実験動物化ナキウサギの反芻獣消化管内線虫に対する感受性

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Susceptibility of Laboratory-Reared Afghan Pika, *Ochotona rufescens rufescens* (Lagomorpha: Ochotonidae), to Gastro-Intestinal Nematodes of Ruminants

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ABSTRACT. It was shown that laboratory-reared Afghan pika, *Ochotona rufescens rufescens*, was susceptible to gastro-intestinal nematodes of ruminants; *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus axei* and *T. colubriformis*. *T. axei* and *T. colubriformis* were recovered up to 70 days post-inoculation and their infection rates were high. Only two gravid females of *O. circumcincta* were recovered from the pikas necropsied on day 20 post-inoculation. *H. contortus* was recovered until 20 days post-inoculation but the recovery rate was very low and mature worm was not found. No worm was detected from 45 day onward. *Strongyloides papillosus, Bunostomum trigonocephalum, Chabertia ovina, Mecistocirrus digitatus* failed to become established in the pika.—KEY WORDS: *Haemonchus, Ochotona rufescens rufescens, Ostertagia,* susceptibility, *Trichostrongylus.*

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Experiments using domestic ruminants are cost-consuming, therefore, not many defined experiments on nematodes of ruminants have been carried out. Various efforts searching for adapted nematodes of domestic ruminants to small laboratory animals have been attempted, but the results are not encouraging. Excluding the works on *Strongyloides papillosus* in rabbit [14, 15], *Trichostrongylus axei* and *T. colubriformis* in rabbit and several species of laboratory rodents, i.e., hamster, Mongolian gerbil, guinea-pig and multimammate rat [3, 7, 9, 10, 23], the recovery rates of nematodes administered in the other attempts were very low. And the hosts showed what were called "nonpermissive" as advocated by Knopf [8] even if nematodes were established in respective hosts [1, 6, 12, 21, 22, 24, 26].

In recent years, the Afghan pika, *Ochotona rufescens rufescens,* belonging to the order Lagomorpha has been established as a laboratory animal [13]. In the present study, the susceptibility of the pika to various species of gastro-intestinal nematodes of ruminants has been determined.

MATERIALS AND METHODS

Pika: The pikas, *Ochotona rufescens rufescens*, used in the experiments have originated from three males and three females given by Dr. Puget (Laboratoire de Pharmacologie et Toxicologie Fondamentales, France) in 1974 [18, 19], and bred in the Central Institute for Experimental Animals, Kawasaki, Japan [13].

Parasite: Infective larvae used for mixed infection were obtained by culturing the feces of goat naturally infected with several species of gastro-intestinal nematodes. Identification of infective larvae followed the method as described by Levine [11].

Infective larvae of *Mecistocirrus digitatus* used for pure infection were obtained by

culturing the eggs derived from adult female worms collected from an abomasum of cattle.

Infective larvae of *S. papillosus* used for subcutaneous infection were obtained by culturing the feces of heavily infected sheep. More than 90% of these infective larvae were *S. papillosus,* but a few larvae of *Haemonchus contortus* and *Trichostrongylus* spp. were contaminated.

Culturing method describes as following. Feces or crushed adult female worms were mounted on a 6 cm diameter and 8 mm thick circular unglazed tile with the exception of 5 mm in width from the rim of the tile. The tile was put into a 10 cm diameter petri dish, and distilled water was added to almost the top of the tile. After putting on the lid, the petri dish was incubated at 26°C for 7 days. At the end of incubation, nearly all larvae had migrated to water. After counting and identification, larvae were held at 4°C for 4 to 8 hours.

Worm recovery: Pikas were sacrificed under anesthesia, and their stomachs, small and large intestines were removed into physiological saline, and opened longitudinally. From the solution containing gastrointestinal wall and contents, the worms were collected and counted under the dissection microscope, and fixed with 10% formalin solution. Then, the specimens were identified and their degree of development were examined under the microscopy.

RESULTS

In mixed infection, five 2-month old and four 13-month old male pikas were orally infected with 20000 and 10000 infective larvae consisting of several species, respectively. Dosage consisting of infective larvae and results of mixed infection are shown in Tables 1 and 2, respectively. Four species of nematodes, *H. contortus, Ostertagia circumcincta, T. axei* and *T. colubriformis* were able to be established in pikas. Except for *T. colubriformis* which was recovered from the small intestine and also from the large intestine, the other 3 species were recovered from the stomach. Recovery rates of *H. contortus* were very low (0–1.5%) and no worm was recovered from day 45 onward. All fifth stage worms recovered up to 20 days post-inoculation were immature. *T. axei* and *T. colubriformis* were recovered throughout the course of the experiments and recovery rates were high (15.4–70.4%). Female worms of *T. axei* and *T. colubriformis* had mature eggs in their uterus from day 18 and 14 onward, respectively. Despite that infective larvae of *O. circumcincta* were not detected in an aliquot of infective larvae suspension used for mixed infection, two gravid female worms were recovered from the pikas inoculated 20000 infective larvae. It was thus considered that a few infective larvae of *O. circumcincta* were contaminated in the infective larvae suspension used for the mixed infection. No *S. papillosus,* Bunostomum trigonocephalum and *Chabertia ovina* was recovered from the pikas with mixed infection.

For the infection with *M. digitatus* alone, one 2-month old male pika was orally infected with 1600 infective larvae. For subcutaneously infection with *S. papillosus*, 3000 infective larvae were injected subcutaneously into the neck region of one 2-month old male pika. Both animals were necropsied 60 and 40 days post-infection,

Table 1. Dosage consisting of infective larvae for mixed infection[a)]

Species	%
Haemonchus contortus	67.1
Trichostrongylus spp.	12.9
Strongyloides papillosus	12.9
Bunostomum trigonocephalum	5.0
Chabertia ovina	0.7
Rhabditis sp.	1.4

a) 140 infective larvae were examined.

