NATURAL INFECTIONS OF BLACKFLIES WITH LARVAE OF ZOONOTIC *ONCHOCERCA* SPP. IN NORTHEAST JAPAN

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Abstract: Dissections of female blackflies collected while attacking a cow at the Omyojin Farm, Iwate, northern Japan showed that 4.4% of 1,104 *Simulium daisense*, 0.8% of 913 *S. aokii*, 0.5% of 741 *S. iwatense* and 0.9% of 340 *S. nikkoense* examined were naturally infected with filarial larvae. There were at least two types of third-stage larvae assignable to *Onchocerca*, of which type A found in *S. daisense* and *S. aokii* measured 914-1,212 μm long by 26-29 μm wide while type B found in *S. daisense* had its body size of 369-477 μm long by 14.9-17.9 μm wide. Morphometric observations suggest that types A and B each correspond to types I and III previously recorded in Kyushu, southwest Japan as the third-stage larvae of bovine *Onchocerca* (i.e., *O. sp.* and *O. lienalis*). This is the first record for natural infection of blackflies in Honshu (main island of Japan) with zoonotic *Onchocerca* larvae.

INTRODUCTION

In relation to the transmission of bovine and equine onchocerciasis in Japan, Hosoya *et al.* (1956) dissected several species of *Simulium* and *Culicoides* collected in the endemic areas, such as Tottori in western Honshu, Fukushima and Aomori in northern Honshu, but failed to detect any larvae of *Onchocerca*, though they found some flies infected with larvae of non-filarial nematodes bearing a sharply pointed tail. Ueno *et al.* (1956) also examined about 16,000 females of four *Culicoides* species caught by light traps in Tokyo and found no *Onchocerca* larvae. There was since then no investigation on the vectors of these zoonotic onchocerciasis in Japan. Recently, we found that several blackfly species collected at cattle sheds in Kyushu, southwest Japan were naturally infected with three types of *Onchocerca* larvae, of which two (types II and III) were each suspected as *O. gutturosa* Neumann and *O. lienalis* Stiles parasitizing cattle while the last (type I) remained unidentified (Takaoka and Bain, 1990). Subsequent infection experiments showed that microfilariae from the skins of slaughtered cattle or from the midgut of freshly blood fed blackflies successfully developed in blackflies to the third-stage larvae (L_3) almost identical to those of types I and III, (Takaoka, 1990). From the results of these and other works, it is now clarified that *S. bidentatum* (Shiraki) and *S. arakawae* Matsumura are the natural vectors of type I (*O. sp.*),

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the former also serves as a vector of type II (probably *O. gutturosa*) and the latter as well as *S. kyushuense* Takaoka is also a natural vector of type III (= *O. lienalis*) in Kyushu (Takaoka, in preparation).

The present work was done in Iwate, northeast Japan to determine whether some other blackfly species are also involved in the transmission of zoonotic onchocerciasis. In this paper, two more blackfly species are added as a natural vector of zoonotic *Onchocerca* spp. (probably *O. sp.* and *O. lienalis*).

MATERIAL AND METHODS

Collections of adult blackflies were carried out at the Omyojin Farm (at about 40° N lat. and 141° E long.; 230–270 m in altitude), Faculty of Agriculture, Iwate University, at Shizukuishi-machi, Iwate Prefecture, in northeast Japan, from April to November in 1989. About 80 cattle were bred in the pasture (ca. 45 ha) of this farm. An hourly 10-minute collection of flies attacking a cow tethered to the wooden fence was made once a month using an insect net by one man, for 24 hrs starting from 11:00 hr. Air temperatures were hourly measured at the end of each collection. This regular collection was attempted to determine the seasonal pattern in biting activity of blackflies. Females captured were preserved in small vials with 80% ethanol solution, and sent to the laboratory in Oita. After being identified to species, these specimens were dissected in 5% Giemsa solution on glass slide under dissecting microscope and examined for filarial larvae. In addition to samples from regular collections, portions of female blackflies captured by a cow-baited trap and/or a CO₂ trap which were set at the same place and time were, if necessary, also dissected in the similar manner. Morphometric observations were microscopically made for all filarial larvae detected. Generic diagnosis of L₃ larvae followed that of Bain and Chabaud (1986).

