# Food Habits of Feral Dogs and Red Foxes in a New Endemic Area of Echinococcus multilocularis

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# Food habits of feral dogs and red foxes in a new endemic area of *Echinococcus multilocularis*

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**Abstract.**    *Echinococcus multilocularis* is a zoonotic parasite for which canid species are the definitive hosts. Its distribution in Japan has been limited to Hokkaido. Recently, however, feral dogs in the Aichi Prefecture have been continuously infected with *E. multilocularis*. This suggests that *E. multilocularis* is becoming endemic to Honshu, but its life cycle in this region is unclear. The feeding habits of the definitive host can be the key to understanding these details. In this study, we investigated the diet of feral dogs, known as the definitive hosts, and red foxes, a possible definitive host, on the Chita Peninsula, Aichi Prefecture, and examined their relationship with the life cycle of *E. multilocularis*. Dietary analysis showed that feral dogs fed mainly on plant matter including fruits, concentrated feed, and birds without consuming rodents that can act as intermediate hosts. In contrast, red foxes consumed *Microtus* voles, which are suitable intermediate hosts for *E. multilocularis*. Therefore, the route of infection of feral dogs with *E. multilocularis* remains unknown, but there is concern that the disease may spread to red foxes via *Microtus* voles.

**Key words:** canids, definitive host, diet, echinococcosis, zoonosis.

*Echinococcus multilocularis* is a zoonotic helminth distributed throughout the Northern Hemisphere, including Asia, that causes fatal alveolar echinococcosis in humans. In Japan, the life cycle of *E. multilocularis* is maintained with red foxes (*Vulpes vulpes*) as the primary definitive host and wild rodents, including grey red-backed voles (*Myodes rufocanus*) and northern red-backed voles (*M. rutilus*), as the intermediate hosts (Takahashi et al. 2005). The endemic area of *E. multilocularis* in Japan has been restricted to Hokkaido for almost 50 years since its life cycle was first identified in eastern Hokkaido in 1965 (Yagi 2017; Morishima 2018).

However, *E. multilocularis* was discovered in a feral dog (*Canis familiaris*) on the Chita Peninsula in Aichi Prefecture, mainland Honshu, Japan, in 2014 (Tomaru et al. 2014; Morishima et al. 2016), and infection has been continuously confirmed in feral dogs in the surrounding area (Morishima 2018). This suggests that *E. multilocularis* is becoming endemic to the Chita Peninsula. On the Chita Peninsula, 250, 265, and 305 feral dogs were captured by local animal protection centers in 2019, 2020, and 2021, respectively (Aichi Prefectural Animal Protection Center 2020, 2021, 2022). Their presence has also been confirmed in 10 of 28 4-km grids throughout the peninsula (Tsukada 2022). By 2022, 953 fecal samples from feral dogs have been examined for parasite eggs and subjected to PCR tests, and five samples have been confirmed positive for *E. multilocularis* (Aichi Prefectural Institute of Public Health 2023). In addition, the red fox, a suitable definitive host for *E. multilocularis*, is found throughout the peninsula (Fukuda and Washizawa 2013; Tsukada 2022), although its population declined in the mid-1960s (Aichi 2000). A total of 18 fecal samples from red foxes have also been examined for *Echinococcus* infection by 2022; however, all samples were negative for *E. multilocularis* (Aichi Prefectural Institute of Public Health 2015, 2020, 2022).

The local government, Aichi Prefecture, has continuously reported the results of the survey on the status of *Echinococcus* infection and warned the residents of the

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prefecture to prevent infection. Although the definitive host is limited to feral dogs, the intermediate hosts are unknown, and the detailed life cycles of *E. multilocularis* have not been clarified in this area. It is necessary to understand its life cycle to develop effective control measures for *E. multilocularis* in this endemic area. However, evaluating intermediate hosts requires the capturing and killing large numbers of individuals and setting up a secure facility for handling infected animals, since the infection rate is very low, in general, compared to definitive hosts. In contrast, analysis of the feces of the definitive host can provide clues to the life cycle of *E. multilocularis* because the feces of the definitive host contain traces of the consumption of the intermediate host.

