

# Dermatophytosis is uncommon in asymptomatic wild eastern cottontail rabbits in Illinois, Midwestern United States

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## OBJECTIVE

To investigate the presence of dermatophytes on the haircoat of wild eastern cottontail rabbits (ECR) (*Sylvilagus floridanus*) with and without skin lesions.

## ANIMALS

2-week-old or older ECR admitted to a Wildlife Medical Clinic (WMC) in central Illinois, Midwest United States, from September 2021 to August 2022.

## METHODS

ECR were surveyed over a 1-year period to assess the prevalence and seasonality of dermatophytosis. A Wood's lamp exam was performed over the haircoat. Hairs were sampled with a sterile toothbrush and plated on Sabouraud dextrose agar. The plates were photographed twice weekly for 3 weeks, and colonies were identified as contaminants or dermatophytes.

## RESULTS

523 ECR were admitted to WMC, 141 ECR met the age inclusion criteria, and 121 samples were plated. ECR presented as a litter were sampled together. None of the sampled ECR presented skin lesions other than acute traumatic wounds. No fluorescence was observed on any ECR during the Wood's lamp examination. Based on culture colony morphology, 115/121 of the samples were identified as contaminants and no growth was observed in 6/121 of plates. Dermatophytes' colonies were not identified on any of the culture plates.

## CLINICAL RELEVANCE

Dermatophytes are zoonotic fungi and can potentially be carried by wild animals. The fungal infection poses a health concern to humans and domestic pets through direct interaction. Our current results suggest that dermatophytosis may not be prevalent in asymptomatic wild rabbits in the studied areas of the Midwestern United States.

**Keywords:** dermatophytes, dermatophytosis, eastern cottontail rabbit, *Sylvilagus floridanus*, wildlife

**D**ermatophytosis is a superficial fungal infection of keratinized skin structures caused by zoophilic, geophilic, or anthropophilic fungal organisms known as dermatophytes. The clinical presentation depends on several factors including the dermatophyte species involved, the host, and the geographic location. Due to the infectious and contagious nature and zoonotic potential, dermatophytosis is an important disease in veterinary and human medicines.<sup>1</sup>

Dermatophyte species in animals are isolated and identified as belonging to the genus *Microsporum* and *Trichophyton* based on macroscopic and microscopic

characteristics of the organism grown in culture.<sup>1</sup> *Trichophyton mentagrophytes* is the most common dermatophyte species reported in rabbits, but *Microsporum canis* and *Microsporum gypseum* are also commonly isolated.<sup>2-4</sup> Previous European studies identified dermatophytes including *T mentagrophytes* and *M canis* in wild rabbits without clinical signs of the fungal infection.<sup>5-8</sup> *T mentagrophytes* spores can be isolated from infected rabbits, asymptomatic carriers, rabbits' nests, and surrounding environments, constituting an important source of infection and indirect transmission to animals and humans.<sup>9-10</sup>

The goal of the current study was to investigate the presence of dermatophytes on the haircoat of wild eastern cottontail rabbits (ECR) (*Sylvilagus floridanus*) with and without skin lesions admitted to the Wildlife Medical Clinic (WMC) at the University of Illinois in central Illinois, Midwest United States, over a period of 12 months. We hypothesize that wild rabbits in the Midwest United States may develop cutaneous lesions due to dermatophytosis or may be asymptomatic carriers of dermatophytes. Infected rabbits can pose a potential risk of transmitting the infection to other animals and humans.

## Methods

This study was approved by the Institutional Animal Care and Use Committee at the University of Illinois, protocol 21151.

### Sample collection

Sample collectors were veterinarians and veterinary students of the WMC. They were given a written protocol and access to an instructional video for correct and consistent sampling. ECR were classified into 3 groups: neonates, juveniles, and adults based on their estimated ages. Life stages were estimated based on previous training and an age estimation chart.

All ECR admitted to the WMC at the University of Illinois from September 2021 to August 2022 that were at least 2 weeks old were enrolled in the study. Sampling was not possible in younger rabbits still developing a full haircoat.

A Wood's lamp exam was performed over the entire haircoat to look for dermatophytes producing fluorescence on hair shafts. Pictures were taken of any suspected fluorescence for review.

