NATURAL INFECTIONS OF *SIMULIUM BIDENTATUM* (DIPTERA: SIMULIIDAE) WITH LARVAE OF *ONCHOCERCA* SPP., IN RELATION TO A HUMAN ZONOTIC ONCHOCERCIASIS IN OITA, JAPAN

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Abstract: A total of 579 wild female flies belonging to eight species of *Simulium* were collected on human baits, in relation to the transmission of a human zoonotic onchocerciasis found in Oita, Japan. *Simulium bidentatum* was the most dominant species. Natural infections with filarial larvae were found in 11 of 449 *S. bidentatum* (or 6.5% of the parous flies) and in 1 of 23 *S. aokii* (or 10% of the parous flies). All the infected flies had 1-4 either first-, second- or early third-stage larvae in the thorax. Examination of third-stage larvae shows that *S. bidentatum* is infected with at least two species of *Onchocerca*. Our result represents the first record of natural *Onchocerca* infections in Japanese Simuliidae, and also suggests the possibility of *S. bidentatum* as the potential vector of zoonotic *Onchocerca* spp. to humans.

INTRODUCTION

Four human cases of zoonotic onchocerciasis have been reported from various areas of the world (Azarova *et al.*, 1965; Siegenthaler and Gubler, 1965; Beaver *et al.*, 1974; Ali-Khan, 1977). An additional case was recently found in Oita, southern Japan (Hashimoto *et al.*, 1990). Examination of histological sections of a female nematode found suggested that, as in all the previous cases, its morphological features resemble those of *Onchocerca gutturosa* Neumann, 1910 and *O. cervicalis* Railliet et Henry, 1910 infecting cattle and horses respectively (Beaver *et al.*, 1989).

Several *Onchocerca* spp. including *O. gutturosa* and *O. cervicalis* have been reported from Japan (Sato *et al.*, 1954; Suzuki *et al.*, 1982). However, no transmission studies of these filarial nematodes have been carried out, although biting midges (*Culicoides* spp.) or blackflies (*Simulium* spp.) have been known as the vectors of certain *Onchocerca* spp. elsewhere (Bain and Chabaud, 1986).

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The objective of this study is to investigate the transmission of zoonotic onchocerciasis which may be acquired by humans. In this paper, we report natural *Onchocerca* infections of *S. bidentatum* Shiraki collected in one of the suburban residential areas of the city of Oita, where a human zoonotic onchocerciasis occurred.

MATERIALS AND METHODS

The patient infected with a zoonotic *Onchocerca*, a two-year-old Japanese girl, was an inhabitant of Tabaru, one of the suburban residential areas (altitude ca. 30 m above sea level; coordinates 131°35'E; 33°10'N), situated in the western part of the city of Oita. It was presumed that the zoonotic *Onchocerca* infection might have been acquired by this child at or near her residential area, because, living in one of the housing complexes (ca. 0.01 km²), she used to spend her time with her mother during the daytime at the recreation ground (ca. 1,200 m²) located on the west within their premises, and had never left the city, according to her mother. It was therefore attempted to collect biting midges or blackflies at the corner of this recreation ground. Adjacent to this place on the west lies another huge housing area recently developed (with an extension of about 0.4 km²). There is a low hill with a shrubbery just behind these living areas on the south. On the north, a flat farmland mainly used for rice cultivation widely opens and in its center the Oita River runs eastwards which is, at the nearest, about 400 m away from the collection site. There are several cattle sheds but no horses are raised within a 4-kilometer radius from the collection site.

Collections were made between 16.00 and 18.00 hours during September–December 1988. Flies landing on or flying around the body surface of the human baits were captured by an aspiration tube or by an insect net, and held overnight in small plastic containers. The following day, flies were dissected in saline on a glass slide, and parity was determined by the presence of follicular relics (Detinova, 1962). Parous females were microscopically searched for filarial larvae. The larvae found were preserved in formalin–glycerol (Wharton, 1959) for morphometric observations.

About 170 blackflies collected in December were individually maintained with sucrose solution at 25°C for seven days, and dissected for the presence of third-stage filarial larvae. Generic diagnosis of third-stage larvae followed that of Bain and Chabaud (1986).

RESULTS

Four collections carried out from September to November 1988 yielded a total of eight blackfly species, but no *Culicoides* species was captured. The majority of blackfly species were *Simulium bidentatum*, followed by *S. arakawae* Matsumura, *S. quinquestriatum* (Shiraki) and *S. aokii* (Takahasi) (Table 1).

Natural infections with filarial larvae were found in 2.5% of 449 *S. bidentatum* collected (or 6.5% of the parous flies), and in 4.3% of 23 *S. aokii* captured (or 10% of the parous flies). Eight of 11 infected *S. bidentatum* had 1 to 4 first-stage larvae, two flies each harbored one second-stage larva, and one fly had two third-stage larvae probably just after the second moulting (Fig. 1). One infected *S. aokii* had one first-stage larva (Fig. 2). All the larvae were found in the thorax of the flies.