The aim of this study was to clarify the feeding habits of feral dogs, a known definitive host of *E. multilocularis*, and red foxes, a possible definitive host of the parasite, to understand the life cycle of *E. multilocularis* in the Chita Peninsula, Aichi Prefecture, a newly endemic area of the parasite.

## Materials and methods

This study was conducted across the Chita Peninsula, Aichi Prefecture, a new endemic area for *E. multilocularis*. From 2018 to 2020, we collected feces from feral dogs and red foxes near 30 livestock farms with frequently identified feral dog populations to efficiently obtain dog feces. To ensure equal sampling of feces throughout the study area, we divided the study area into 28 4-km grids and established one or two survey plots within each grid. The survey plots were not completely random within the grid but were selected from agricultural areas, including livestock farms and large forested areas where feral dogs and red foxes were expected to occur. We collected feces from both dogs and foxes in the plots. These surveys were conducted in December 2018 and 2019 and in February and September 2020. During the 2018 and 2019 surveys, one to three investigators searched for and collected feces along roads and agricultural fields in each study plot. In February 2020, one investigator walked and collected feces along a 1–2 km survey route established for each survey area at 30 plots. In September 2020, two investigators walked along the 1–2 km survey route established for each survey area in 56 plots and collected fecal samples. The feces were identified as those from a feral dog or red fox based on their shape and the circumstances of def-

ecation. In general, feces with a maximum diameter of ≥ 2 cm were considered to be from feral dogs, and thinner feces were considered to be from red foxes (Takeuchi 1995). For fresh feces, the characteristic scent of red foxes was also a clue to distinguish between species. The feces of feral cats were distinguished as those covered and hidden by sand, etc., and the feces of Japanese weasels were distinguished as those less than 0.7 cm in diameter (Tsuji et al. 2011). Up to four fecal samples per species were collected on each survey route, placed in 50 mL centrifuge tubes, and returned to the laboratory.

Fecal samples were frozen at –70°C for at least one week to kill *E. multilocularis* eggs and eliminate the risk of infection with the parasite eggs. The fecal samples were thawed and divided into two. One half was used for parasite egg examination and DNA testing, and the other half was used for dietary analysis. To clarify feeding habits, undigested material in the feces was analyzed using the point frame (PF) method (Tsukada et al. 2020). Each fecal sample was heated at 70°C for ≥ 12 hours and washed with water through a 0.5–1 mm mesh sieve, and the remaining undigested material was preserved in 70% ethanol. We classified undigested items into ten food categories: mammals (hair), bones, birds (feathers, eggshells, and skin), insects, concentrated feed, plants, fruits, seeds, garbage, and unidentified food. To compare the diet composition, we calculated the proportion of PF values (%PF) and frequency of occurrence (%FO) for each item. We calculated %PF using the following formula:

$$\%PF = \sum PFi/n,$$

where $PFi$ is the PF value of food item $i$ divided by the total number of points counted, and $n$ is the number of fecal samples analyzed. We also calculated %FO using the following formula:

$$\%FO = 100Fi/n,$$

where $Fi$ is the number of fecal samples, including food item $i$, and $n$ is the number of fecal samples analyzed.

In addition, we identified the species from the surface structure of mammalian hair using the sump method (Murai et al. 2011) and evaluated the species consumed by feral dogs and red foxes. From each sample in which mammalian hairs were detected using the PF method, ten hairs were collected, the species were identified,

and the number of hairs was recorded; the percentage of the identified species in each fecal sample was multiplied by the PF value of mammals to obtain the exact PF value of each mammalian species. If their own hair was detected, it was excluded from the %PF and %FO calculations.

Half of the fecal samples were examined for parasite eggs using the formalin-ethyl acetate sedimentation technique (Young et al. 1979). We extracted copro-DNA using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) and performed PCR amplification of a fragment of the 12S ribosomal RNA gene (Dinkel et al. 1998). The first primer set non-specifically amplifies DNA of the Cyclophyllidea. Therefore, prior to sequencing, a portion of the initial PCR product was amplified using *E. multilocularis*-specific nested primers to rule out any other cyclophyllid species infection than *E. multilocularis*, as in Dinkel et al. (1998). We performed this rapid test to confirm *E. multilocularis* infections.

