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METAZOAN PARASITES OF SIKA DEER FROM EAST HOKKAIDO, JAPAN AND ECOLOGICAL ANALYSES OF THEIR ABOMASAL NEMATODES

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ABSTRACT: Metazoan parasites of 50 sika deer (*Cervus nippon yezoensis*) collected in March 1991 in the Ashoro District in east Hokkaido, Japan, were evaluated. Ten species of helminths and three species of ectoparasites were obtained. Estimated abundance of males of two species of abomasal nematodes, *Spiculopteragia houdemeri* and *Rinadia andreevae*, were positively correlated with each other, and were overdispersed; *S. houdemeri* followed Poisson's and *R. andreevae* followed a negative binomial distribution. No significant relationship was detected between the estimated abundance of males of these two nematode species and nutritional condition of the hosts. Using a general linear model, the fourth-root transformed estimated abundance of male *S. houdemeri* was influenced by the main effects of host sex and age. This phenomenon was attributable to the ecological and behavioral features of the deer. The low diversity of the abomasal nematode community was regarded as the result of the extinction of some species of nematodes on Hokkaido Island.

Key words: Sika deer, *Cervus nippon yezoensis*, metazoan parasite fauna, abomasal nematode, *Spiculopteragia houdemeri*, *Rinadia andreevae*, ecological analysis.

INTRODUCTION

Although there are many studies on the metazoan parasite fauna of sika deer, *Cervus nippon,* including Ovcharenko (1963), Kotrla and Kotrly (1977), and Yokohata and Suzuki (1993), little information is available on the metazoan parasite fauna of one subspecies of the sika deer in Hokkaido Island, *C. nippon yezoensis* (Ohbayashi, 1966; Yamaguchi et al., 1977). These reports include two species of abomasal nematodes specific to cervids, *Spiculopteragia houdemeri* and *Rinadia andreevae.* The abomasal nematodes of cervids have been studied with respect to pathogenicity in the deer, interactions between their abundance and host population density, and relationships between the abundance and host or habitat characteristics (Gray et al., 1978; Moore and Garner, 1980; Demarais et al., 1983; Waid et al., 1985, Stubblefield et al., 1987; Pence, 1990). However, these studies were concerned only with the abomasal nematodes of cervids in North America; studies on the abomasal nematodes of other cervids often have been limited to taxonomy.

Herein we examined the helminth and ectoparasite fauna of wild sika deer shot in Ashoro District in east Hokkaido (Suzuki and Ohtaishi, 1993; Suzuki et al., 1996, Yokoyama et al., 1996). Results of an ecological analysis on two species of abomasal nematodes also are evaluated.

MATERIALS AND METHODS

Twenty-one male and 29 female sika deer were shot by hunters between 13 and 18 March 1991 in Ashoro District, in the eastern part of Hokkaido, Japan (43°15′N, 143°30′E). Elevation varies from 80 to 1,636 m in this district. Most (84%) of the area is mountainous and covered with forest, including natural deciduous broad-leaved forest dominated by *Quercus mongolica, Ulmus davidiana, Betula ermanii* and *Acer monomaxim*, by natural mixed stands including *Abies sachalinensis, Picea jezoensis* and *P. glehmii*, and by artificial forest of *A. sachalinensis, Larix leptolepis* and *P. jezoensis*.

The body cavity, hocks, trachea, lungs, bile duct, liver and esophagus of each deer were examined macroscopically on the day it was shot. The digestive tract from rumen to colon of each deer was isolated from other viscera by ligation, removed and frozen for examination in the laboratory. Ectoparasites were collected with visual examination and fixed in 70% alcohol.

In the laboratory, the mucosa of the rumen, reticulum and omasum were examined macroscopically for parasites. One-tenth or one-twentieth of the contents of the 48 abomasa available and each of upper, middle and lower parts of the five small intestines were examined for helminths. Two abomasa were punctured when the deer were shot. Trematodes and cestodes were fixed in 70% ethanol, stained with acetocarmine (Ash and Orihel, 1987) and mounted in Canada balsam. Nematodes were fixed in 10% formalin and cleared in glycerin alcohol. Ectoparasites were washed in 10% potassium hydroxide (37 C, 1.5 to 2 hr), neutralized with about 10% acetic acid and mounted in gum chloral. Representative parasite specimens collected in this study were deposited in the collection of the Laboratory of Parasitology, School of Veterinary Medicine, Hokkaido University, Sapporo 930, Japan (Accession numbers: 708–713 and 2901–2911).

