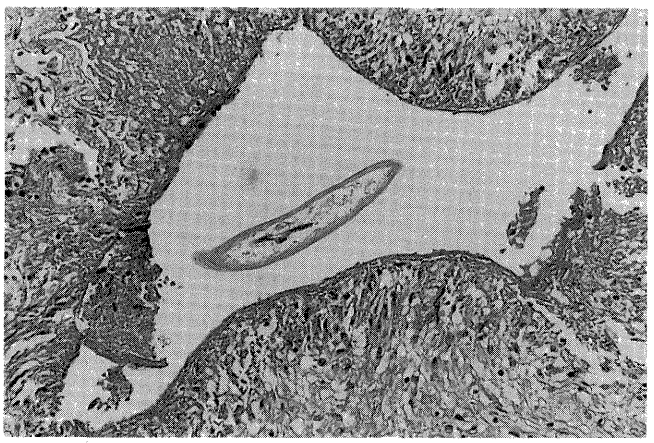

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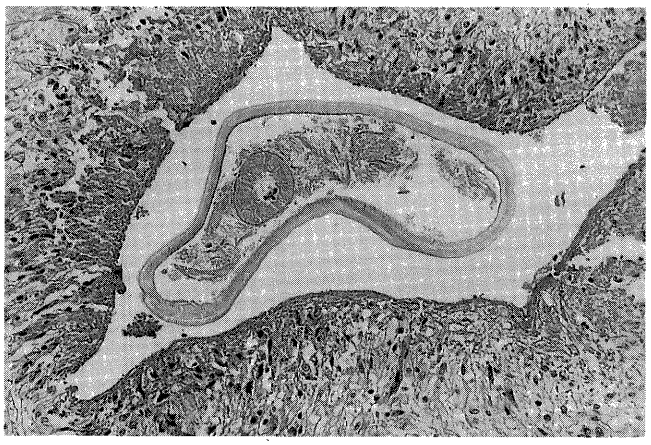

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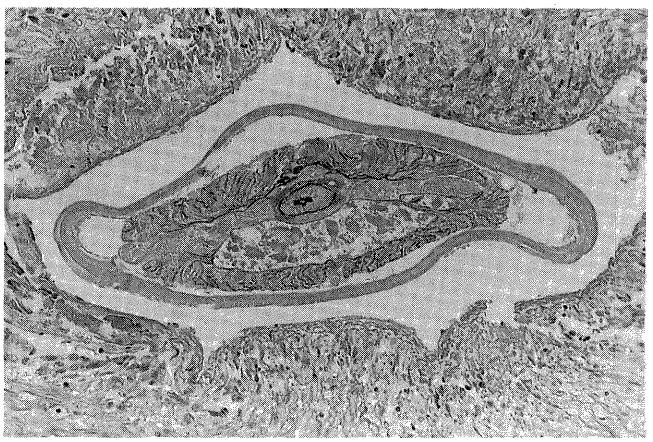

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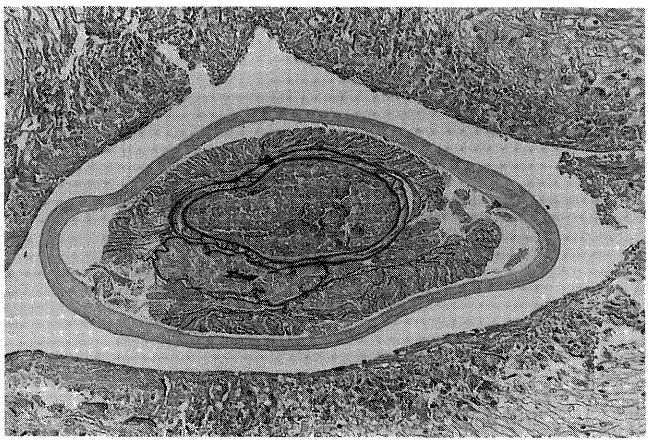

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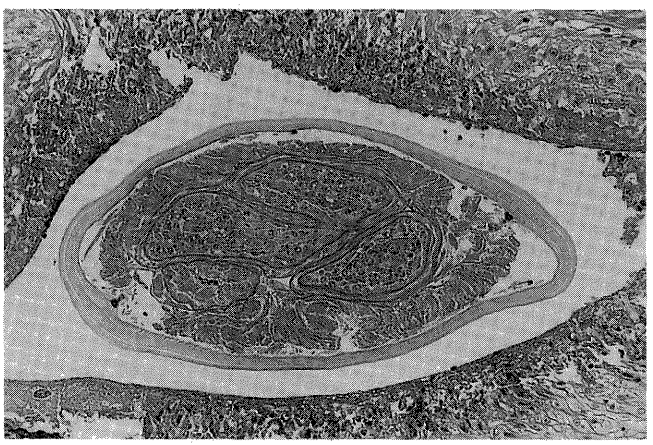