For the %PF results, we used a value for each food item in each fecal sample and evaluated interspecies differences pooled over the entire period using Welch's *t*-test and seasonal differences within a species by multiple comparison tests using the Steel–Dwass method. For the %FO results, we evaluated interspecies differences using Fisher's exact probability test for each food item. We also compared seasonal differences within a species using Fisher's exact probability test for the contingency table of each food item between seasons, using

the Benjamini and Hochberg method for multiple comparisons. All statistical analyses were performed using R statistical software (ver. 4.2.2) in the MASS and RVAideMemoire packages.

## Results

A total of 105 fecal samples from feral dogs (66, 28, and 11) were collected in December 2018 and February and September 2020, respectively. A total of 89 red fox fecal samples (17, 51, and 21) were collected in December 2019 and February and September 2020, respectively. No positive *Echinococcus* specimens were identified among the samples examined.

Plants, concentrated feed, fruits, and birds were the primary food sources of feral dogs (Table 1). Plants had the highest % PF of feral dogs, followed by concentrated feed (containing mechanically cut plant pieces, grains, and fibers), fruits, birds, bones, and garbage. The order of %PF was very similar to that of %FO in feral dogs, except that the order of concentrated feed and fruits was reversed (Table 2). The consumption of mammals was not observed. Plants, fruits, and insects were the primary food sources for red foxes, with some mammals consumed (Table 1). Plants had the highest %PF of red foxes, followed by fruits, insects, birds, mammals, and concentrated feed. The order of %FO in red foxes was plants, fruits, insects and birds, seeds, and mammals. A total of 21 fox fecal samples included mammals, of which 19 samples

**Table 1.** Food habits of feral dogs and red foxes in the Chita peninsula, Aichi, Japan, determined using the point-frame method

| %PF | Feral dog | | | | Red fox | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | Dec-18 | Feb-20 | Sep-20 | Total | Dec-19 | Feb-20 | Sep-20 |
| *n* | 105 | 66 | 28 | 11 | 89 | 17 | 51 | 21 |
| Mammals | 0.0 † | 0.0 | 0.0 | 0.0 | 6.6 † | 4.4 | 5.8 | 10.2 |
| Bones | 4.7 | 6.0 | 3.6 | 0.0 | 2.3 | 0.0 | 3.5 | 1.4 |
| Birds | 8.5 | 9.6 | 9.4 | 0.0 | 7.0 | 4.2 a | 9.8 a | 2.4 b |
| Insects | 2.7 † | 1.9 b | 0.1 b | 14.7 a | 11.9 † | 3.0 | 0.1 | 48.8 |
| Concentrated Feed | 25.3 | 24.8 | 29.2 | 18.3 | 6.2 | 5.1 | 7.1 | 4.9 |
| Plants | 33.0 | 28.8 | 33.2 | 57.8 | 33.2 | 40.0 a | 40.1 a | 10.4 b |
| Fruits | 20.5 | 23.7 a | 18.0 a | 7.6 b | 21.3 | 39.5 a | 21.5 a | 5.9 b |
| Seeds | 0.9 † | 1.1 | 0.1 | 1.6 | 5.3 † | 2.7 | 6.0 | 5.9 |
| Garbage | 3.6 | 3.1 | 6.4 | 0.0 | 3.0 | 1.2 | 4.8 | 0.0 |
| Others | 0.7 | 1.1 | 0.0 | 0.0 | 3.0 | 0.0 | 1.2 | 10.0 |

†Welch's *t*-test (*P* < 0.05).
a, b, c: the different letters indicate statistically significant differences (Steel–Dwass test, *P* < 0.05).