RESULTS

Table 1 shows the results of regular collections. At least 12 blackfly species belonging to *Prosimulium*, *Cnephia* and *Simulium* were collected. The biting activity of *Pro. yezoense* Shiraki was high in May but very low or nil in the following months. On the other hand, biting activity of most *Simulium* species became marked after May, continued to be relatively high until August with their peak in June or July and decreased in September. Thereafter, biting activity was still observed in several species but in small numbers except *S. aokii* (Takahasi). Among *Simulium* species, the most abundant were *S. aokii* and *S. daisense* (Takahasi), followed by *S. iwatense* (Shiraki) during the summer months from June to August.

In view of the critical air temperature of ca. 17°C below which larval development of *O. volvulus* (Leuckart) in the intermediate blackflies would cease (Takaoka *et al.*, 1982), dissections for natural infections with any filarial larvae were made only for samples of four *Simulium* species which were relatively abundant during a period from June to September when air temperatures were for the most part beyond 17°C (Fig. 1). The results of dissections are presented in Table 2.

All the four species dissected harboured filarial larvae in their thoracic and/or cephalic regions. The overall infection rates varied from 0.5% to 4.4% depending on the species.

Table 1 Number of female blackflies captured by a sweeping net on cattle at the Omyojin Farm, Iwate, northeast Japan, in 1989*

Blackfly species	Months of collection							
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.
<i>Prosimulium yezoense</i>	0	398	9	1	0	0	0	0
Other <i>Prosimulim</i> spp.	26	49	0	0	0	0	0	0
<i>Cnephia</i> sp.	2	19	0	0	0	0	0	0
<i>Simulium uchidai</i>	0	0	4	1	0	0	0	0
<i>S. aokii</i>	9	10	146	281	207	12	55	189
<i>S. arakawae</i>	0	3	0	11	16	0	2	1
<i>S. daisense</i>	0	0	722	201	108	9	5	0
<i>S. iwatense</i>	43	2	132	91	28	0	6	2
<i>S. japonicum/kawamurae</i>	4	50	2	8	0	2	34	0
<i>S. nacojapi</i>	0	0	0	1	0	0	0	0
<i>S. nikkoense</i>	0	1	16	71	15	0	0	1
<i>S. rufibasis</i>	0	0	3	3	1	0	0	0
Total	84	532	1,034	669	375	23	102	193

*: An hourly 10-minute collection was made for consecutive 24 hrs by one man, once a month.

Table 2 Natural filarial infections of blackflies collected on cattle at the Omyojin Farm, Iwate, northeast Japan

Blackfly species	Months of collection				Total
	Jun.	Jul.	Aug.	Sep.	
<i>S. aokii</i>					
No. infected/no. dissected	3/210	1/247	2/310	1/146	7/913(0.8%)
No. and stage ^a of larvae	4L ₁	1L ₃	2L ₁	4L ₂	
No. larvae/infected fly					
Median (Range)	1(1-2)	1(1)	1(1)	4(4)	
<i>S. daisense</i>					
No. infected/no. dissected	17/569 ^b	29/202	1/281	1/52	48/1,104(4.4%)
No. and stage of larvae	54L ₁ +1L ₂	6Mf+56L ₁ +29L ₂ +39L ₃	2L ₁	3L ₂	
No. larvae/infected fly					
Median (Range)	2(1-13)	4(1-27)	2(2)	3(3)	
<i>S. iwatense</i>					
No. infected/no. dissected	0/176	3/499 ^b	1/66	—/—	4/741(0.5%)
No. and stage of larvae	—	2Mf+2L ₁ +2L ₂	2L ₁		
No. larvae/infected fly					
Median (Range)		2(1-3)	2(2)		
<i>S. nikkoense</i>					
No. infected/no. dissected	—/—	2/312	1/28	—/—	3/340(0.9%)
No. and stage of larvae		8L ₁ +2L ₂	23L ₁		
No. larvae/infected fly					
Median (Range)		4(1-9)	23(23)		

a. Mf, microfilaria; L₁, L₂ and L₃ represent first-, second- and third-stage larvae, respectively

b. A larva of non-filaria nematode was found in the thorax of one *S. daisense* and one *S. iwatense*

Table 3 Measurements of third-stage larvae (L_3) of *Onchocerca* spp. found in *Simulium aokii* and *S. daisense* collected at the Omyojin Farm, Iwate, northeast Japan

