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The Blood Source of *Tabanus nipponicus* in Hokkaido, Japan (Diptera : Tabanidae)

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Abstract

All the 4,413 individuals of *Tabanus nipponicus* was collected by CO_2-lured, cow-baited and chicken-baited mosquito-net traps and sweeping at the three pastures in Hokkaido during pasturing periods of 1990 and 1991. Of these, 203 individuals (4.6%) were engorged.

The ELISA method was applied to determine the blood meal source and 44 blood meals (21.7%) were determined their sources. Of these sources, cattle was the most utilised host (39 indiv., 88.6%). Other domestic animals were not so numerous and no wildlife except the sika deer was chosen as the host of *T. nipponicus*. The results suggest that the role of *T. nipponicus* in the mechanical transmission of some pathogenic organisms such as the leukemia virus and *Babesia* of pasturing cattle will be important. Therefore, a more detail survey on the role of *T. nipponicus* in epidemiology is required.

Introduction

Blood meal identification for tabanid flies and other haematophagous Diptera is important in the study of arthropod-born diseases and bionomics of of these dipterous insects.

Several serological techniques have been used to detect host-specific blood meals. Of these techniques, enzyme-linked immunosorbent assay (ELISA) is considered to be the most sensitive and useful method and is applied to determine their blood meals for many kinds of haematophagous Diptera such as mosquitoes[1),2)] and black flies[8)].

Even though tabanid flies include serious vector species of various arthropod-born diseases such as *Chrysops* spp. of loiasis, few studies have been done on determination of their blood meal sources or hosts[9),5),3)].

In hokkaido, Japan, the role of tabanid flies as a vector of diseases in both humans and domestic animals is not very high, but some species are thought to be the mechanical transmitters of several diseases and parasites, such as leukemia of cattle[6)] and bovine *Babesia* sp[10)]. On the other hand, it is well known that the tabanid flies cause reduction of daily gain in both beef cattle and raising cattle and milk production for dairy cattle due to both blood loss and irritation[4)]. These serious effects for animal husbandry are seen especially in the large pastures near the foot of mountains.

In the present paper, the authors report the results from blood meal analyses performed on 203 engorged samples out of collected 4,413 individuals of *T. nipponicus*, the most predominant tabanid species in the pastures of Hokkaido, Japan.

Materials and Methods

1. Study area

The investigation was concentrated on three pastures, namely Yachiyo, Naitai and Shizunai. Yachiyo and Naitai are situated in the Tokachi district (central part) and Shizunai is located in the

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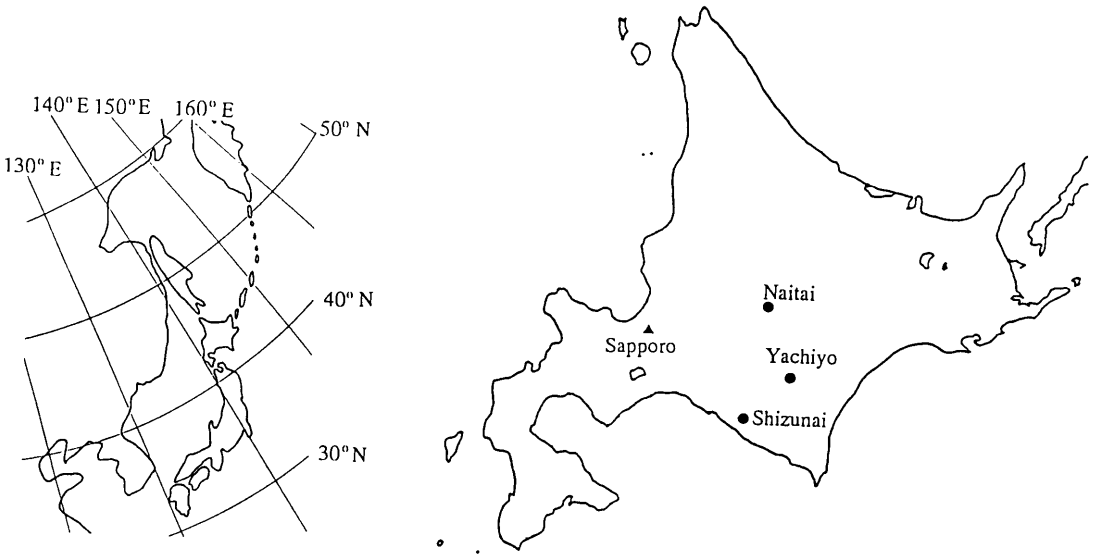

Fig 1. Map of Hokkaido, Japan showing the location of the study areas.

Hidaka district (southeastern part) of Hokkaido (Fig.1). It covered the pasturing periods in 1990 and 1991 at Yachiyo and in 1991 at Naitai and Shizunai.

2. Collecting method of the fly and blood meal

CO_2 lured mosquito-net traps[7] were mainly used to collect *T. nipponicus* at the three pastures. The cattle baited and chicken baited mosquito-net traps were also used at Shizunai. Collection of resting flies by a sweep net was performed at Yachiyo.

The collected flies were kept at low temperature in an ice box and transferred to the laboratory and dissected to remove their mid guts that containing blood meals. Removed mid guts containing blood meals were put into micro tubes individually and kept in a deep freezer at -80℃ until the assay.