**Table 2.** Food habits of feral dogs and red foxes in the Chita Peninsula, Aichi, Japan, based on the frequency of occurrence (%FO)

| %FO | Feral dog | | | | Red fox | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | Dec-18 | Feb-20 | Sep-20 | Total | Dec-19 | Feb-20 | Sep-20 |
| *n* | 105 | 66 | 28 | 11 | 89 | 17 | 51 | 21 |
| Mammals | 0.0 [§] | 0.0 | 0.0 | 0.0 | 23.6 [§] | 23.5 | 27.5 | 14.3 |
| Bones | 14.3 | 18.2 | 10.7 | 0.0 | 10.1 | 0.0 | 13.7 | 9.5 |
| Birds | 26.7 | 28.8 | 32.1 | 0.0 | 28.1 | 35.3 [a] | 35.3 [a] | 4.8 [b] |
| Insects | 8.6 [§] | 6.1 [b] | 3.6 [b] | 36.4 [a] | 28.1 [§] | 29.4 [b] | 3.9 [c] | 85.7 [a] |
| Concentrated Feed | 51.4 | 48.5 [ab] | 71.4 [a] | 18.2 [b] | 22.5 | 29.4 | 27.5 | 4.8 |
| Plants | 79.0 | 77.3 | 89.3 | 63.6 | 83.1 | 100 [a] | 100 [a] | 28.6 [b] |
| Fruits | 61.0 | 63.6 | 75.0 | 9.1 | 67.4 | 88.2 [a] | 82.4 [a] | 14.3 [b] |
| Seeds | 3.8 [§] | 3.0 | 3.6 | 9.1 | 24.7 [§] | 5.9 | 25.5 | 38.1 |
| Garbage | 7.6 | 7.6 | 10.7 | 0.0 | 12.4 | 11.8 | 17.6 | 0.0 |
| Others | 2.9 | 4.5 | 0.0 | 0.0 | 12.4 | 0.0 | 7.8 | 33.3 |

[§]Fisher's exact test ($P < 0.05$).
a, b, c: the different letters indicate statistically significant differences (Steel–Dwass test, $P < 0.05$).

were dominated by rodents, *Microtus montebelli* (or *Alexandromys montebelli*; 90.5%) and one each by rodents, genus *Apodemus* and *Lepus brachyurus* (4.8% each) (Appendix 1). Significant differences in food utilization between feral dogs and red foxes were found regarding mammals, insects, and seeds for both %PF and %FO; red foxes consumed significantly more of these food items than feral dogs (%PF: Welch's *t*-test, $P <$ 0.05; %FO: Fisher's exact test, $P < 0.05$).

Regarding the differences in food consumption by month, feral dogs showed significant variation in %PF for insects and fruits (Table 1). The former was consumed significantly more in September than in December or February (Steel–Dwass test, $P < 0.05$), whereas the latter was consumed significantly more in December and February than in September (Steel–Dwass test, $P <$ 0.05). Regarding the %FO of feral dogs, significant monthly variations in food consumption were found for insects and concentrated feed (Table 2). The former was consumed significantly more in September than in December or February (Steel–Dwass test, $P < 0.05$), whereas the latter was consumed significantly more in February than in September (Steel–Dwass test, $P < 0.05$).

Seasonal changes were more pronounced in red foxes than in feral dogs. The %PF in red foxes showed significant monthly variations in the consumption of birds, plants, and fruits (Table 1), with all food items consumed significantly more frequently in February than in September or December (Steel–Dwass test, $P <$ 0.05). The %FO in red foxes showed significant

monthly changes in the consumption of insects, birds, plants, and fruits (Table 2): the first food item was consumed—significantly in the order of most to least frequently—in September, December, and February (Steel–Dwass test, $P < 0.05$), whereas the remaining three food items were consumed significantly more frequently in December and February than in September (Steel–Dwass test, $P < 0.05$).

