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Black flies that suck blood from Japanese deer (*Cervus nippon*) and filarial infection in the flies

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Abstract: In a study of whether black flies transmit pathogens from deer to livestock, black flies were collected with a light trap at a deer shed in Morioka, Iwate Prefecture, Japan, and examined for natural infection with filariae. A total of 970 black flies belonging to the following five species were captured: *Simulium bidentatum, S. iwatense, S. aokii, S. nikkoense*, and *S. daisense*. All but *S. daisense* sucked blood from deer. *S. bidentatum, S. iwatense*, and *S. nikkoense* had sucked blood from both cattle and deer. *S. bidentatum* was naturally infected with first-stage filarial larvae in the pectoral muscle. The black flies may carry pathogens between deer and cattle.

INTRODUCTION

Recently, the number of Japanese deer (*Cervus nippon*) has been increasing in many regions, causing severe crop damage. On the other hand, deer ranches have been opened to promote industry in villages. These deer ranches are often close to cattle pastures, and encounters among deer, livestock, and people are becoming more common. Many Japanese deer possess a high level of antibodies to livestock-disease pathogens, such as blue tongue virus and Akabane virus, and deer may spread disease to livestock (Imada *et al.*, 1996).

Bloodsucking insects such as black flies can transmit pathogens (Anderson *et al.*, 1961; Kolstrup, 1975; Schulz-Key and Wenk, 1981; Takaoka *et al.*, 1989, 1992; Takaoka, 1999), but there have been few reports on black flies that sucked blood from deer (Schulz-Key and Wenk, 1981; Sasaki *et al.*, 1986, 1988).

The purpose of this research was to clarify the possibility that black flies transmit pathogens of deer to livestock. Therefore, we surveyed the species of black flies that may suck blood from deer and examined the flies for natural infection with filariae.

The results indicated the possibility of black flies to carry pathogens between deer and cattle.

MATERIALS AND METHODS

Black flies were collected at a deer shed in an animal-breeding facility of Tohoku National Agricultural Experiment Station in Morioka, Iwate, Japan, from June 1 to November 1 or 30 in 1995 and 1996, respectively. The shed stands in grassland with deer and sheep paddocks. A cattle paddock was about 100 m away, and some ten adult cattle were kept there. Woods and a small stream were nearby.

Three adult deer were being kept in the deer shed adjoining a sheep shed in the facility. About 30 adult sheep were always kept in the sheep shed. The deer shed had a room for feeding and resting (length 3 m,

width 4 m, height, 3 m) and a paddock with a concrete floor (length 3 m, width 10 m). The deer and insects, such as black flies and mosquitoes, could freely pass through the opening (width 1 m, height 1.2 m) between the room and the paddock. There was a window covered with a 1-inch wire mesh on the north wall of the deer shed facing the grassland.

A light trap with a black light bulb (Toshiba light trap FLM-601G, 20 W) was suspended at a height of 2 m in the center of the feeding room. The light trap was left on continuously, and the bag for collecting insects was changed every morning during the survey. The sampling of each time was carried out over two days in this way. All of the black flies that were collected in the morning of the 2nd day were recorded as the individuals that had been captured on the 1st day, for the sake of convenience.

The flies collected were preserved in a freezer at −30℃ until the capture of the flies ended in November. The head, thorax, and genital segment of the flies were sent to Oita Medical University. After the species of the flies were identified (Bentinck, 1955), these specimens were dissected in a 5% Giemsa solution on a glass slide under a dissecting microscope and examined for filarial larvae (Takaoka *et al.*, 1992). In Morioka, blood in the abdomens of the engorged flies collected in 1995 and 1996 was analyzed for its source by the direct enzyme-linked immunosorbent assay (ELISA). In brief, the abdomens of the frozen black flies were put into phosphate-buffered saline (PBS) and crushed to release any blood in the middle gut. The emulsion obtained was poured into plates for ELISA as the antigen. The antigen was adsorbed onto the wells of the plates and the plates were incubated at 4℃ overnight. Thereafter, chicken serum was added as the coating buffer, and goat anti-bovine, goat anti-cervine, or goat anti-ovine serum was poured into the wells. Horseradish peroxidase conjugated with rabbit anti-goat serum was put into the wells, and the plates were incubated at 37℃ for two hours. These sera and the peroxidase were commercial products of ICN Pharmaceuticals, Inc. (Costa Mesa, CA, U.S.A.). The emulsion in the wells was colored with a substrate solution (a mixture of *o*-phenylene diamine, citric acid-Na_2HPO_4 buffer, and H_2O_2), and the reaction was stopped by the addition of 4-N sulfuric acid. The absorbance was measured by a microplate reader. PBS containing 0.5% Tween 20 was used as a washing buffer after each treatment.

