Association of sarcoptic mange with kinship and habitat use in raccoon dogs (Nyctereutes procyonoides)

Natsuko SUGIURA1,2)*, Aki TANAKA1), Kazuhiko OCHIAI2), Toshiaki YAMAMOTO3), Tatsushi MORITA4), Takuya KATO1), Yoshi KAWAMOTO1), Toshinori OM1) and Shin-ichi HAYAMA1)

1)Laboratory of Wildlife Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan
2)Department of Basic Science, School of Veterinary Nursing and Technology, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan
3)Department of Applied Science, School of Veterinary Nursing and Technology, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan
4)Laboratory of Veterinary Parasitology, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan

ABSTRACT. Although kinship (parent-offspring or siblings) contact has been suggested as a driving factor for sarcoptic mange epizootic in raccoon dogs (Nyctereutes procyonoides), no effect has been reported. In contrast, habitat fragmentation caused by urbanization may result in a high occurrence of sarcoptic mange, because habitat fragmentation may promote contact infection by increasing the population density of raccoon dogs. The habitat distribution of raccoon dogs may therefore influence epizootic sarcoptic mange. The genetic relationship between raccoon dogs was analyzed to examine Sarcoptes scabiei transmission between kin. The relationship between S. scabiei infection and the habitat of raccoon dogs was also investigated. Seventy-five raccoon dogs from Takasaki, Gunma prefecture, were examined from 2012 to 2018; 23 were infested with S. scabiei. The genotypes were determined using 17 microsatellite loci, and the relationships were categorized into four patterns by the ML-Relate software. There was no significant difference between infested pairs and other two pairs (Chi-squared test: $\chi^2=0.034$, $df=1$, $P=0.85$). Although it was difficult to predicate because the mortality rate was unclear in this study, kinship contact does not seem to be an important factor for sarcoptic mange epizootic. S. scabiei infection rates were significantly associated with the location of village sections (OR=1.55, 95% CI=1.11–2.17, $P=0.011$). It is suggested that direct/indirect contact between individuals living closely together is an important factor for the transmission of S. scabiei.

KEY WORDS: habitat, kinship, raccoon dog, raccoptic mange, Sarcoptes scabiei

Sarcoptic mange is a parasitic skin disease caused by the mite Sarcoptes scabiei and has been reported in more than 100 mammal species [1, 12]. In Japan, there have been numerous reports on sarcoptic mange in wild mammals including wild boar (Sus scrofa), red foxes (Vulpes vulpes) [7, 17], and raccoon dogs (Nyctereutes procyonoides) [5, 7, 11, 17, 18, 21, 22]. Infested raccoon dogs often become severely debilitated and die of asthenia [5]. Previous studies analyzed the effects of epizootic sarcoptic mange on the population dynamics of raccoon dogs [5, 18, 20]. However, the main transmission factor of epizootic sarcoptic mange in raccoon dogs is still unknown.

Sarcoptes scabiei were transmitted by both direct and indirect contact among wildlife [1]. Shibata [17] suggested that direct contact between family members is one of the driving factors associated with epizootic sarcoptic mange. Several populations were probably comprised of S. scabiei infested families and pregnant females, which can be attributed to behavioral ecology. Both raccoon dog parents were responsible for raising their offspring [4, 23], and the pens were shared by parents and pups [23, 25]. Home ranges were small (about 0.5–2 km$^2$) compared to most other canids [10, 13, 24], and dispersion was relatively low (about 1–10 km) [13, 26]. Because of these behavioral characteristics, direct/indirect contact among kin (parent-offspring or siblings) was hypothesized to influence epizootic sarcoptic mange in raccoon dogs [17, 21]. Direct/indirect contact transmission of S. scabiei between kin might increase if multiple related individuals inhabit areas close to each other. To understand these transmissions, it is...
necessarily to examine the kinship of raccoon dogs affected by sarcoptic mange epizootic in a limited area.

Habitat fragmentation caused by urbanization might result in a higher occurrence of sarcoptic mange in raccoon dogs [15]. The habitat of raccoon dogs is comprised of both urban areas and rural areas [10, 14, 19]. Raccoon dogs in urban green areas had a small home range, with an overlap in home ranges and core areas [6]; the progression of habitat fragmentation might increase the direct/indirect contact between raccoon dogs. Thus, it is possible that epizootic sarcoptic mange of raccoon dogs occurred in a limited area as a result of habitat fragmentation.

The objective of this study was to investigate whether direct/indirect contact transmission of *S. scabiei* between kin was a driving factor of epizootic sarcoptic mange in raccoon dogs. The genetic relationships among raccoon dogs, *S. scabiei* infection, and the number of kin were analyzed. The effect of habitat on epizootic sarcoptic mange was also investigated for the raccoon dogs examined in this study.

