

Trichobilharzia brevis Basch, 1966, as a Cause of an Outbreak of Cercarial Dermatitis in Japan

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In 1967, an endemic dermatitis of unknown etiology occurred among farmers working in paddy fields in Saitama Prefecture, an eastern district of Japan (Ozu *et al.*, 1968, 1972).

The dermatitis exhibited clinical manifestations quite similar to the already known avian schistosome dermatitis. It was characterized by erythematopapulo-vesicular eruptions accompanied with severe itching on the parts exposed to the water, especially the legs and arms.

In order to ascertain the cause of the dermatitis, the vector snails for the avian schistosome were investigated in the endemic areas. As a result, a species of apharyngeal furcocercous cercaria was found from *Austropeplea ollula* (Synonym: *Lymnaea ollula*) collected in paddy fields. Experimental infections with the cercariae on the forearm skin of the present authors were made and exactly the same symptoms resulted as those paddy field dermatitis prevailing in the endemic areas.

The present study was undertaken to identify the species of the cercaria based on the

morphological and some biological characteristics of developmental stages by working out the life cycle of this avian schistosome in the laboratory.

Epidemiological considerations in Saitama Prefecture

In 1967, 38 cases of the dermatitis were reported from the areas under the Kounosu Health Center and 40 cases from the Oomiya Health Center, Saitama Prefecture (Table 1). In the early summer of the same year, 7,333 *Austropeplea ollula* and 6,293 *Physa acuta* were collected from the paddy fields in the endemic areas and held for 24-hours to check the shedding of cercaria, and no cercaria belonging to Schistosomatidae was found (Table 2). In 1968 and 1969, a total of 395 and 533 cases of the dermatitis were reported in the prefecture.

In the same years, many snails of *A. ollula* were examined for the avian schistosome cercariae, with a positive result of 25 infected snails out of 4,102 in 1968, and 1 out of 1,785 in 1969.

In 1970, 122 cases of the dermatitis were found in 3 localities. Among the 2,709 *A. ollula* examined, the same cercariae were found in 2, but avian schistosome cercariae were found in none of the 2,990 *P. acuta* (Suzuki *et al.*, 1973). In 1971 there were

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Table 1 Reported cases of cercarial dermatitis in Saitama Prefecture

Localities*	Year				
	1967	1968	1969	1970	1971
Oomiya	40	75	45		
Kounosu	38	75	51		
Kasukabe		69	42	20	
Gyoda		8	10		22
Kazo		127	220	100	50
Yoshikawa		114	150	2	80
Satte		2	15		
Kawagoe		45			
Total	78	395	533	122	152

* Localities are indicated by the name of the Health Center.

Table 2 Incidence of apharyngeal furcocercous cercariae in *Austropeplea ollula* in Saitama Prefecture

Localities*	Year				
	1967	1968	1969	1970	1971
Oomiya	0/7,333	25/4,102			
Kounosu				0/1,523	
Kasukabe					6/2,479
Gyoda		0/490	2/1,186	0/1,290	
Yoshikawa					3/1,805
Satte		1/922			
Kawagoe		0/373			
Total	0/7,333	25/4,102	1/1,785	2/2,709	9/5,574

* Localities are indicated by the name of the Health Center.

152 cases reported and the avian schistosome cercariae were revealed in 9 *A. ollula* of the 5,574 examined.

Materials and Methods

The cercariae obtained from *A. ollula* of an endemic area in Saitama Prefecture were given to domestic ducklings. The birds were left for about 15 minutes in water of a few centimeters of depth which contained the cercariae collected from several snails. A total 300-1,000 cercariae were used for each bird.

Embryonated eggs which belong to an ocellata group were initially detected in the feces of ducklings 17, 18 and 19 days after contacting the cercariae. The miracidia were examined unstained or stained with neutral red. Silver nitrate was also used for detecting the epidermal plates.