3. ELISA methods for determination of the blood meals

The detection performed by the method described by Sasaki[8] with slight modifications. Briefly, test material and both positive (species serum) and negative controls (heterologous serum) were diluted with washing buffer (0.1% Tween 20 PBS solution), and 40 μl were poured into the wells of the micro-titre plate. The plate was incubated over night at 4℃. Subsequently, coating buffer (0.2 % heterologous serum in washing buffer) was added for neutralization of polystyrene material not covered by antigen. After rigorous washing with washing buffer, 40 μl of antiserum at working dilution was added and incubated 2 hours at 37℃. Forty μl of conjugate (goat-anti-rabbit IgG-PO) at working dilution was added after rigorous washing and incubated 2 hours at 37℃. Then the wells were emptied and washed rigorously. After washing, 40 μl of substrate (40 mg of *o*-phenylen diamine dissolved in 100 ml of citric acid-Na_2HPO_4 buffer plus H_2O_2) was added and incubated 45 minutes in a dark box at room temperature. The results were assessed either visually or by an ELISA meter (Sanko Junyaku Co., My Reader) after stopping the reaction by the addition of 4N sulfuric acid.

Seven kinds of forensic commercial antisera produced from rabbit, namely anti-brown bear, -sika deer, -cattle, -horse, -goat, -sheep and -human IgG (Cappel Co.), were used in this study.

Results and Discussion

All the 4,413 individuals of the fly were collected by the trap and sweeping methods. Of these methods, most individuals were collected by CO_2 lured mosquito-net traps (n=3,963, 89.8%). Chicken baited mosquito-net traps were not effective to collect the fly (only 6 indiv. collected). Even the authors

included the nonhungry flies with the engorged ones in the present study, only 203 individuals (4.6%) were classified to be engorged. The engorged ratios ranged from 1.90 (CO_2 lured mosquito-net trap at Yachiyo in 1990) to 14.3% (sweeping at Yachiyo in 1990) for each method (omitting the flies collected the chicken baited traps) (Table 1).

The engorged ratio obtained in this study was not very high but it seems to be a reasonable result. The CO_2 lured mosquito-net trap method is one of the most popular and effective methods to collect the haematophagous dipterous insect in Japan, however, this trap has the characteristic of luring mainly hungry individuals.

Table 1. Number of *T. nipponicus* collected, engorged and determined their blood meal sources.

Year	1990		1991		1991	1991	1991		Total
Locality	Yachiyo		Yachiyo		Naitai		Shizunai		
Method*	CO_2	Sw	CO_2	Sw	CO_2	CO_2	Cow	Chicken	
Collected	2524	7	223	17	55	1158	420	6	4413
Engorged	48	1	7	2	5	111	29	0	203
%	(1.9)	(14.3)	(3.1)	(11.8)	(9.1)	(9.6)	(6.9)	(0)	(4.6)
Determined	20	1	2	1	1	12	6	0	44
%	(41.7)	(100)	(28.6)	(50)	(20)	(10.8)	(20.7)		(21.7)

*CO_2 : CO_2 lured mosquito-net trap ; SW : sweeping ; Cow : cow baited mosquito-net trap Chicken : chicken baited mosquito-net trap.

A total of 44 blood meals out of 203 (21.7%) was determined their origin (Table 2). The ratios of determination were varied from 10.8% to 100% according to the collecting methods and year of survey. The sweeping method showed the highest ratio (66.7%) and CO_2 lured mosquito-net trap method showed the lowest (20.5%).

Cattle (n=39, 88.6%) was the most utilized as blood source animal for the fly at the surveyed pastures. Other domestic animals were not used so much and wild animals except sika deer were not used as the blood source of *T. nipponicus* at the pastures (Table 2).

Table 2. Number of *T. nipponicus* determined their blood meal sources.

Blood source	Namber	(%)
cattle	34	(77.3)
horse	1	(2.3)
sheep	2	(4.5)
deer	3	(6.8)
cattle+horse	2	(4.5)
cattle+sheep	1	(2.3)
cattle+deer	1	(2.3)
Total	44	(100.0)

This can may have been due to the abandunce of domestic animals and wild animals in and surroundings of the investigated pastures.

The low determination rates obtained in this study suggest the possibility that *T. nipponicus* get their blood meal from other wild animals that detectable procedure. However, other factors such as the very small volume of examined blood meal may also be relevant.

The fact that the determined main blood source animal was cattle suggests that the role of *T. nipponicus* in the mechanical transmission of some pathogenic organisms such as virus of leukemia and *Babesia* of pasturing cattle will be important. Therefore, more detailed surveys on the role of *T. nipponicus in* epidemiology are required.

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要　約

1990年と91年に、炭酸ガス誘引・ウシおとり・ニワトリおとり各蚊帳トラップならびにすくい捕り法で得られた、放牧地における吸血昆虫の優占種であるニッポンシロフアブ 4,413 個体を調査し、4.6% にあたる 203 個体から blood meal (消化管内血液) を採取した。

それらの blood meal の由来を酵素抗体法 (ELISA) で検討し、21.7% にあたる 44 個体分を判明することができ、ウシが 39 個体 (88.6%) と最も多く吸血源動物として利用されていたことが判った。野生動物では、エゾシカが利用されていることが判明したが、わずかであった。

以上のことから、ウシの白血病やピロプラズマ症などのmechanical transmitter (機械的伝播者) としてのニッポンシロフアブの役割が推測され、今後の疫学的調査の必要性が示唆された。