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Photos. 6—11. *Dirofilaria immitis,* transverse sections of adult male in the pulmonary artery. 6, At anterior tip, showing transverse striations of the smooth cuticle. 7, Near anterior end. 8, Between anterior end and nerve ring. 9, At level of nerve ring, showing small dark spots at the internal longitudinal ridges of the cuticle, and distinct dorsal and ventral muscular layers. 10 and 11, Between the nerve ring and esophagus-intestine junction, showing distinct dark spots at the internal longitudinal ridges, and one or two reproductory tubes, as well as layered cuticular body wall, and muscular layers. Scale, 100$\mu$m.

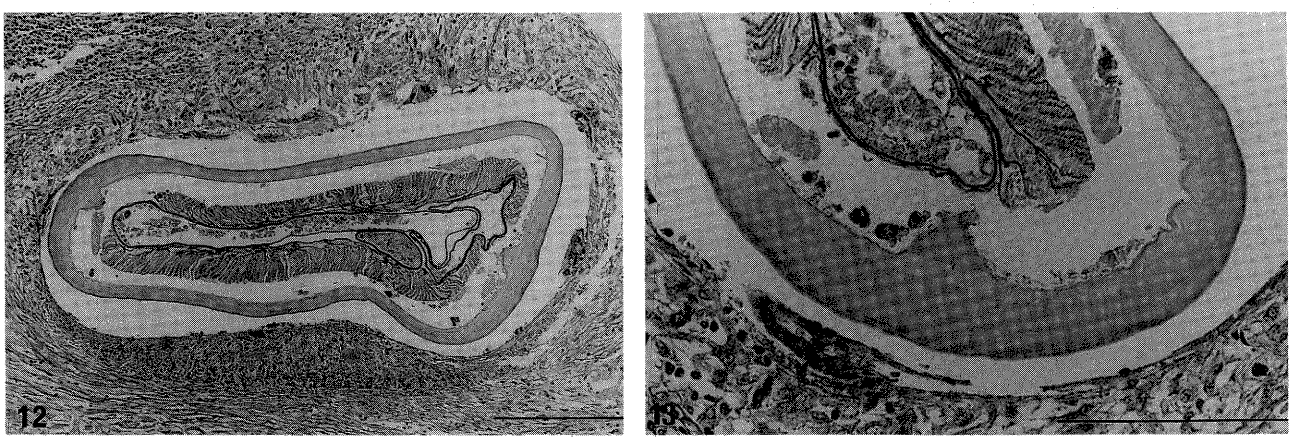
略す）が認められた。

病理観察のための切片標本とは別に，肺病変部の一部を5 μmの厚さで連続切片を作製し，2つの血管に存在した虫体の断面像をH・E染色およびPAS染色で観察した。第1の血管内の虫体像は，虫の前端部から食道の部分に相当し，第2のものは，食道・腸分岐部より後方の一部分と思われる。第1の食道部を含む虫体部分は切片標本で71枚，また第2の中腸部を含む部分は83枚について観察できた。