Young laboratory-raised snails of 4-5 weeks old were exposed to miracidia. The snails were put individually into the dish with 3 miracidia and left for about 10 hours under an electric light.

Cercariae from experimentally infected snails as well as from naturally infected ones were observed alive, and then fixed in hot 10% formalin for measurements.

The adult worms were obtained mostly from the mesenteric veins around the intestine of the infected birds. The birds were killed soon after an injection of 1% sodium citrate through the heart to prevent blood coagulation. Although it was difficult to obtain uninjured specimens, as the flukes are fine and fragile, 2 almost complete male worms and several fragments of males and females could be obtained from a total of about 30 birds.

Results

Egg

The egg is crescent-shaped, with a thin colorless shell. Measurements of 17 mature eggs obtained from an experimentally infected duckling were as follows: the average width $44.9 \pm 1.5 \mu\text{m}$, ranging from 39.1 to $50.0 \mu\text{m}$; the average length $197.3 \pm 11.0 \mu\text{m}$, ranging from 178.3 to $219.6 \mu\text{m}$. One end is blunt and the other finely pointed. The miracidium is clearly seen within (Fig. 1).

Miracidium

The miracidium, is cylindrical and highly contractile and swims very rapidly.

There are two pairs of flame cells, one located at the level of the penetration glands and another at about one-third of the body length from the posterior end (Fig. 2). The germ cells occupy the major portion of the posterior half of the body. They are con-

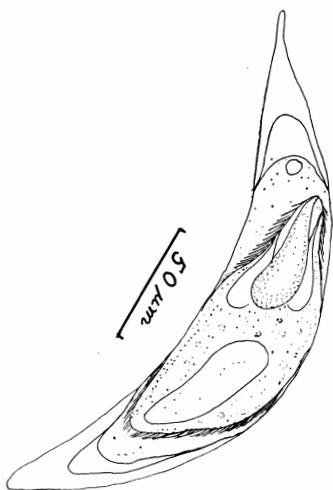


Fig. 1 Egg.

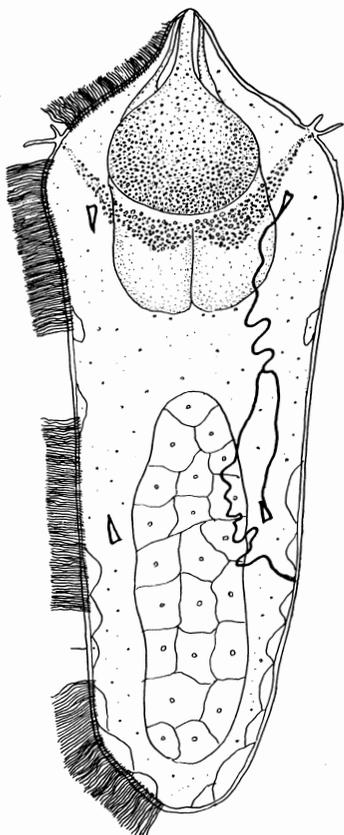


Fig. 2 Miracidium.

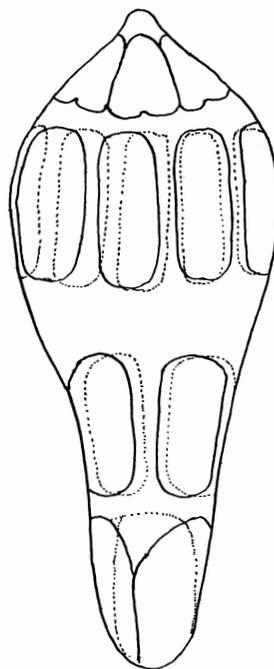


Fig. 3 Pattern of ciliated plates of miracidium.

tained in an elongated, elastic sac which retain its saclike structure, even while its shape changes according to contraction and elongation of the miracidium. The tegument of the miracidium consists of 22 ciliated epidermal plates. The arrangement of the epidermal plates is: 6 plates in the first row, 9 in the second, 4 in the third and 3 in the fourth row (Fig. 3).