L_3 no.	Type of L_3	Host <i>Simulium</i> spp. and body parts	Landmarks*				
			BL	BW	OL/BL	TL	TL/TW
1	A	<i>S. aokii</i> thorax	1,212.7	26.6	0.49	40.0	1.79
2	A	<i>S. daisense</i> thorax	914.2	28.0	—	—	—
3	A	" "	947.8	29.1	0.46	33.5	1.29
4	A	" "	1,059.7	26.9	0.49	43.7	1.83
5	A	" "	1,179.1	26.1	0.43	—	—
6	B	" head	369.4	14.9	0.66	—	—
7	B	" "	373.1	17.9	0.67	—	—
8	B	" "	403.0	16.8	0.62	33.5	2.25
9	B	" "	410.5	16.8	0.61	—	—
10	B	" "	440.3	16.8	—	33.5	2.01
11	B	" "	447.8	17.9	0.62	—	—
12	B	" "	447.8	16.8	0.65	29.1	2.05
13	B	" "	447.8	15.7	0.58	—	—
14	B	" "	451.5	15.7	0.69	29.8	1.95
15	B	" "	462.7	16.8	0.66	—	—
16	B	" "	462.7	—	0.68	—	—
17	B	" "	466.4	16.4	0.68	—	—
18	B	" "	477.6	16.4	0.63	33.5	2.25

* : BL, body length; BW, maximum body width; OL/BL, length ratio of oesophagus/whole body; TL, tail length; TL/TW, ratio of tail length/tail width.

— : not measured.

Filarial larvae found were at various developmental stages (Fig. 2a-h), of which L_3 larvae were found only in 1 or 0.4% of 247 *S. aokii* and 7 or 3.5% of 202 *S. daisense* examined in July.

There are apparently two types of L_3 larvae judging from the body size and relative length of oesophagus to whole body, as shown in Table 3. The longer type (designated as type A; Fig. 2h) was found in both of *S. aokii* and *S. daisense* whereas the shorter one (type B; Fig. 2f) was detected only from the latter blackfly species. Of the seven *S. daisense* infected with L_3 larvae, three had type A, while the others harboured type B. One of the four females with L_3 of type B was also infected with two preinfective larvae assignable to type A (Fig. 2g) due to long body size (630.6 μm and 570.9 μm) and short oesophagus ($0.36\times$ and $0.34\times$ body length).

The L_3 larvae of type A were all found in the thorax and their number per infected fly was one or two, whereas those of type B were mostly recovered from the cephalic region and their number varied from 1 to 27. In one female infected with 27 L_3 larvae, all but one were found in the head, in particular, clustering within labrum of the mouthpart (Fig. 3).

Several large second-stage larvae (L_2) (e.g. Fig. 2g) measuring ca. 400–780 μm in body length (i.e., advanced L_2 or preinfective) found in *S. daisense* were evidently the same species as the L_3 designated type A. However, classification was impossible for most of other smaller L_2 (body length ca. 200–290 μm and oesophagus about $1/2\times$ the body length) including four L_2 larvae found in *S. iwataense* and *S. nikkoense* Shiraki (Figs. 2d and 2e) as well as first-stage

larvae (L_1) (ca. 90–170 μm long) (Figs. 2b and 2c).

Microfilariae (Fig. 2a) were found in the thorax of one *S. iwatense* and three *S. daisense*. In the latter three females, L_1 and/or L_2 larvae were also found. All these microfilariae were similar in size (i.e., 186.6–223.9 μm long by 5.0–5.9 μm wide), were unsheathed and had a slender pointed tail.

Two larvae of non-filarial nematode were each found in the thorax of *S. iwatense* and *S. daisense*. Both of these larvae had a sharply pointed tail (Fig. 2i) and measured 492.5 μm long by 14.9 μm wide and 856.0 μm long by 31.3 μm wide, respectively.

DISCUSSION

The present work shows that four blackfly species attacking cattle in northeast Japan were naturally infected with at least two types of filarial larvae developing in the fly's thorax, which are probably assignable to the genus *Onchocerca* by the very small, caudal lappets of L_3 larva.

Out of two types, type A found in *S. daisense* and *S. aokii* is characterized by the very long body (average 1,062.7 μm) and the short oesophagus $\{(0.43\text{--}0.49) \times \text{the body length}\}$. With these characters type A does not fit with any known *Onchocerca* species (Bain and Chabaud, 1986), and reminds us of type I, L_3 of *Onchocerca* sp. (body length, 1,075–1,380 μm ; ratio of oesophagus/body length, 0.45–0.50) which was reported from wild caught *S.*

Figure 1 Daily fluctuation of air temperatures by months at the Omyojin Farm, Iwate, northeast Japan. Horizontal line shows a critical air temperature of ca. 17°C for development of *Onchocerca volvulus* larvae in the vector blackfly. Data on air temperatures from 21:00 to 04:00 hr in November were not available due to the cessation of the collection during the night.