第1の虫体は前端から約0.355mmに相当し，横断像は部位により違いが見られた。最初の11枚の標本では，横紋をもった角皮のみが見られた（Photo. 6）。虫体の横断像は12枚目から出現したが，17枚目までは断面の直径も小さく（約0.130mm），角皮も約4 μmと薄かった（Photo. 7）。内部には食道内壁をなすと思われる角皮が濃染して認められた。筋細胞やi. l. r.は見られなかった。18枚目で虫体断面の直径が0.330mmと急に大きくなるが，後は徐々に大きさを増し，最大0.400mmに達した。食道は18枚目から厚い筋質部で囲まれた管状を呈する（Photo. 8）。内腔は狭く，三放射対称をなすが，すぐに背腹に偏平で不整形になった。食道断面の直径は64～76μmであった。29枚目前後に食道を囲んだ神経輪が観察された（Photo. 9）。また，側索は全体にわたって不明瞭であったが，神経輪およびその前後の部位では中心部に向かって突出していた（Photo. 9）。角皮は3～4層から成り，10～30μmの厚さであった。i. l. r.は20枚目から微かに認められるようになるが，71枚目でもそれほど顕著ではなかった。ただ，神経輪より後方ではi. l. r.に濃く染まるスポットが現れるので，位置の確認は容易であった（Photos. 10 & 11）。体壁筋層は側索部を境に背腹に二分され，ほぼ等しい高さの多数の細い細胞からなる，いわゆる多筋細胞型polymyarian-coelomyarian musculature typeを示した。しかし，神経輪より前方では個々の筋細胞の区別は出来なかった。後方の虫体断面では4分の1区画当たり35から44の筋細胞が認められた。生殖管は41枚目に1本の大きな管（直径0.180mm）として出現し（Photo. 10），47枚目で2本となり（Photo. 11），49枚目から61枚目では5～8本となった。また，それ以降は3本の断面として認められた。生殖管はPAS染色でよく染色される2重の管壁からなり，内部は生殖細胞で充満していた（Photos. 10 & 11）。第2の虫体部分はどの標本でも，厚い角皮，発達したi. l. r.，多数の筋細胞，1本の腸管および2本の生殖管が認められた（Photo. 12）。側索や背腹の中央線は一部を除いて不明瞭であった。虫体断面の直径は大部分が0.670～0.680mmであったが，最後の10枚の標本では0.720～0.891mmと幾分大きくなっていた。角皮は20～55μmの厚さで4層からなり，中2層は特に厚く斜走構造が認められた。両側索に対応する部位の角皮が最も厚く，その内層から概ね二等辺三角形をなしてi. l. r.が15～27μmの高さで内側に突出していた（Photo. 13）。一部の標本ではi. l. r.が角皮内層から遊離したり，全くなくなっていた。第1の虫体部分の標本で認められたi. l. r.の濃染スポットは見られなかった。筋細胞数は4分の1区画で52～75であった。腸管は断面の直径が70～85μmと小さく，内部は非細胞物で詰まっていた。2本の生殖管は大きさが異なり，断面の直径は，大きい管で0.350～0.450mm，小さい管で0.245～0.320mmであった。2つの生殖管の大きさの比は1.2～1.7と虫体の部位によって違いがあった。生殖管は管壁が厚さの異なる2重の層からなり，内腔は大部分が中空で，8～10μmの大きさの楕円形をした生殖細胞が散在していた。

病理観察に用いた標本に見られた虫体の断面像（Photo. 3）も，大きさ（直径約0.700mm）および内腔に2つの生殖管と1つの腸管を持つ点，基本的には上記の第2の連続切片に見られた虫体部分と同じ形態であった。

第2の虫体部分の横断面に見られる層状の厚い角皮，角皮内の斜走繊維構造，角皮内層に発達したi. l. r.，および多筋細胞型の筋層，さらに摘出された虫体部分に見られた




Photos. 12 and 13. *Dirofilaria immitis*, transverse sections of adult female in the pulmonary artery. 12, Through midbody, showing thick cuticular body wall with marked internal longitudinal ridges, two reproductory ducts, intestine, and muscles. Scale, 200μm. 13, Portion of transverse section of body wall, showing four-layered thick cuticle with a distinct internal longitudinal ridge. Scale, 100μm.

平滑で横紋線を有する角皮の表面などの形態的特徴から犬糸状虫 *Dirofilaria immitis* と同定した。我国からは別の犬糸状虫 *D. repens* の人体例が1例だけ沖縄から報告されているが（MacLean et al., 1979），この種は角皮の表面に多数の縦走する隆起列があるので本症例の虫体とは異なる。また *D. repens* の場合，人の皮下に寄生する例がほとんどであり，我国の犬や猫からはまだ見いだされていない（吉田ら，1984）。我国の熊には *D. ursi* の寄生が知られているが，*D. repens* 同様，角皮表面の縦走隆起を持つ点より，今回の虫体とは区別される。今回の虫体は断面の大きさと大きな生殖管を備えることから，既に成虫にまで発育していたものと思われる。また，断面に腸管を持っていた摘出虫体部と連続切片にした第2の虫体部分は大きな生殖管を2本持っていることから雌であると思われる。一方，虫体断面に食道が見られた第1の虫体部分は神経輪のすぐ後方に2本以上（最大8本）の生殖管が認められることなどから，雄の虫体と思われる。すなわち，神経輪のすぐ後ろで，後方から伸びてきた1本の輸精管がUターンし，その先端部分の精巣が何度か折り重なり合っている状態が推測される。