Cercaria

The cercaria is apharyngeal furcocercous type with two pigmented eye spots. The entire surface is covered with minute spines. The head organ is a muscular one, particularly so at its posterior margin. The acetabulum is muscular and can project and retract like a telescope. Two eye spots are located between the head organ and the acetabulum on the dorsal side. They are cup-shaped and composed of brownish-black pigments. There are 5 pairs of penetration glands. Two pairs of circum-acetabular glands are more granular and the other three postacetabular pairs appear more homoge-

neous (Fig. 4).

The excretory system consists of 7 pairs of flame cells, 6 pairs in the body and 1 pair in the base of the tail. The flame cell formula is thus $2[3+3+(1)]=14$. There are

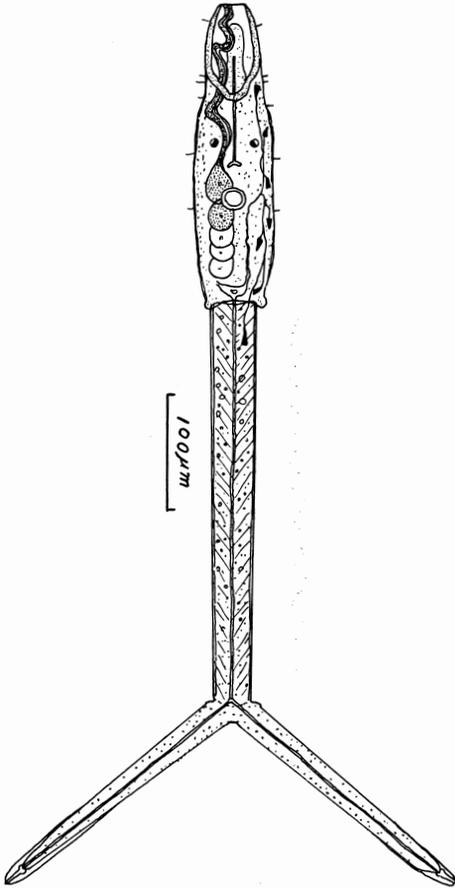


Fig. 4 Cercaria.

a pair of anterior and a pair of posterior collecting tubes connecting with each flame cell by a fine capillary tubule. The two collecting tubes on the same side of the body unite at a short distance behind and lateral to the acetabulum to form a common collecting tube. The common collecting tube passes backwards and inwards, lying ventral to the penetration glands. Each opens into a small bladder, lying at the posterior extremity of the body. The bladder discharges through two small tubes, around "island of Cort", which then unite to form a single excretory tube passing posteriorly through the tail stem. Just anterior to the base of furcae, it divides and discharges exteriorly through the tips of the furcae.

The measurements of the cercariae are shown in Table 3.

Table 3 Measurements of 35 cercariae of the present species (μm)

	The present species	<i>T. brevis</i> *
Length of body	254.2±27.6	237
Width of body	63.2± 8.6	80
Length of head organ	84.7± 6.9	
Width of head organ	48.9± 6.1	
Length of acetabulum	23.0± 1.4	
Distance of acetabulum to posterior end of body	91.6±11.9	
Length of tail stem	342.3±20.1	304
Width of tail stem	41.7± 4.8	41
Length of tail furca	209.7±29.5	218
Width of tail furca	25.7± 5.4	

* by P. F. Basch (1966)

Table 4 Number of days from exposure to miracidia until the first release of cercaria from snails (Figures in the table indicate the number of snails which began to shed cercariae on the specified day)

Days	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
The present species*																				
22-24 C								2	6	3			1							1
24-25 C					21	2				5	1									
† <i>T. brevis</i> † (room temp.) (about 28 C)	1		2	3	1	2	7	3	5	4	1	3			1					

* from *Austropelea ollula*

† *Lymanea rubiginosa* (by P. F. Basch (1966))