## Discussion

We did not identify any *Echinococcus*-positive individuals among the feral dogs in this study. This is likely due to the low rate of *Echinococcus* infection among feral dogs on the Chita Peninsula in previous studies (only 5 out of 953; Aichi Prefectural Institute of Public Health 2023), which did not allow for sufficient sample size to detect infection. However, our results can reflect the feeding habits of the feral dog population, including that of *Echinococcus*-positive individuals, because the sites studied included locations where *Echinococcus*-positive individuals were identified in previous surveys (Morishima et al. 2016). Dietary analyses showed that feral dogs did not consume mammals that could serve as intermediate hosts for *Echinococcus* but instead exhibited strong vegetative omnivorous characteristics, including plant matter and concentrated feeds. The diets of these feral dogs differ significantly from those previously reported in Japan, such as in Nikko (Ban et al. 1995) and Amami Oshima (Watari et al. 2007),

where a high proportion of mammals are consumed, as well as reports from other regions (Butler and Du Toit 2002; Mitchell and Banks 2005; Silva-Rodríguez et al. 2010; Glen et al. 2011), but are similar to a plant-based diet, as reported in Warsaw (Krauze-Gryz and Gryz 2014). Reflecting these feeding habits, feral dogs were frequently observed near dairy farms and in agricultural fields, where they likely used concentrated feed and field residues as their primary food source.

In contrast to the diet of feral dogs, red foxes consumed relatively frequently *Microtus* rodents, which can act as intermediate hosts for *E. multilocularis*. Small arvicolines including *Microtus* rodents have predominantly become important intermediate hosts throughout the northern hemisphere where *E. multilocularis* is endemic, e.g., *Microtus arvalis* and *M. agrestis* in temperate Europe, *M. agrestis*, *M. obscurus*, *M. oeconomus*, *M. gregalis*, and *M. limnophilus* in temperate Asia, and *M. pennsylvanicus* in temperate North America, *M. oeconomus* and *M. gregalis* in Arctic and subarctic regions (Romig et al. 2017). Red foxes also consume small mammals including arvicoline rodents as an important food source in many other areas of Japan (Tsukada 1997; Hisano et al. 2022). In previous surveys on the Chita Peninsula (Aichi Prefectural Institute of Public Health 2023), no *Echinococcus*-positive individuals were found among red foxes. Conversely, red foxes are more frequently infected with *E. multilocularis* than feral dogs in Hokkaido, an area endemic for this parasite in Japan (Irie et al. 2019), and red foxes are infected with *E. multilocularis* by consuming wild arvicoline rodents, such as *M. rufocanus* (Takahashi et al. 2005). Assuming that arvicoline rodents are the source of infection in feral dogs on the Chita Peninsula like other endemic regions including Hokkaido (Takahashi et al. 2005; Romig et al. 2017), *E. multilocularis* infection in red foxes, which feed on rodents more frequently than the feral dogs do, is likely to be confirmed. Therefore, the results are puzzling; we may not have found feces from feral dogs consuming intermediate host species because such dogs were not abundant. Further research on the diet of feral dogs is required to identify potential intermediate hosts.

In contrast, red fox feces were collected from the same sites where feral dog feces were collected, confirming that the two species are sympatric. Thus, *Microtus* rodents can be infected with *E. multilocularis* eggs shed in the feces of feral dogs infected with the parasite. Failure to control *E. multilocularis* in feral dogs can result in the

spread of the parasite to red foxes through preying on *Microtus* rodents infected. If the parasite spreads to foxes, there is concern that areas contaminated with *E. multilocularis* could expand rapidly, as was the case in Hokkaido, Japan (Takahashi et al. 2005; Yagi 2017). At the current stage of the epidemic, when *E. multilocularis* is limited to feral dogs, it is preferable to quickly implement anthelmintic baiting programs, which have been shown to reduce the prevalence of *E. multilocularis* in red foxes in Hokkaido (Tsukada et al. 2002; Inoue et al. 2007; Takahashi et al. 2013; Uraguchi et al. 2022).

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## Appendix  1.

Frequency of mammal species recovered from red fox feces

| Species | Total | Dec-19 | Feb-20 | Sep-20 |
|---|---|---|---|---|
| *Microtus montebelli* | 19 | 4 | 13 | 2 |
| *Apodemus* rodents | 1 | 0 | 0 | 1 |
| *Lepus brachyurus* | 1 | 0 | 1 | 0 |