**MATERIALS AND METHODS**

**Study area and Samples**

Capture survey for raccoon dogs: Twenty-eight raccoon dogs were captured in the Misato, Miyazawa, and Jumonji districts of Takasaki, Gunma Prefecture, Japan, between July 2012 and March 2018. Thirty-six single-door cage traps (81 × 25 × 30 cm; model #: 1089, Havahart, Woodstream Corp., Lititz, PA, USA) were used. Captured raccoon dogs were immobilized with medetomidine (0.04 mg/kg) and midazolam (0.2 mg/kg). Each animal was weighed, and the sex and age were determined by tooth wear and reproductive condition. Blood and mange-compatible skin lesions were collected, and microchip transponders were injected for identification of the individual animals. Atipamezole (0.25 mg/kg) and flumazenil (0.01 mg/kg) were injected to recover the animals from anesthesia and the animals were released at the capture site. This method of capture survey followed the guidelines of The Mammal Society of Japan.

Raccoon dog carcasses obtained by population control measures: Forty-seven raccoon dog carcasses were collected in the Misato, Miyazawa, and Jumonji districts of Takasaki between March 2014 and September 2018. The animals were captured as a part of pest control measures for preventing agricultural damage and were euthanized by licensed hunters on behalf of the district of Takasaki.

Each animal was dissected, and the sex and tooth wear were determined. The canine teeth were collected for age estimation if all the permanent teeth had erupted, and muscle tissue samples and mange-compatible skin lesions were also obtained. The samples were frozen at −20°C until further analyses were performed.

The age and sex of the target animals are shown in Table 1, while the months the raccoon dogs were captured are shown in Fig. 1. The capture site of each raccoon dog was plotted on a map using QGIS 2.14.

Detection of sarcoptic mange

A scalpel was used to scrape off mange-compatible skin lesions. The samples were placed in a 15% potassium hydroxide (KOH) and 40% dimethyl sulfoxide (DMSO) solution, and then mounted on glass slides for microscopic observation. Isolated mites were determined based on Fain’s criteria [3]. Sarcoptes scabiei infestation in raccoon dogs was confirmed by isolating *S. scabiei* from skin lesions. In cases with only mild symptoms, it was hard to isolate *S. scabiei* [16]. Therefore, even if mites were not detected in the skin sample, the raccoon dog was included from all further analysis as an infested individual.

Kinship analysis

The DNA was extracted from blood and muscle tissue samples using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), and DNA extracts were stored at 4°C. The Canine Genotypes Panel 1.1 Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to amplify 18 microsatellite loci with a polymerase chain reaction (PCR). The PCR was performed with 4.5 µl Primer Mix, 4.5 µl Master Mix (Buffer, dNTP, Phusion Hot Start DNA Polymerase), and 1 µl DNA extract (1 ng/µl). The thermal cycling conditions were as follows: 98°C for 3 min; 30 cycles of 98°C for 15 sec, 60°C for 75 sec, and 72°C for 30 sec; and 72°C for 5 min. The fluorescent PCR amplicons were analyzed in an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the microsatellite genotypes were determined by Gene Mapper v.4.0 (Applied Biosystems).

The number of alleles, the observed heterozygosity (*H*<sub>o</sub>), and the expected heterozygosity (*H*<sub>e</sub>) for each locus were calculated using the software Cervus 3.0. The software ML-Relate, which calculates the maximum likelihood estimates of pairwise genetic relatedness and relationships, was used to categorize each relationship in one of four patterns: parent-offspring (PO), full-siblings (FS), half-siblings (HS) and unrelated (U).

The relationships categorized as PO or FS were defined as kinships. A chi-squared test was performed to compare the relationship between *S. scabiei* infection and the kinships using the statistical software R 3.6.3. The kinship relationships of each infested individual were shown on a map using QGIS 2.14.

**Table 1.** The sex and age of the target raccoon dogs

|        | Non-Infested | Infested | Infested (without mites) | Total |
|--------|--------------|----------|--------------------------|-------|
| Sex    | Male         | 29       | 7                        | 1     | 37    |
|        | Female       | 23       | 11                       | 4     | 38    |
| Age    | Young        | 21       | 6                        | 0     | 27    |
|        | Adult        | 31       | 12                       | 5     | 48    |

Age was determined from tooth wear. If all permanent teeth were not fully developed, the raccoon dogs were determined as “young”.

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Habitat analysis

Village sections were categorized by each capture point of the raccoon dogs. The village sections with a very limited population size were integrated with adjacent village sections; a total of seven sections were analyzed. A logistic regression was performed to analyze the association between *S. scabiei* infection (the response variable) and the location of village sections (the explanatory variable) to determine whether the habitat of raccoon dogs was associated with epizootic sarcoptic mange. The statistical package Stata 14 was used for this analysis.

RESULTS

Detection of sarcoptic mange

In total, 23 out of 75 raccoon dogs showed clinical signs of mange-compatible lesions. Mites were isolated from 18 of the raccoon dogs with clinical signs. All mites were determined as *S. scabiei* (Fig. 2).