We estimated the host age as fawn, yearlings, or more than 2 yr old according to Ohtaishi (1980). The method of Ohtaishi (1980) and Koike and Ohtaishi (1985) was used to distinguish the age of those deer >2-yr-old.

The terms prevalence, intensity, and abundance follow the definitions of Margolis et al. (1982).

Chi-square (Yonezawa et al., 1988) and Mann-Whitney's *U* (Kasuya and Fujita, 1984) statistics were used to determine the significant differences in the prevalence and abundance of each parasite, respectively, among different host age groups and between both host sex groups. Kendall's tau rank correlation coefficient (Kasuya and Fujita, 1984) was used to evaluate the relationships between the estimated abundance of male *S. houdemeri* and that of *R. andreevae*; adult females of these two species are indistinguishable from each other, so that the females were counted only on the hosts from which one species was obtained on the male nematodes. A parasite was considered overdispersed when the variance was significantly greater than the mean (chi-square analysis); this was measured by the Morisita's Iδ index (Morisita, 1959) and the negative binomial parameter *k* (Bliss and Fisher, 1953). If the distribution pattern did not follow the negative binomial distribution (k-value$\rightarrow\infty$), adaptation to Poisson's distribution was examined by a chi-square statistic. The estimated abundance of males of the two species of nematodes was evaluated with Kendall's tau coefficients on the relationship with nutritional condition of each deer; nutritional condition was based on the concentration of bone marrow fat (%) in the femur (Yokoyama et al., 1996). For estimated abundance, the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., 1985) was performed to detect the main and interactive effects of two qualitative and one quantitative factors: sex (male and female); age (fawn, yearlings, 2 yr, 3 yr, and ≥4-yr-old); and total body weight (kg) of the hosts. The models included three main effects of these factors and all four possible interaction effects. The analysis using GLM was performed based on the transformed estimated abundance with the fourth root transformation; this was done only with species for which the transformed estimated abundance had the normal distribution pattern, based on a chi-square analysis. Use of the term significant or significantly in this study refers to statistical significance at $P < 0.05$.

RESULTS

One species of trematode, one cestode, eight nematodes and three ectoparasites were collected (Table 1). No parasites were recovered from body cavity, hocks, trachea and lungs of each deer. All deer were infected with one to four species of mature helminths. On Anoplocephalidae gen sp., only one fragmental strobilla was obtained from an intestine which was broken accidentally. Both *Gongylonema* sp. recovered were female. From the 49 abomasa, 0 to 1,820 4th stage nematode larvae were obtained. Twenty 5th stage larvae and 10 Ancylostomatidae larvae each were detected from one abomasum, respectively. No parasites differed significantly in prevalence or abundance from different host age groups and between both host sex groups, except for *R. andreevae,* which had a lower prevalence in younger deer (fawn and yearlings combined, 46%) than in older hosts (≥2-yr-old combined, 77%; df = 1, $\chi^2 = 5.03$) (Table 2).

Spiculopteragia houdemeri and *R. andreevae* had very high prevalences (Table 1), and there was a positive rank correlation between the estimated abundance of males of these two species in each of the 48 hosts (Kendall's $\tau = 0.34$). Mean abundance and its variance of male *S. houdemeri* were 171.0 and 17929.2, respectively, so that the ratio of variance to mean is 1: 104.8 ($k\rightarrow\infty$). Morisita's Iδ of males of this species was 1.61, and the estimated abun-

TABLE 1. Prevalence, intensities and abundance of species of metazoan parasites from the sika deer in Ashoro District in Hokkaido, Japan, March 1991.

Species of parasites	Infection sites	Prevalence	Intensity Mean ± SE	Intensity Range	Abundance Mean ± SE	Abundance Total individuals
Trematodes						
Dicrocoelium dendriticum	bile duct	6/50 (12)[a]	1.8 ± 0.4	1–4	0.2 ± 0.1	10
Cestodes						
Anoplocephalidae gen. sp.	intestine	unknown	unknown		unknown	
Nematodes						
Gongylonema sp.	cervical esophagus	2/35 (5.7)	1.0 ± 0.0	1	0.1 ± 0.0	2
Spiculopteragia houdemeri[b]	abomasum	48/48 (100)	171.0 ± 19.5	30–620	171.0 ± 19.5	8,210
Rinadia andreevae[b]	abomasum	30/48 (63)	33.4 ± 4.8	10–160	21.7 ± 4.5	1,040
Ostertagia ostertagi	abomasum	1/48 (2.1)	NC[c]	20	0.4 ± 0.4	20
Mecistocirrus sp.	abomasum	1/48 (2.1)	NC[c]	60	1.3 ± 1.3	60
Nematodirus helvetianus	upper intestine	1/5 (20)	NC[c]	20	4.0 ± 4.0	20
Capillaria bovis	lower intestine	2/5 (40)	190.0 ± 10.0	180–200	76.0 ± 46.6	380
Trichuris sp.	cecum and colon	1/5 (20)	NC[c]	10	2.0 ± 2.0	10
Ectoparasites						
Haemaphysalis japonica	skin	15/29 (52)	unknown		unknown	
Solenopotes sp.	skin	10/29 (35)	unknown		unknown	
Trichodectidae gen. sp.	skin	1/29 (3.4)	unknown		unknown	