One of the two third-stage larvae (Fig. 1) recovered from September collection showed

Table 1 Species composition, parity and natural infections of blackflies collected in Tabaru, one of the suburban residential areas, where a human case of zoonotic onchocerciasis occurred, in Oita, Japan

Simulium spp.	Date of collection														
	Sept. 23			Oct. 6			Oct. 13			Nov. 17			Total		
	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.
<i>aokii</i>	0	—	—	4	3	1	7	4	0	12	3	0	23	10	1
<i>arakawae</i>	33	11	0	0	—	—	0	—	—	3	1	0	36	12	0
<i>bidentatum</i>	88	30	4	43	9	0	174	69	4	144	62	3	449	170	11
<i>japonicum</i>	0	—	—	0	—	—	1	0	0	1	0	0	2	0	0
<i>nikkoense</i>	0	—	—	0	—	—	0	—	—	1	0	0	1	0	0
<i>quinquestriatum</i>	0	—	—	1	0	0	2	0	0	30	10	0	33	10	0
<i>rufibasis</i>	1	0	0	3	2	0	7	2	0	1	1	0	12	5	0
<i>takahasii</i>	0	—	—	2	1	0	0	—	—	21	1	0	23	2	0

the following measurements: total body length (BL) 743 μm , body width (BW) 26 μm , length of oesophagus (LE) 464 μm , length of tail (LT) 35 μm , width of tail (WT) 21 μm .

In dissections of flies maintained for seven days after collection, one of 144 *S. bidentatum* examined had one third-stage larva (Fig. 3) in the thorax, but all of the 22 *S. aokii* were negative. The third-stage larva recovered (Fig. 3) showed following measurements: BL 1,280 μm , BW 25 μm , LE 620 μm , LT 45 μm , WT 20 μm . This larva, as well as two early third-stage larvae mentioned above, seemed to belong to the genus *Onchocerca* by possessing the very small, almost indiscernible caudal lappets.

It is generally impossible to identify the young third-stage larvae. However, the early third-stage larva found in September seemed to be distinct from the longer third-stage larva found in December because the ratio of LE/BL, as well as body length, was clearly different (i. e., 0.62 vs. 0.48). This conclusion is supported by our observations (unpublished data) that early third-stage larvae of the latter (long type *Onchocerca*) bear the short oesophagus (less than 1/2 of body length) like mature third-stage larva, and measure over 850 μm in body length.

It remained to be determined whether the filaria infecting *S. aokii* belongs to *Onchocerca*.

DISCUSSION

Beaver *et al.* (1989) suggested that the human zoonotic onchocerciasis found in Oita was probably caused by *O. gutturosa* or *O. cervicalis* which have been commonly known in cattle and horses respectively around the world. Our result shows the least possibility that the causative worm was *O. cervicalis*, because its transmitter, *Culicoides*, was not captured during the daytime in the study area. This is also supported by the fact that horses, the definitive host of this parasite, were not raised around this area.

On the other hand, it is demonstrated that there were several anthropophilic blackfly species in the study area, and that *S. bidentatum*, the most dominant species, was naturally infected with larvae of *Onchocerca*. Examination of the third-stage larvae recovered shows

Figure 1 Early third-stage larva of *Onchocerca* sp. found in *S. bidentatum* dissected soon after collection.

Figure 2 First-stage larva of unidentified filaria found in *S. aokii* dissected soon after collection.

Figure 3 Third-stage larva of *Onchocerca* sp. found in *S. bidentatum* maintained for seven days after collection.

All scale bars indicate 100 μ m

that *S. bidentatum* was infected with at least two *Onchocerca* species. One of these two fits in with the body length of third-stage larva of *O. gutturosa* given by Bain (1979), but more specimens of mature stage are needed to compare with the bovine parasite. The other one, though only one specimen is available, may belong to a new species because it has a very long body, as compared to those of other known *Onchocerca* species (Bain and Chabaud, 1986). At the present, it is thus difficult to conclude that the zoonotic *Onchocerca* acquired by a Japanese child was *O. gutturosa*, although it might be one of the two *Onchocerca* species found in *S. bidentatum*.

There were many cattle (both Holstein and Japanese Black) at several sheds set up in the farmland near the study area, and microfilariae of *Onchocerca* spp. were already found in blood-fed females of *S. bidentatum*, *S. aokii* and *S. arakawae* collected at one of five cowsheds examined. Further, three types of third-stage larvae of *Onchocerca* spp. were commonly discovered in the same blackfly species collected at the same cowshed (Takaoka and Bain, unpublished data). One of these three types of *Onchocerca* appears to be the same species as the longer third-stage larva found in this survey, and the remaining two types resemble third-stage larvae of *O. gutturosa* and *O. lienalis* Stiles, 1908 respectively, although both of the latter two bovine filariae have been not yet recorded in Oita. Whether all these types of *Onchocerca* spp. recovered from *Simulium* spp. are parasites of cattle or of other animals should await future studies.

In summary, our findings indicate that there exists a situation, under which the zoonotic *Onchocerca* infection may be acquired by humans through biting of the local blackfly species, in the western suburbs of Oita.

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大分におけるキアシツメトゲブユの *Onchocerca* 幼虫自然感染
—特に動物寄生性 *Onchocerca* による人への伝播に関して—

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大分市の西部郊外において見いだされた、動物寄生性 *Onchocerca* 線虫による人体例の伝播に関して、患者の居住区において人囹法によって吸血ブユの採集を行い、フィラリア幼虫の感染の有無を調べた。その結果、8種のブユが採集され、そのうちキアシツメトゲブユが優先種であることが分かった。また本種ブユでは、調べた449個体のうち11個体の雌に、フィラリア幼虫の感染が見つかった。第3期幼虫の形態的観察により、*Onchocerca* 属に属する2種が含まれていることが分かった。これらの結果から、動物寄生性 *Onchocerca* の人体への感染について考察を行った。

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