Results

1. *Species of black flies*

The species of captured black flies were the same in 1995 and 1996; *Simulium bidentatum, S. iwatense, S. aokii, S. nikkoense,* and *S. daisense* (Table 1). In both years, *S. bidentatum, S. iwatense*, and *S. aokii* were caught more abundantly than *S. nikkoense* or *S. daisense. Simulium bidentatum, S. iwatense*, and *S. aokii* were abundant in autumn and only *S. nikkoense* was abundant in midsummer. Only one *S. daisense* individual was captured, in October of each year.

2. *Source of ingested blood*

Of the 970 individuals captured, there were 100 flies engorged with blood (Table 1). Engorged individuals of *S. bidentatum* and *S. iwatense* were caught only in autumn, and their rates of engorged individuals were 8.1% (31/383), 9.9% (29/292), respectively. In contrast, engorged individuals of *S. nikkoense* were captured only in midsummer and the rate was 81.8% (18/22). Those of *S. aokii* were captured in summer and autumn and the rate was 8.1% (22/271). In 81 of the engorged flies, the source of the blood ingested was examined by ELISA, and blood sources of 20 flies were identified (Table 2). Seventeen flies belonging to four species, *S. bidentatum, S. iwatense, S. aokii,* and *S. nikkoense*, had sucked blood from

Table 1. Number of black flies collected with a light trap at a deer shed in Morioka, Iwate, Japan, in 1995 and 1996.

1995

Species	June	July	August	September	October	Total
S. bidentatum	0	0	0	3	362*(31)	365 (31)
S. iwatense	4	0	0	4	117 (15)	125 (15)
S. aokii	6	2 (2)	0	1	177 (15)	186 (17)
S. nikkoense	1	8 (8)	11 (10)	0	1	21 (18)
S. daisense	0	0	0	0	1	1
Total	11	10 (10)	11 (10)	8	658 (61)	698 (81)

1996

Species	June	July	August	September	October	November	Total
S. bidentatum	2	0	0	0	8	8	18
S. iwatense	0	1	1	0	128 (13)	37 (1)	167 (14)
S. aokii	0	0	0	0	27 (2)	58 (3)	85 (5)
S. nikkoense	0	0	0	0	1	0	1
S. daisense	0	0	0	0	1	0	1
Total	2	1	1	0	165 (15)	103 (4)	272 (19)

* Four black flies infected with filariae are included.
Numbers of engorged individuals are in parentheses.

Table 2. Source of blood ingested by black flies collected with a light trap at a deer shed in Morioka, Iwate, Japan, in 1995 and 1996.

Species	Number of individuals collected	Number of engorged individuals	Blood source identified as			Blood source not identified
			Deer	Cattle	Sheep	
S. bidentatum	383	31 (27)	2	1	0	24
S. iwatense	292	29 (18)	1	1	0	16
S. aokii	271	22 (18)	4	0	0	14
S. nikkoense	22	18 (18)	10	1	0	7
S. daisense	2	0 (0)	no test	no test	no test	no test
Total	970	100 (81)	17	3	0	61

Numbers of individuals examined are in parentheses.

deer and all but *S. aokii* sucked blood from cattle as well. No black flies had sucked blood from sheep.