## 考　　察

本症例は，胸部X線検査により陰影が指摘され，術後摘出された肺組織内の虫体から診断された人体肺犬糸状虫症である。病理学的には，病変は虫体による動脈塞栓であった。大分からは，血清診断による人体肺犬糸状虫症が1例報告されているので（明石ら，1983），今回の症例が2例目であるが，前述したように，我国における犬糸状虫による人体寄生例，特に今回の症例に似た肺への寄生例の報告が近年増加しているので（真喜屋，1990），人獣共通寄生虫症の一つとして注目する必要がある。犬糸状虫が肺に寄生した場合，肺癌との鑑別が重要であるが，胸部X線で陰影が見つからない腫瘤摘出例も報告されており（真喜屋ら，1988），開胸術前に免疫学的診断法などだけで確定診断を行うことは困難である（吉村，1988）。

手術によって摘出された肺組織中の寄生虫の種の鑑別に関しては，Beaver and Orihel (1965)，Orihel and Beaver (1965) および Neafie and Piggott (1971) などに詳しく検討され，横断面で他の糸状虫から *Dirofilaria* 属を同定する基準も指摘された。これらの形態的特徴の一つとして側索基部に突出した i. l. r. の存在が重要視されている。今回の虫体の横断面では，ほとんどの標本で顕著な i. l. r. が認められたが，虫体の前端部近くでは全くないか，あってもそれほど発達していなかった。また，後方の断面像で腸管が見られる部位の一部の標本では，発達した i. l. r. が離れかかったり，完全に離れてなくなっていた。これは，成長した虫体でも部位によって i. l. r. の発達の度合に差があること，また虫体の変性過程が進めば i. l. r. がなくなる場合もあることを示唆しており，少ない断面像から *Dirofilaria* 属を同定するような場合には留意すべきであろう。また，食道を含む虫体の幾つかの切片標本において，i. l. r. の位置に H・E および PAS 染色で濃く染まる部位が認められた。このような性質は *D. immitis* だけに特異的なものかどうかは今後検討する必要がある。

犬糸状虫の宿主である犬における感染は北海道から沖縄まで全国的に見られ，九州では平均25.3%の感染率が示されている（真喜屋，1990）。犬から犬，および犬から人への媒介は蚊によって行われ，アカイエカ *Culex pipiens pallens*，ヒトスジシマカ *Aedes albopictus*，コガタアカイエカ *Culex tritaeniorynchus* など数種が重要な媒介種である（末永，伊藤，1973）。本症例の発生した臼杵市を含め，大分県における犬の感染状況や媒介者についてはまとまった資料がなく，今後の調査を待たなければならない。ただ，大分市で1985年に我々が行った予備的調査では，検査した8頭のうち4頭の犬が *D. immitis* の仔虫を血中に保有しており，また1988年9月に人囮法で採集したヒトスジシマカからも低率（279雌個体中1個体陽性または0.4%）ではあるが *D. immitis* の幼虫が検出されているので（未発表），人への感染が起きる状況は十分整っていると思われ，予防の面で注意を喚起する必要がある。

## 結　　語

大分県臼杵市において発症をみた人体肺犬糸状虫症の一例を報告した。本症例は，胸部X線撮影で右肺中葉下部に銭型陰影を認め，術後，肺動脈より摘出された虫体によって診断されたものである。本報告では，摘出虫体の他に，組織切片標本中に種々の虫体断面像が比較的多数得られたので，犬糸状虫の形態学的特徴についての検討もおこなった。

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**Case Report**

# A HUMAN CASE OF PULMONARY DIROFILARIASIS IN OITA WITH SPECIAL REFERENCE TO MORPHOLOGICAL CHARACTERS OF THE CROSS-SECTIONED WORM

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A Japanese woman, 39, in Oita, Japan who showed a "coin lesion" in the lung on a routine X-ray examination, though asymptomatic, was diagnosed as a pulmonary dirofilariasis due to *Dirofilaria immitis* by the morphological characteristics of cross-sectioned worm specimens in the resected tissue. Some comments on an internal longitudinal ridge of the worm's cuticle, one of key characters for identification of the genus *Dirofilaria*, were given.

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