Table 5 Number of days from exposure to cercariae until the first appearance of eggs from ducklings (The figures in the table indicate the number of duckling began to be positive on the specified day)

Days	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
The present species*							3	1	3							
<i>T. brevis</i> †	1	3	2	4	4	4	2	2	2		1			1	1	

* The appearance of eggs was proved by the hatching test for the miracidium

† by P. F. Basch (1966)

The snails began to release cercariae from 24 to 35 days after exposure to miracidia when reared at 22-24 C and from 21 to 27 days when kept at 24-25 C after exposure (Table 4). The number of cercariae shed from *A. ollula* varied greatly both from snail to snail and from day to day. Differences of several hundred were common between one day and the next day. The cercarial shedding usually took place until a few days before the death of snail.

The cercariae adhere strongly to the wall of the glass container, hanging by their

acetabula in characteristic postures.

Tests were made to determine whether the present cercaria could cause a dermatitis. About 15 cercariae were placed in the Petri dishes of 3 cm diameter and the author's left forearm was made to touch the water with the flexor surface down. After about 10 minutes, strong itching was felt on the spot of contact. Pinpoint-sized red maculae appeared. This experimental dermatitis exhibited characteristics almost like to those of the paddy field dermatitis prevailing in the endemic areas. Thus it was definitely

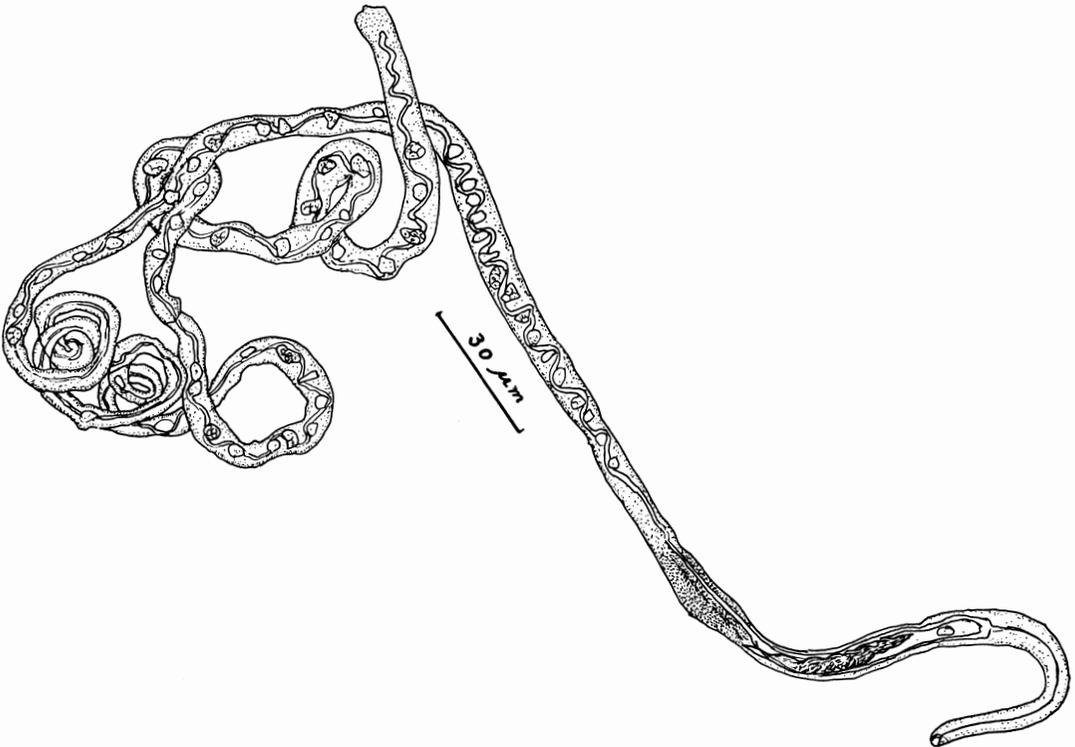


Fig. 5 Adult male worm.