All ECR had their haircoats sampled for dermatophyte investigation within 24 hours of admission; regardless of presentation complaint or condition. Sampled animals had no direct contact with other animals at the facility before sampling. Using the "Mackenzie brush technique," each rabbit was sampled individually by brushing the entire ventrum, dorsum, face, ears, tail, limbs, and paws with a sterile toothbrush. The toothbrush was then placed in a new, self-sealing plastic bag and submitted to the Dermatology service at the University of Illinois for plating.<sup>11</sup> Rabbits presented as a litter were sampled together using the same sterile toothbrush and their hairs were submitted as a single sample.<sup>1</sup>

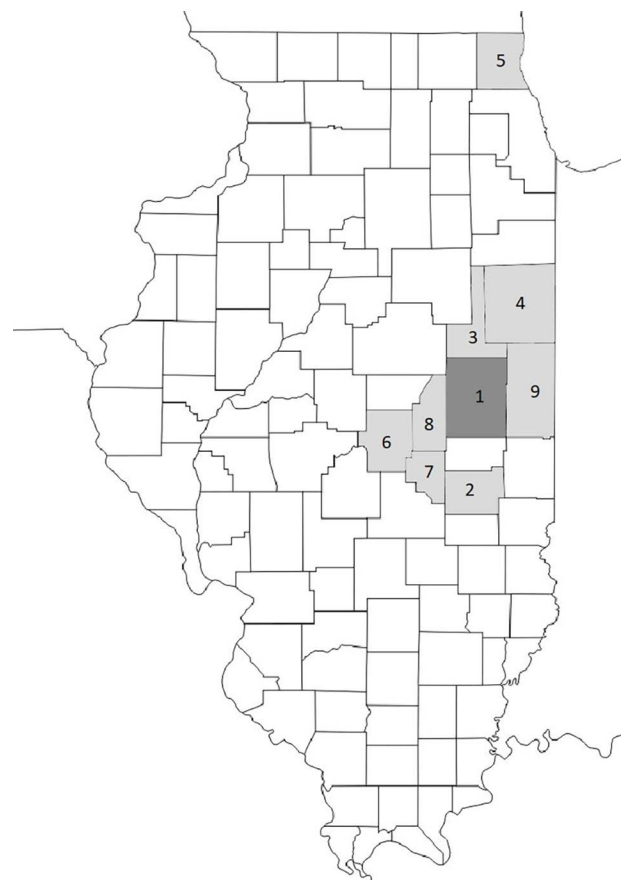
### Sample plating

Sabouraud dextrose agar pH 5.6 plates were inoculated by gently pressing the toothbrush into the agar. Any hairs remaining on the toothbrush and in the plastic bag were collected with sterile tweezers and also pressed onto the plate. The samples were left to grow at room temperature for 3 weeks. The plates were photographed twice weekly and colonies were identified as contaminants or dermatophytes by colony characteristics, as described in St-Germain & Summerbell, 2011.<sup>12</sup>

If no fungal growth was seen within 3 weeks, the sample was considered negative. All samples were plated and checked by a certified veterinary technician who routinely processes and analyzes dermatophyte samples at the Dermatology service at the University of Illinois. Descriptive statistics were used to summarize the data obtained from the study.

## Results

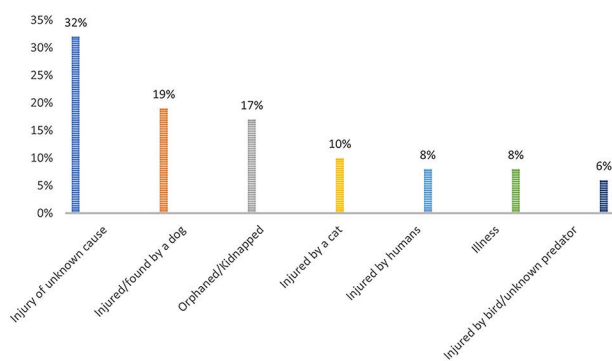
During the 12-month study period, 523 ECR, consisting of 239 individuals and 72 litters, were admitted to WMC. ECR were sampled throughout the year to address possible infection difference with seasonality. A total of 141 of all life stages ECR were sampled (6 neonates, 87 juveniles, 31 adults, and the life stage was not estimated for 17 ECR), and 123 samples were produced. Of the 123 samples, 113 were from ECR sampled individually and 28 ECR were sampled as part of 10 litters. Two samples were lost during transportation from WMC to the



**Figure 1**—Distribution by Illinois county of eastern cottontail rabbits enrolled in the study from September 2021 to August 2022. The counties represented are: (1) Champaign County with 98 samples, (2) Coles County with 2 samples, (3) Ford County with 2 samples, (4) Iroquois County with 2 samples, (5) Lake County with 1 sample, (6) Macon County with 1 sample, (7) Moultrie County with 1 sample, (8) Piatt County with 1 sample, (9) Vermillion County with 6 samples, and 7 samples had no associated county reported on intake paperwork.

Dermatology service. In total, 121 of the 123 samples collected were plated.

Sampled ECR were brought to the WMC from 9 Illinois counties. Champaign County accounted for 81.0% of samples plated (98/121) (Figure 1). The most common reason for admission given by finders when bringing wild ECR to the WMC was an interaction between the rabbit and a dog, cat, bird, or another unidentified animal, followed by an unknown cause of injury (Figure 2). ECR injured from lawn mower, fence, or trap, or hit by a car were included in the group injured by humans. None of the sampled ECR presented skin lesions other than acute traumatic wounds.



**Figure 2**—Reasons for admission of eastern cottontail rabbits to the Wildlife Medical Clinic of the University of Illinois enrolled in the study from September 2021 to August 2022.