[a] Number of infected hosts/number of hosts examined (% infected).
[b] Only males were counted.
[c] Not counted.

TABLE 2. Prevalence and estimated intensity and abundance of male worms of two species of abomasal nematodes in different age and sex groups of sika deer from Ashoro District in Hokkaido, Japan, March 1991.

Age of hosts in yr	Number sampled	*Spiculopteragia houdemeri* Prevalence	*Spiculopteragia houdemeri* Abundance (mean ± SE)	*Rinadia andreevae* Prevalence	*Rinadia andreevae* Abundance (mean ± SE)
			Males		
0	5	5 (100.0)[a]	84.0 ± 14.7	4 (80.0)	12.0 ± 4.9
1	6	6 (100.0)	158.3 ± 40.4	1 (16.7)	3.3 ± 3.3
2	6	6 (100.0)	166.7 ± 44.6	4 (66.7)	38.3 ± 16.8
3	1	1 (100.0)	460.0	1 (100.0)	80.0
≥4	3	3 (100.0)	273.3 ± 174.9	2 (66.7)	56.7 ± 51.7
			Females		
0	6	6 (100.0)	153.3 ± 50.6	4 (66.7)	15.0 ± 6.2
1	5	5 (100.0)	74.0 ± 22.5	1 (20.0)	4.0 ± 4.0
2	4	4 (100.0)	210.0 ± 96.0	4 (100.0)	20.0 ± 7.1
3	5	5 (100.0)	236.0 ± 61.4	4 (100.0)	80.0 ± 11.2
≥4	7	7 (100.0)	178.6 ± 38.2	5 (71.4)	24.3 ± 8.1

[a] Number of infected hosts (%).

dance of them followed a Poisson's distribution (χ^2 = 9.80, df = 6). Mean abundance, its variance and the ratio of them of *R. andreevae* were 21.7, 955.4 and 1:44.0 (k = 0.21), respectively. Morisita's Iδ of males of this species was 3.15. The index of nutritional condition of the hosts did not indicate no significant rank correlation to the estimated abundance of male of both nematode species (Kendall's τ = −0.06 on *S. houdemeri*; τ = −0.05 on *R. andreevae*).

Forty to 580 female nematodes were obtained from the hosts in which male *R. andreevae* were not collected. We obtained 2,210 male *S. houdemeri* and 3,410 females from these hosts; the sex ratio differed significantly from 1:1 (χ^2 = 12.96). If all of these females were *S. houdemeri*, the sex ratios were ranged from 1:0.8 to 1:3.6 ($\bar{x}$ ± SE = 1:1.74 ± 0.80).

TABLE 3. Factorial ANOVA (GLM) with main and interactive effects of sex, age and total body weight of host individuals for forth-root transformed estimated abundance of males of *Spiculopteragia houdemeri* in sika deer from Ashoro District in Hokkaido, Japan, March 1991.

Factor	Degrees of freedom	*F* value
Sex	1	4.18[a]
Age	4	2.88[a]
Sex-age	3	1.14
Total body weight	1	0.07
Sex-total body weight	1	3.30
Age-total body weight	4	2.69
Sex-age-total body weight	3	0.70

[a] Significant at $P < 0.05$.

The fourth-root transformed estimated abundance of male *S. houdemeri* had normal distribution pattern (χ^2 = 7.60, df = 3), but that of *R. andreevae* did not (χ^2 = 120.03, df = 3). Based on the factorial analysis of variance, the general linear models used were significant for the transformed estimated abundance of male *S. houdemeri* (F = 2.37). The only two main effects of the host sex and age were significantly related to the transformed abundance of male *S. houdemeri* (Table 3).