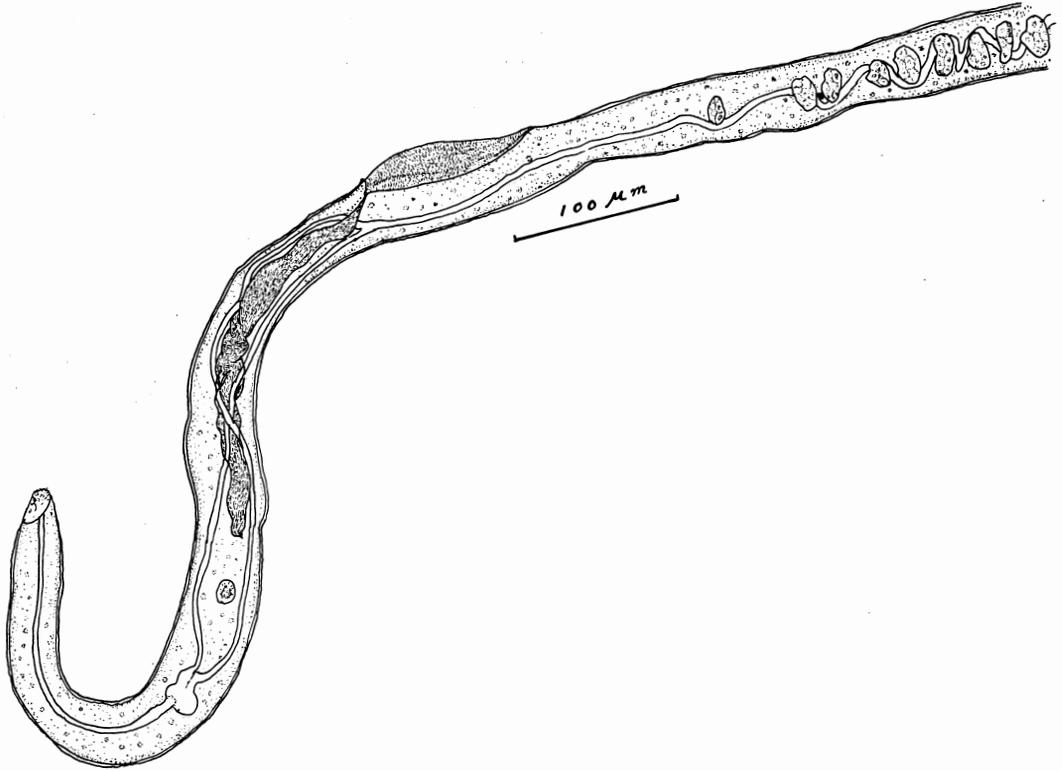


Fig. 6 Anterior portion of male.

established that this species of cercaria was the dermatitis producing agent.

Adult male

The experimental infection with the cercaria was conducted with white mice, canaries, quails, and ducklings. The first 3 species of animals failed to become infected, and only ducklings were proved susceptible. The first appearance of eggs in ducklings occurred from the 17th to the 19th day after exposure (Table 5).

Although the experimental infections were extensively tried with several dozens of domestic ducklings, only 2 complete male worms and the fragments of both male and female worms could be recovered (Figs. 5, 6).

The measurements of 2 complete and 8 incomplete male adults are summarized in Table 6. As shown in the table, the body length of the complete worm is 3.6 mm in No. 5 and 3.9 mm in No. 6. The greatest body width is at the gynecophoral canal,

which is set with spines on the ventral surface. The body width near the acetabulum varies from 30 to 44 μm . The oral sucker measures 21 to 40 μm in length and from 26 to 44 μm in width. The acetabulum varies from 12 to 25 μm in diameter.