No fluorescence was observed on any rabbit during the Wood's lamp examination. Based on culture colony morphology, 95.0% (115/121) of the samples were identified as contaminants and no growth was observed in 5.0% (6/121) of plates. Dermatophytes' colonies were not identified on any of the culture plates.

## Discussion

Dermatophytosis is a superficial fungal infection that poses a health concern to humans and domestic pets. Different species of dermatophytes infect animals that may develop cutaneous clinical signs. Some animals can be asymptomatic carriers and may be a source of infection for other animals or humans.<sup>1,7,13</sup> ECR are found throughout the United States and have been documented to live in dense populations, including urban settings (9.4–16.3/ha) in Illinois. ECR have been reported to utilize green spaces, such as parks and gardens, for food sources.<sup>14</sup> Suburban areas in particular have reportedly seen an increase in human-wildlife interactions.<sup>15</sup> A previous study, which surveyed domestic dog owners within a suburban forest, noted that 64% of owners indicated wildlife was seen near their homes. And 23% of owners reported that their pet had a negative interaction with wildlife.<sup>16</sup> Increased human-wildlife

and pet-wildlife interactions may lead to increased potential for subsequent disease transmission.

To the authors' knowledge, this is the first time ECR were investigated as a carrier of dermatophyte in the United States. Other surveys have been conducted previously in Europe showing different results. A study in Piedmont, Italy collected 216 hair samples using the Mackenzie brush technique from wild rabbits from September 1999 to July 2000. Dermatophytes were identified in 26.4% of samples, and *T mentagrophytes* and *M canis* were species identified consistently throughout the year. Even though none of the ECR showed signs of a dermatological infection,<sup>7</sup> Dermatophytosis caused by other geophilic fungi was found to have a statistically significant seasonal prevalence observed between the months of May and September. This correlated to increased temperatures in that region.<sup>7</sup> A separate study conducted in Bologna, Italy, collected samples from 1,481 wild animals using sterilized carpet pieces, of which 415 came from European hares (*Lepus europaeus*).<sup>5</sup> Of these samples, 39 were positive for dermatophytosis. Only 1 sample was positive for *T mentagrophytes* and there were no *M canis* positive samples. A single hare exhibited clinical signs of dermatophytosis.<sup>5</sup> In Northern Portugal, a survey of 71 captive wild rabbits was conducted from August to December of 2019 and dermatophytes identified as *T mentagrophytes* complex were diagnosed in 5 (7%) individuals. The samples were collected using the Mackenzie brush technique and the positive rabbits had no evidence of dermatologic lesions.<sup>8</sup> The present study used a similar methodology for sampling and culturing the ECR hairs as used by other European studies. All regions on both continents have similar climates. Northern Italy and Portugal are temperate zones and Illinois is temperate to subtropic.

Dermatological lesions were not an inciting cause of admission for any of the ECR sampled. The ECR presented with skin lesions and had wounds suspected to be associated with trauma. Also, dermatophytosis mainly causes superficial lesions and the fungal infection would not be expected to incapacitate or negatively affect a rabbit's ability to escape injury or avoid capture, which could have created a bias for our sampled animals.

The present study sampled 27% of the rabbits admitted to the WMC due to the exclusion of rabbits that had not yet grown in a haircoat, which occurs within the second week of life.<sup>17</sup> Before this study, an attempt to sample hairs from ECR without a haircoat (most neonates less than an estimated 2–3 weeks of age) was proven unsuccessful. On these young ECR the hairs did not come out while brushing and could not be collected for sampling. Therefore, ECR that had not grown in a haircoat were excluded from the study. However, we understand that this age selection may have interfered with the results. There is a possibility that 2-week-old rabbits could be carrying dermatophytes. In experimental models of *M canis* infection in cats, the period between inoculation and development of clinical lesions was 7 to 14 days.<sup>1,18</sup>

Similar studies conducted in rabbits have not been reported. The times between sample collection and processing varied due to the availability of personnel to transport the samples and the schedule of the Dermatology service at the University of Illinois. Some human laboratories recommend submitting dermatophytes samples within 72 hours of collection. However, it has not been reported how long dermatophytes may live after collection with a limited keratin source. Most samples for the present study were plated within 5 days (an average of 4.5 days) from the time the sample was collected.

One of the goals of this study was to determine if dermatophytosis in ECR is present in the Illinois region. It may become a public health concern through direct human interaction or through transmission to domestic animals that cohabitate closely with humans. It was noted that 29% of the ECR in this study came into direct contact with a domestic animal (cat or dog) and 100% of them came into direct contact with a human (the finders who brought the animal for medical care and WMC staff). No dermatophyte colony was identified in the studied ECR. Although this does not rule out the presence of dermatophytosis in the wild population in the region studied, it can be speculated that rabbits without cutaneous lesions are unlikely carriers of dermatophytes in the studied areas of the Midwestern United States.

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