Table 2. Species and number of nematodes recovered at necropsy from pika simultaneously infected with several species of caprine gastro-intestinal nematodes[a)]

No. of larvae dose	Days post inoculation	*Haemonchus contortus*					*Trichostrongylus* spp.			*O. circumcincta*
		eL4	lL4	L5	Total	Recovery(%)	*T.a.*	*T.c.*	Recovery(%)#	
20000	8	ne	ne	ne	–	–	ne	1368	–	ne
20000	14	22	27	0	49	0.4	134	1185	51.1	0
20000	18	4	97	41	142	1.1	327	1139	56.8	0
20000	20+	0	23	1	24	0.2	502	1314	70.4	1
20000	20+	0	137	71	208	1.5	313	1150	56.7	1
10000	16	14	12	24	50	0.8	91	462	42.9	0
10000	20+	0	0	2	2	<0.1	50	472	40.5	0
10000	45	0	0	0	0	0.0	89	275	28.2	0
10000	70	0	0	0	0	0.0	38	161	15.4	0

eL4: early fourth stage, lL4: late fourth stage, L5: fifth stage.
T.a.: *T. axei.*, *T.c.*: *T. colubriformis*, *O. circumcincta*: *Ostertagia circumcincta*.
ne: not examined, +: dead.

$$\# \text{ Recovery}(\%)=\frac{\text{No. of } T. \textit{ axei} \text{ and } T. \textit{ colubliformis}}{\text{No. of infective larvae of } \textit{Trichostrongylus} \text{ spp.}}\times 100.$$

a) Dosage consisting of infective larvae is shown in Table 1. Larvae of *Ostertagia* was not detected in 140 larvae.

respectively. However, no worm was detected in the two pikas.

DISCUSSION

The results presented in this study showed that the pika was susceptible to *H. contortus, O. circumcincta, T. axei* and *T. colubriformis*.

H. contortus is a parasite of ruminants, but spontaneous infection in man [5] and in ground squirrel [25] has been reported. Attempts to infect with *H. contortus* in small laboratory animals; Mongolian gerbil, mouse, rabbit and chinchilla have been made. Mongolian gerbil was not susceptible [17], and in mice the worm could grow no more than the fourth stage [22]. In rabbit, *H. contortus* was established with low infectivity but only artificially exsheathed larvae were infective and a dose of at least 50000 larvae was required to produce a significant level of infection [6]. When administered exsheathed larvae, *H. contortus* completed its development in rabbit, but as a slower rate than that in sheep [6]. Following doses of up to 10000 larvae, nearly all worms were lost from days 16–21 post-inoculation [12]. Chinchilla could be infected with ensheathed larvae even small dose (2500 L3–12000 L3) and eggs were detected in feces in some animals [2].

Susceptibility of the pika to *H. contortus* resembles that of rabbit; with respect to recovery rate, development and that nearly all worms were expelled on day 20 post-inoculation. But the pika is considered to be more susceptible than the rabbit, when ensheathed larvae and smaller dose were administered.

The pika is also thought to be very susceptible to *O. circumcincta* because very small dose of larvae could produce gravid worms. It was reported that rabbit and guinea-pig were susceptible to *O. circumcincta* but the worms could not become mature [26].

Although hosts of *T. axei* and *T. colubriformis* are ruminants, experimental animals such as rabbit, Mongolian gerbil, guinea-pig

and other rodents are also susceptible [11]. It appeared in this study that the pika was very susceptible to both species of *Trichostrongylus*. As indicated by the long parasitic and patent period, moreover spontaneous cure which occur in guinea-pig inoculated *T. colubriformis* larvae after 15 days post-inoculation [20] was not observed in the pika.

There are few reports of experimental infection with *B. trigonocephalum* and *C. ovina* in laboratory animals. And it was reported that infection with *M. digitatus* in small experimental animals had not been successful [4]. We confirmed that these parasites could neither establish themselves in the pika. It has been reported that the rabbit was permissive to *S. papillosus* [14, 15]. In the present study, however, no worm was recovered from gastro-intestinal tract of only one pika examined. It could not be excluded the possibility that once established worms were expelled until day 40, or the migrated larvae were arrested in the muscles. Nwaorgu & Connan [16] reported that arrested larvae remained longer than 4–5 weeks in the muscles of rabbits. Further study is required to elucidate the susceptibility of the pika to *S. papillosus*.

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要　　約

実験動物化ナキウサギの反芻獣消化管内線虫に対する感受性：岡本宗裕・神谷正男・奥　祐三郎・大林正士・松崎哲也[1)]（北海道大学獣医学部家畜寄生虫病学講座，[1)]実験動物中央研究所）——実験動物化されたナキウサギに，反芻獣由来消化管内寄生線虫を感染させたところ，*Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus axei, T. colubriformis* で感染が成立した。*T. axei* と *T. colubriformis* は感染率が高く，感染後70日でも patent infection が持続し，spontaneous cure は見られなかった。*O. circumcincta* は，感染後20日に剖検したナキウサギから虫卵を保有した雌２虫体が回収されただけであった。*H. contortus* は，感染率は低いながらも感染後20日までは回収されたが，感染後45日以降は回収されなかった。また，回収された虫体はすべて未成熟であった。*Strongyloides papillosus, Bunostomum trigonocephalum, Chabertia ovina, Mecistocirrus digitatus* は，感染が成立しなかった。