The intestinal bifurcation is seen to be a short distance in front of the acetabulum, and the reunion is immediately anterior to the opening of the genital pore. The cecum passes backward, winding between the testes in contracted specimens and lying straight on one side of the body in well extended specimens. In 3 specimens (No. 3, 5 and 6) the number of testes are countable, being 66, 85 and 95 respectively. The testes are spherical or subspherical in shape and are arranged tandem in well extended specimens. Some randomly selected testes in the extended specimens measure 12.3 to 35.3 μm (average 23.8 μm) long and 27.9 to 40.5 μm (average 31.8 μm) wide.

Table 6 Measurements of adult males of the present species (μm)

Specimen number	1	2	3	4	5*	6*	7	8	9	10	<i>T. brevis</i> †
Length of body					3.6 (mm)	3.9 (mm)					2.1&4.3 (mm)
Width of body											
near acetabulum	34	30			44	33	40		36		60
at gynecophoral canal	43	36			60	53	43		41		90-100
in region of testis		39			36	37	40		30		55
Length of oral sucker	33	21	21	25	21	40	40	33	36		35
Width of oral sucker		31	33	26	31	44	36		37		50
Diameter of acetabulum		25		21	15				12		35×50
Length of esophagus	226	208			220	318	340		309		200-300
Distance between oral sucker and acetabulum	281	350			268	401	447	249	269	262	260-350
Distance between acetabulum and gynecophoral canal	212	270			212	274	248	281	189	307	380
Length of seminal vesicle	148	149	247		194	210	202	248	142	235	
Length of gynecophoral canal	98	94	89	101	100	113		72	96	86	
Intestinal reunion	-----just anterior to the gynecophoral region-----										
No. of testis		88 or more	66			95	85				40 & 51

* These were complete worms. † by P. F. Basch (1966)

The seminal vesicle varies from 142 to 235 μm in length, extending from a short distance behind the acetabulum to the anterior end of gynecophoral canal. The distance between the oral sucker and the acetabulum measures from 249 to 447 μm , and the distance between the acetabulum and the gynecophoral canal varies from 189 to 307 μm . The gynecophoral canal is from 72 to 113 μm in length.

Discussion

From the morphological characteristics and dimensions of both adult and cercaria, it may be concluded that the present species belongs to the Genus *Trichobilharzia*. At least 32 species of *Trichobilharzia* have so far been reported: these are *T. elevae* (Miller, 1923), *T. yokogawai* (Oiso, 1927), *T. kowalewskii* (Ejsmont, 1927), *T. ocellata* (Brumpt, 1931), *T. physellae* (Talbot, 1936), *T. stagnicolae* (Talbot, 1936), *T. querquedulae* (McLeod, 1937), *T. filiformis* (Szidati,

1938), *T. corvi* (Yamaguti, 1941), *T. burnetti* (Brackett, 1942), *T. waubesensis* (Brackett, 1942), *T. horiconensis* (Brackett, 1942), *T. kegonensis* (Brackett, 1942), *T. oregonensis* (MacFarlane and Macy, 1946), *T. cameroni* (Wu, 1953), *T. szidati* (Neuhaus, 1952), *T. taijanae* (Spaekaja, 1954), *T. adamsi* (Edwards and Jansch, 1955), *T. anatina* (Fain, 1955), *T. spinulata* (Fain, 1955), *T. berghei* (Fain 1955), *T. rodhaini* (Fain, 1955), *T. nasicola* (Fain, 1955), *T. shoutedeni* (Fain, 1955), *T. cerylei* (Fain, 1956), *T. aureliani* (Fain, 1956), *T. brantae* (Farr and Blanke-meyer, 1956), *T. littlebi* (Byrd, 1956), *T. alaskensis* (Harkema *et al.*, 1957), *T. indica* (Baugh, 1963), *T. brevis* (Basch, 1966) and *T. maigraith* (Kruatrachue *et al.*, 1968).

They are listed in Table 7 according to the site of the cecal union in male worms with information about egg-shape and the intermediate snail hosts added.