Kinship analysis

The microsatellite genotypes of each individual were determined for 10–17 loci (mean 15.28 loci). The locus *AHTH260* was removed from the analysis because of the presence of multiple peaks. Table 2 shows the number of alleles, *H*<sub>o</sub>, and *H*<sub>e</sub>. The diallel crossing of the 75 raccoon dogs resulted in two PO pairs, 51 FS pairs, 235 HS pairs, and 2,487 U pairs. There was a
significant difference between the kinship ratios of the infested pairs, infested/non-infested pairs and non-infested pairs (Chi-squared test: $\chi^2=116.23, df=2, P<0.001$, Table 3). On the other hand, there was no significant difference between infested pairs and the other two pairs ($\chi^2=0.034, df=1, P=0.85$, Table 3); however, a significant difference was recognized between non-infested pairs and the other two pairs ($\chi^2=14.32, df=1, P<0.001$, Table 3). There were no kinships found in nine out of 23 infested individuals. Out of the 14 remaining individuals, 10 paired with non-infested individuals. Thus, there were few kinships among the infested individuals.

### Habitat analysis

Ten out of 75 individuals were excluded from this analysis because the capture points of these individuals were unclear (Fig. 3). The logistic regression analysis showed a significant association between *S. scabiei* infection and the location of the village sections (odds ratios=1.55, 95% CI=1.11–2.17, $P=0.011$). The mapped distribution of individuals revealed that epizootic sarcoptic mange occurred locally (Fig. 3).

### DISCUSSION

The raccoon dog is generally a monogamous animal, and both female and male are observed rearing their offspring [4, 23]. Moreover, parents and offspring or siblings share their dens [25]. Considering the behavioral ecology of raccoon dogs, there was frequent direct/indirect contact between parents and their offspring, and between full siblings, but not between half-siblings [4, 23, 25]. Thus, the HS group was not taken into consideration. Previous studies have suggested that the direct/indirect contact between parents and offspring was one of the driving factors of epizootic sarcoptic mange in raccoon dogs and was related to their behavioral ecology [15, 21]. However, our genetic analysis indicated that only four pairs of infested individuals had kinships, whereas 230 pairs of infested individuals were unrelated (U). Many infested individuals did not have kinships (Table 3). There was a significant difference between the kinship ratios whether raccoon dogs were infested or non-infested, however, the ratio of kinships with pairs of infested individuals did not significantly differ from that with the two other pairs. Thus, it is assumed that the frequency of transmission of *S. scabiei* between kin was not high. In contrast, 14 out of the 23 infested individuals had kinships.
while 10 infested individuals had kinships with non-infested individuals. This suggested that only certain kinships did not have *S. scabiei* transmitted between individuals. Therefore, *S. scabiei* transmission between kin might not be an important parameter for sarcoptic mange epizootic in raccoon dogs. However, the mortality rate of infested individuals was not observed in this study. There is a possibility that the number of infested individuals was underestimated because infested individuals had already died and were not captured. There was a significant difference between the kinship ratios of non-infested pairs and the other two pairs, which might be influenced by the high mortality rate of sarcoptic mange. Further studies considering the mortality rate of sarcoptic mange are needed to determine the transmissions of *S. scabiei* between kin.

The *S. scabiei* infection rates and the locations of the village sections were significantly associated (Fig. 3). In Central Europe, raccoon dogs avoided settlements [2], but Japanese raccoon dogs were highly sedentary because of their smaller home ranges [10, 14]. It is suggested that the contact between nearby individuals is high, which might cause local epizootic sarcoptic mange in raccoon dogs through contact transmission. In the study area, Mount Haruna is in the northwest and human-modified landscapes (i.e., urban and agricultural areas) are toward the southeast. Many infested individuals were distributed in human-modified landscapes rather than in the forest areas (Fig. 3). Epizootic sarcoptic mange in raccoon dogs might be related to high population densities [5, 17, 20]. The occurrence of sarcoptic mange was high in urbanized areas because of higher population densities [15]. *Sarcoptes scabiei* transmission may be significantly affected by direct/indirect contact between individuals living closely together in areas with high population densities, such as urbanized areas. Needless to say, the kinships of all raccoon dogs inhabiting the study area were not fully observed. There was also a possibility that many infested individuals were not captured because they had died of sarcoptic mange. Moreover, raccoon dogs were not equally captured throughout the year. As a result, there was the possibility that the incidence of epizootic sarcoptic mange in the raccoon dog was underestimated because of seasonal changes. It is necessary to investigate the sampling rate of infested individuals and the association with mortality rate.

In general, the human population, as well as the number of domesticated dogs, was higher in the urbanized area than in the forest area. *S. scabiei* had high host specificity and low cross infectivity [12], however, a recent study using genetic analysis suggested *S. scabiei* transmission occurred between raccoon dogs and domestic dogs [8]. Therefore, contact transmission between raccoon dogs and domestic dogs is a real threat. Other studies reported close genetic relationships among mites of wild mammals in Japan [7, 9]. Raccoon dogs and other animals may induce *S. scabiei* transmission, increasing the risk of epizootic sarcoptic mange. Therefore, it is necessary to focus on the behavioral ecology of various host animals to understand *S. scabiei* transmission.

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