Figure 2 Filarial and non-filarial larvae found in *Simulium* spp. caught on a cow at the Omyojin Farm, Iwate, northeast Japan. a, microfilaria found in *S. iwatense*; b and c, first-stage larvae found in *S. daisense*; d, second-stage larva found in *S. iwatense*; e, second-stage larva found in *S. nikkoense*; f, third-stage larva of *Onchocerca* sp. (type B) found in *S. daisense*; g, preinfective larva of *Onchocerca* sp. (type A) found in *S. daisense*; h, third-stage larva of *Onchocerca* sp. (type A) found in *S. aokii*; i, non-filaria larva found in *S. iwatense*. All larvae were found in the thorax except type B larva (f) which was found in the head. Scale=100 μ m.

Figure 3 The labrum of the mouthpart of *Simulium daisense* clustered with many third-stage larvae of *Onchocerca* sp. (type B). a, anterior tip of two larvae; b, middle part of larval body.

bidentatum and *S. arakawae* (Takaoka and Bain, 1990) and was soon proved to be of bovine origin in Kyushu (Takaoka, 1990). In fact, it is difficult to separate type A from type I. The L_3 larvae of *Dirofilaria ursi* Yamaguti of bears and *D. immitis* (Leidy) of dogs are somewhat similar to those of type A in having a long body size and small caudal lappets but their development takes place in the Malpighian tubules of the blackflies (Addison, 1980; Takaoka and Baba, 1987).

Type B, found in *S. daisense*, seems to conform to type III, L_3 of *O. lienalis* parasitizing cattle, reported from *S. arakawae* and *S. kyushuense* in Kyushu too (Takaoka and Bain, 1990; Takaoka, in preparation). The body length of type B (369.4–477.6 μm , average 435.4 μm) is shorter than that of type III (510–530 μm) reported by Takaoka and Bain (1990). This gap is attributed mainly to the difference in the fixative solution used (ethanol vs. formalin).

The finding that L_3 larvae of type B were found together with preinfective larvae of type A in the same fly, though once only, strongly suggests that both of these types share the same animal as a definitive host.

From these results as well as high biting and infection rates of *S. daisense* caught on the cow, it is likely that both type A and B are a bovine parasite. However, further studies are needed to confirm this observation since no data has been available yet on the *Onchocerca* infection in cattle bred in and near the Omyojin Farm, and also on the biting preference of these blackflies to other animals. Whether or not the filarial larvae found in *S. iwatense* and *S. nikkoense* are a bovine *Onchocerca* remained to be studied.

Our results could add two more blackfly species, *S. daisense* and *S. aokii*, as a natural vector of zoonotic *Onchocerca* in Japan, though the latter species has been reported as an experimental vector of type I in Kyushu (Takaoka, 1990).

The biting activities of blackflies were observed throughout the surveyed period from April to November. And, four of these eight months (i.e., from June to September) were expected as the probable period for *Onchocerca* transmission in and near the Omyojin Farm from the air temperature conditions. However, actual transmission time of these zoonotic *Onchocerca* spp. would be more restricted, because female blackflies carrying L₃ larvae were collected only in July.

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東北地方のブユ成虫におけるオンコセルカ幼虫自然感染

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岩手県雫石町御明神牧場において、1989年4月から11月にかけて、牛囙法で合計12種のブユを得た。6月―8月に比較的捕獲数の多い *Simulium aokii*, *S. daisense*, *S. iwatense* および *S. nikkoense* の4種3,098個体を解剖し、それぞれ0.8% (7/913), 4.4% (48/1,104), 0.5% (4/741), 0.9% (3/340) にフィラリア幼虫の感染を認めた。*S. aokii* を除く他の3種のブユからは、今回初めてフィラリア幼虫が見付かった。第三期幼虫 (L_3) は、7月に採集された *S. aokii* および *S. daisense* から得られた。 L_3 の形態から2種が含まれていることが分かった。type A は両種ブユから見いだされたが、type B は *S. daisense* からのみ得られた。type A および B は、それぞれ九州のブユから得られている *Onchocerca* type I, II, III のうちの type I (牛に寄生する *O. sp.*) と type III (*O. lienalis*) と思われる。他の2種のブユからは L_3 は得られなかったので、フィラリア種の同定は出来なかった。動物寄生性オンコセルカに感染された人体症例が、既に九州から報告されているが、今回の調査により、本州においてもブユの媒介による、同様の人体感染例が発生する可能性が示唆された。

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