Since the site of the reunion of the cecum in the present male worm is posterior to the seminal vesicle and anterior to the genital

Table 7 List of the species of *Trichobilharzia* according to the site of the cecal union in male worms

1) Cecal union anterior to seminal vesicle			
<i>T. physellae</i> †	<i>T. ocellata</i> *	<i>T. stagnicolae</i> *	<i>T. corvi</i> ‡
<i>T. alaskensis</i> *	<i>T. brantae</i> ‡	<i>T. szidati</i> *	<i>T. cerylei</i> ‡
<i>T. anatina</i> ‡	<i>T. spinulata</i> ‡	<i>T. rodhaini</i> ‡	<i>T. elevae</i> *
<i>T. nasicola</i> *			
2) Cecal union posterior to seminal vesicle			
i Cecal union posterior to genital pore			
<i>T. yokogawai</i> ‡	<i>T. tatiana</i> ‡		
ii Cecal union anterior to genital pore			
Egg-shape: oval or spindle			
<i>T. maigraith</i> *	<i>T. indica</i> †	<i>T. filiformis</i> ‡	<i>T. cameroni</i> †
<i>T. oregonensis</i> †	<i>T. querquedulae</i> ‡		
Egg-shape: crescent			
<i>T. brevis</i> *			
Egg-shape: undescribed			
<i>T. kowalewski</i> ‡	<i>T. shoutedeni</i> ‡	<i>T. berghei</i> ‡	
3) Location of cecal union undescribed			
Egg-shape: spindle			
<i>T. waubesensis</i> ‡	<i>T. kegonsensis</i> ‡		
Egg-shape: crescent			
<i>T. aureliani</i> ‡			
Egg-shape: undescribed			
<i>T. littlebi</i> ‡	<i>T. horiconensis</i> ‡	<i>T. burnetti</i> ‡	<i>T. adamsi</i> †

* *Trichobilharzia* with larval stages in Lymnaeid snails

† *Trichobilharzia* with larval stages in Physid snails

‡ Intermediate hosts are unknown.

pore, this species should belong to the group 2)-ii or 3) reported in the table. Because the present species yields eggs of crescent shape, the species which produce oval or spindle eggs can be excluded. Although the shape of eggs of *T. kowalewski* and *T. shoutedeni* are unknown, they can be excluded because of the large size of the adult male which are never less than 5 mm. The gynecophoral canal of *T. berghei* which was obtained from birds in Rwanda by Fain (1955), is longer than that of the present species.

Among 7 species belonging to the group 3) in the Table 7, *T. waubesensis* and *T. kegonsensis* have spindle-shaped eggs. According to Farley (1971), *T. horiconensis* and *T. burnetti* as well as the above mentioned

T. berghei are synonymous with *T. physellae*, which is quite different with the present species. *T. horiconensis* can be distinguished by the beaded appearance of the male worm, by a longer gynecophoral canal of about 150 μ m and by a wider seminal vesicle which usually forms 4-6 loops. *T. burnetti* can also be distinguished by the length of the seminal vesicle, which reaches only one-half of the length of the distance from the acetabulum to the gynecophoral canal. *T. littlebi* has a larger size adult male.

T. adamsi, or which only one immature female worm has been described by Edwards and Jansch (1955), possesses a special body spination, and the cercariae were obtained from the snails belonged to Family Physidae. Thus it seems unlikely that both *T. burnetti*

and *T. adamsi* are identical with the present species.

The worm of *T. aureliani* was described by Fain (1956) from the nasal fossa of birds in Rwanda. It has a longer gynecophoral canal and a much greater number of testes than those of the present species.

The last species to be considered is *T. brevis*. This was firstly described by Basch (1966) in Malaysia. The intermediate host was reported as Lymnaeid snail, *Lymnaea rubiginosa*.