DISCUSSION

With the exception of previous reports of *S. houdemeri, R. andreevae* and *Trichuris* sp. (Ohbayashi, 1966; Yamaguchi et al., 1977), this is the first report of other species of parasites from sika deer in Hokkaido Island. Only two female nematodes of the genus *Gongylonema* were obtained. Since *Gongylonema pulchrum* has been found from another subspecies of sika deer (*C. nippon centralis*) in Honshu Island in Japan (Yokohata and Suzuki, 1993), and also in cattle in Hokkaido Island (Suzuki et al., 1992), it is probable that the present *Gongylonema* sp. is also *G. pulchrum*.

The two common species of abomasal nematodes, *S. houdemeri* and *R. andreevae*, have been reported previously in sika deer from Hokkaido Island (Ohbayashi, 1966; Yamaguchi et al., 1977). The nutritional condition of the sika deer was not significantly related to the estimated abundance of males of these two abomasal nematodes. These results are similar to the findings of Waid et al. (1985) on white-tailed deer (*Odocoileus virginianus*) from the Edwards Plateau in Texas (USA) and of Moore and Garner (1980) on mule deer (*Odocoileus hemionus*) in southwest Texas. Waid et al. (1985) compared the abundance of nematodes with various nutritional conditions including "optimal," "transitional" and "suboptimal," and detected little relationship between the abundance of the nematodes and these nutritional conditions. However, most deer examined in the present study were in optimal nutritional condition (Yokoyama et al., 1996), and the sika deer population in Ashoro District in Hokkaido had high fertility, including a high pregnancy rate, low puberty age and frequent ovulation of yearlings (Suzuki and Ohtaishi, 1993).

Based on the factorial analysis of variance, the estimated abundance of male *S. houdemeri* was related to sex and age of the host. The interpretation of this result was restricted to the males by the uneven sex ratios, but at least on the males, this may be attributable to both physiological differences between the sexes of hosts and to some ecological and behavioral differences between males and females observed in some cervids of the genus *Cervus* (Clotton-Brock, 1982; Koga and Ono, 1994). Based on these studies, the males in their rutting season vary from dominant individuals ocuppying wide territories and enclosing many females in the territories to inferior ones without territories and females so that the social status of males are more diverse than those of females which do not form such hierarchichal social system in the season. These differences affect their patterns of annual habitat utilization and also qualitative and quantitative characteristics of their food habits. Hence, these differences also would affect their chance of becoming infected with nematodes. Furthermore, these behavioral and ecological differences between sexes also may affect the resistance of the hosts to infection by helminths. For example, Demarais et al. (1983) observed the increase in the number of abomasal nematodes of adult male white-tailed deer during rutting and suggested that this phenomenon might be attributable to a breakdown of resistance, possibly associated with their stress during this season.

The abomasal nematode community of sika deer in Hokkaido Island consists of only two common species (*Ostertagia ostertagi* and *Mecistocirrus* sp. are regarded as immigrant species from domestic ruminants). Certain other species of abomasal nematodes are reported in continental sika deer populations (Ovcharenko, 1963; Kotria and Kotrly, 1977). Certain abomasal nematode communities of cervids composed of small number of species have also been reported in some regions in the USA (Prestwood et al., 1976; Gray et al., 1978; Davidson and Crow, 1983; Waid et al., 1985; Davidson et al. 1985, 1987; Stubblefield et al., 1987; Pence, 1990). Gray et al. (1978) and Pence (1990) have regarded this low diversity as the result of semiarid or xeric habitat environment in these regions resulting in very low transmission potentials for species with direct life cycles. However, this is not the case in Hokkaido Island, where humid forests have developed with sufficient precipitation. This low species diversity in the abomasal nematode community of sika deer on this island can be attributed to other reasons, which may include the local extinction of some parasites due to the small population size of this host in Hokkaido Island in the past. The population of sika deer declined almost to extinction due to heavy snow falls in 1897 and 1903 on this island, but it has since recovered (Kaji, 1990). Other temporal decreases in their population size might have occurred on this island which has been separated from the Eurasian Continent since the last glacial age (Kamei et al., 1988). Such extinction has been observed on small islands or at low host densities in certain other mammalian parasite communities (Kisielewska, 1970; Montgomery and Montgomery, 1988; Asakawa et al., 1991). Kotrla and Kotrly (1977) found that the composition of the helminth fauna of some ruminants, including sika deer, introduced into Czechoslovakia were relatively poor compared with those in their native region. Reports on the helminth fauna of sika deer in natural and introduced populations include *Ashworthius sidemi,* which is specific to cervids (Kotrla and Kotrly, 1977). It is probable that some parasite species, such as *A. sidemi,* may have become extinct during the decrease in the population size of this host due to temporal change in Hokkaido Island.

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