3. *Filarial infection*

Four black flies infected with filariae were collected on October 1–2, 19–20, 25–26, and 28–29 in 1995. No black flies infected with filariae were collected in 1996. All filariae found were first-stage larvae, and they were detected only in the pectoral muscle of *S. bidentatum*. The caudal portion of the filaliae curled (Fig. 1). The mean body length was 116.8 μm (range, 80.1 to 133.1 μm), and the mean maximum body width was 10.5 μm (range, 6.0 to 14.3 μm) in nine individuals.

Discussion

Our results showed that *S. bidentatum*, *S. iwatense*, *S. aokii*, and *S. nikkoense* sucked blood from deer and that *S. bidentatum* was naturally infected with first-

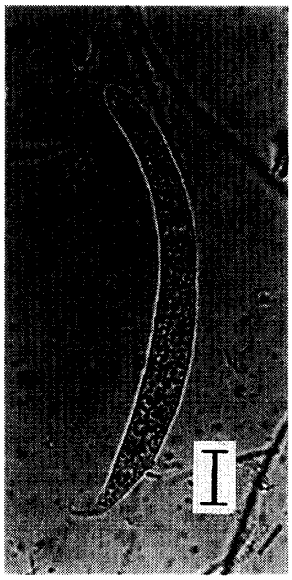

Fig. 1. First-stage larva of a filaria found in the thorax of *S. bidentatum* collected with a light trap at a deer shed in Morioka, Iwate, Japan.
Scale bar indicates 10 μm.

stage filarial larvae in the pectoral muscle.

Sasaki et al. (1986,1988) confirmed with the use of ELISA that *S. japonicum, Twinnia canivora, Distosimulium daisetsense*, and *Cnetha uchidai* sucked blood from deer in Hokkaido. We confirmed four new species that sucked blood from deer. Our result showed that *S. bidentatum, S. iwatense*, and *S. nikkoense* had sucked blood from cattle as well. Deer have pathogens in common with cattle (Imada *et al.*, 1996). These black flies might carry pathogens between deer and cattle.

Many kinds of black flies carry filariae (Takaoka, 1990, 1994; Takaoka *et al.*, 1978, 1989, 1992). In our survey, uninfective-stage larvae of filariae were detected in the pectoral muscles of *S. bidentatum* collected in October 1995. This is the first report of natural infection with filarial larvae in a population of black flies that probably sucked blood from Japanese deer, furthermore, this is the first report of such natural infection of *S. bidentatum* in Iwate Prefecture. A previous survey at Shizukuishi-machi, a town near Morioka City, showed that *S. iwatense, S. aokii, S. nikkoense*, and *S. daisense* were infected with *Onchocerca* larvae (Takaoka *et al.*, 1992). Therefore, with the addition of *S. bidentatum*, a total of five kinds of black fly in the vicinity of Morioka may have been infected with filarial larvae.

As to whether *S. bidentatum* becomes a vector of this filaria, it is necessary to confirm whether filariae can grow to the infective-stage in the body of the black fly before the bloodsucking activity of the black fly stops. *S. bidentatum* are common species in Honshu and Kyushu areas (Takaoka, 1994; Takaoka *et al.*, 1989, 1992; Shogaki *et al.*, 1956), and more detailed work on them in the epidemiology of filariae is requred.

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摘　要

ニホンジカから吸血するブユ相とフィラリア感染

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ニホンジカを加害するブユの種類相とブユの病原媒介能を明らかにするため，1995年と1996年に岩手県盛岡市東北農試場内のニホンジカを飼育しているシカ舎でライトトラップを用いてシカから吸血するブユを捕獲し，そのフィラリア感染状況を調査した．*Simulium bidentatum, S. iwatense, S. aokii, S. nikkoense, S. daisense* の5種類，計970個体のブユが捕獲され，これらのうち *S. daisense* を除く4種はシカから吸血した．*S. bidentatum, S. iwatense, S. nikkoense* はシカと牛から吸血した．*S. bidentatum* の胸筋でフィラリア幼虫が確認された．ブユがシカとウシの間で病原を媒介する可能性がある．