According to Basch (1966), the duck infected with *T. brevis* yields crescent-shaped eggs, the site of the reunion of the cecum in the male worm is posterior to the seminal vesicle and anterior to the genital pore, and the distribution pattern of the miracidial plates is 6-9-4-3, exactly the same as the present species. In addition, the measurements and the shape of both eggs and cercariae of the present species are quite similar to those of *T. brevis*. Moreover, Margono (1968) reported that the cercarial dermatitis in Djakarta, Indonesia was caused by this species from *Lymnaea javanica*.

Judging from these findings the authors came to the conclusion that the species found in Saitama Prefecture, the causative agent of the paddy field dermatitis, is to be identified as *T. brevis*.

In Japan, Tanabe (1948) reported that the disease known locally as "Koganbyo" or "Lake-side disease" was due to an avian schistosome, *Gigantobilharzia sturniae*. Subsequently, surveys carried out by Oda (1956, 1958) and other researchers showed that the cercarial dermatitis due to cercariae of either *T. physellae* or *T. ocellata*.

Therefore, up to now, three species have been recognized as producing the cercarial dermatitis in Japan. These are *T. ocellata*, *T. physellae* and *G. sturniae*. Now, these are four with the addition of *T. brevis*.

Similar cases of cercarial dermatitis have been reported in many prefectures of Japan since the authors found the cercariae from *A. ollula*; Aichi (Kumada *et al.*, 1971), Chiba (Yokogawa *et al.*, 1976), Kagoshima

(Suzuki *et al.*, 1976), Ibaragi (Yasuraoka *et al.*, 1977), Gifu (Kobayashi *et al.*, 1977), Tokushima (Ookubo *et al.*, 1978) and Shizuoka (Ito & Mochizuki, 1978). All these cercarial dermatitis are suspected to be due to the avian schistosome cercariae released from *A. ollula*. It is assumed that these cercariae are the same as *T. brevis*.

Summary

The farmers working in paddy fields in the early summer of 1967-1970 were suffering from dermatitis. The circumstances suggested that it may be caused by an invasion of the cercaria of avian schistosome.

Investigation of the snail intermediate host was carried out and cercariae of an aphyaryngeal brevifurcate furcocercous type were found from the fresh water snail, *Austropeplea ollula*. The experimental exposure of human skin to the cercariae resulted in dermatitis of the same characteristics as those observed on the farmers.

Experimental infections in domestic ducklings using the cercariae showed that the birds passed crescent shaped schistosome eggs in about 17 to 19 days after infection.

Male adult worms, eggs, miracidia and cercariae were studied and identified as those of *Trichobilharzia brevis* Basch, 1966.

Finally, consideration was given to the recent reports on the occurrence of cercarial dermatitis caused by an avian schistosome cercaria from the same snail host, *A. ollula*.

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わが国においてセルカリア性皮膚炎をおこす *Trichobilharzia brevis* Basch, 1966

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1967年の初夏に埼玉県東部で水田作業中の農民の手足に皮膚炎が発生し、1971年までに少なくとも1,280名の患者を認めた。調査の結果、ヒメモノアラガイを中間宿主とする鳥類住血吸虫のセルカリアの皮膚侵入によっておこることが明らかにされた。

このセルカリアは、眼点を有し、咽頭を欠き、岐尾の短い岐尾セルカリアであり、5対の侵入腺細胞をもち、その焰状細胞式は、 $2[3+3+(1)=14]$ である。このセルカリアはアヒルに感染し、17~19日後にはその糞便中に三日月形の虫卵を排出した。ミラシジウムの epidermal plate の配列は 6-9-4-3 であった。ミラシジウムは

ヒメモノアラガイによく侵入し、約 24 C で 21 日目にセルカリアの遊出がはじまる。モノアラガイには感染しない。

成虫の雄の腸管は口吸盤の前方で分岐し、genital pore 開口部の直前で再び結合する。

これら虫卵、ミラシジウム、セルカリア及び成虫等の形態から、本虫が1966年にBaschがマレーシアにおいて見出した *Trichobilharzia brevis* Basch, 1966 にきわめて近似していると考えた。本種による皮膚炎はその後、国内各地で発生していることが報告されている。