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Prevalence and zoonotic risks of *Trichophyton mentagrophytes* and *Cheyletiella* spp. in guinea pigs and rabbits in Dutch pet shops

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ABSTRACT

Young rabbits and guinea pigs are often purchased as pets for children and may be infected with zoonotic skin infections. To assess the risk of acquiring such an infection from rabbits or guinea pigs, this study investigated the prevalence of the fungus *Trichophyton mentagrophytes* and the fur mite *Cheyletiella parasitovorax* in asymptomatic rabbits and guinea pigs in Dutch pet shops. In 91 pet shops a total of 213 rabbits and 179 guinea pigs were sampled using the Mackenzie technique and cultured. Clean cultures were examined microscopically and a PCR was performed on at least one sample from each pet shop. All animals were investigated for fur mite using a flea comb, a magnifying glass and white paper. From the fur of 3.8% (8/213) of the rabbits and 16.8% (30/179) of the guinea pigs, *T. mentagrophytes* was isolated. From 1 guinea pig (0,6%) *Chrysosporium keratinophilum* was isolated. Dermatophyte-positive rabbits and guinea pigs originated from 5.6% (5/90) and 27.3% (24/88) of the investigated pet shops, respectively. Fur mites were not found.

Pet shops can play an important role in preventing transmission of zoonotic ringworm infections (dermatophytosis) and educating their customers. Specific preventive measures such as routine screening examinations and (prophylactic) treatment of rabbits and guinea pigs are recommended next to regular hygiene when handling animals.

1. Introduction

Dermatophytosis is one of the most common and important zoonotic skin diseases of pets (Moriello, 2003). Rodents or rabbits are mainly infected with *T. mentagrophytes*, while *M. canis* is primarily found in dogs and cats (Drouot et al., 2009). Infection occurs by direct or indirect contact with infected hair, scales or materials (Chermette et al., 2008). Reported prevalence in rabbits varies between 0 and 49% (Cafarchia et al., 2010; Kraemer et al., 2012) and in guinea pigs between 1 and 35% (Balsari et al., 1981). Dermatophytes may cause infections of the keratinized structures of the skin, hair and nails of the living host (Donnelly et al., 2000). The teleomorphs of the zoophilic isolates of the *T. mentagrophytes* complex are *T. benhamiae, Arthroderma simii* and *T. mentagrophytes* (Kano et al., 2011). Most of the dermatophytes in guinea pigs and rabbits are associated with *T. benhamiae* (Gräser et al., 2008). Infectious microconidia can persist for many months in the environment or on fomites (Foster and Foil, 2003; Scott et al., 2000).

Signs of animal dermatophyte infection may range from asymptomatic carriers, via mild to severe (Chomel et al., 2007; Vermout et al., 2008) and start usually on the head and spread over the back, the flanks and the limbs. The lesions are often pruritic and may consist of focal circular areas of alopecia, scaling, and erythema (Chermette et al., 2008). Infected exotic pets (e.g. rabbits, rodents, guinea pigs, ferrets) are significantly younger than non-infected animals (D'Ovidio and Santoro, 2015). Most of the infected guinea pigs show no clinical signs and are contagious asymptomatic carriers (Quesenberry and Carpenter, 2012).

Human dermatophytosis is usually acquired directly or indirectly by exposure to typical reservoir hosts or their immediate environment and not by human to human transmission (Scott et al., 2000; Mignon and Monod, 2011). It is associated with the typically ringworm-lesion, and pruritus is common (Chermette et al., 2008). In contrast to *M. canis*, *T. mentagrophytes* infections frequently cause highly inflammatory lesions

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in humans (Hay, 1992). In New Zealand, zoonotic human dermatophytes account for around 22% of all human dermatophytes (Pier et al., 1994). Asymptomatic exotic pet animals often represent a source for fungal infections in people, even if the contact time is short (D'Ovidio and Santoro, 2015). In one study *T. benhamiae* was found in 9 isolates from 8 children and 1 adult with dermatophytosis. Eight of these individuals had previous contact with rodents, mostly guinea pigs (Fumeaux et al., 2004).

Cheyletiella is a non-burrowing fur mite, which is common in dogs (*C. yasguri*), cats (*C. blakei*), and rabbits *(C. parasitovorax*) and is transmitted by direct contact. The various species are not extreme host specific and are zoonotic (Quesenberry and Carpenter, 2012). In guinea pigs the mite is very rare (Scott et al., 2000). In one Italian study the parasite was found in 3% of 455 investigated rabbits and none of the 93 guinea pigs in pet shops (D'Ovidio and Santoro, 2014). The obligate parasite causes a disruption of the stratum corneum which may facilitate a dermatophyte infection (Scott et al., 2000). In rabbits the mite may cause crusts on the back, trunk, and flank with increased scaling and alopecia. In the human, *Cheyletiella* can cause intensely itchy papules with necrotic areas on locations in contact with the animal (Dobrosavljevic et al., 2007).

Young rabbits and guinea pigs are often purchased in pet shops as pets for children. If these animals have zoonotic skin infections, they can potentially spread the illness to other animals within the shop and to many new owners (Halsby et al., 2014). To assess the risk of acquiring a zoonotic infection from these animals, in this study the prevalence of dermatophytes and fur mites on rabbits and guinea pigs in pet shops in the Netherlands has been investigated.

2. Material and methods

2.1. Pet shops

A power analysis based on the total of 1232 pet shops in the Netherlands was performed. The prevalence of *T. mentagrophytes* infection in rabbits was estimated at 5%. With a confidence level of 95% and accepted error of 5%, it was calculated that the involvement of a minimum of 69 pet shops was required for the study. A randomized list of 125 pet shops was provided by the Dutch branch organisation Dibevo from which 91 pet shops were visited. From these shops, 88 were selling guinea pigs (in average 2.0 cages per shop) and 90 rabbits (in average 2.4 cages per shop). None of the pet shops was notified in advance of the visit.

2.2. Questionnaires

Each pet shop owner was asked to fill out a questionnaire focused on the origin of the animals and the eventual presence of (hygiene, prophylactic therapy) protocols when new animals arrive (Table 1).

Table 1
Questionnaire used for the investigated pet shops.

1. How many rabbits and guinea pigs are sold per year in this pet shop?
2. What is the origin of the rabbits and guinea pigs?
3. Is a veterinarian associated with the pet shop?
4. Are the rabbits vaccinated? If yes, against which disease(s)?
5. Are the rabbits and/or guinea pigs prophylactic treated at arrival in the pet shop? If yes, which treatment?
6. How often are the cages cleaned? What products are used?
7. Are the eating and drinking bowls used in the same cages with the same animals?
8. Are the animals mixed during their stay?
9. What education concerning animal management/care taking is required?
10. Is the pet shop certified?

2.3. Sample collection

From every sampled animal, the pet store, species, sex, age, coat type, estimated adult size, cage size, the number and species per cage, the bedding and hygiene was documented. The estimated adult sizes of the rabbits were divided into dwarf, < 2.5 kg; medium, 3–4 kg; and greater than 5 kg. A fur sample was taken from one animal out of each present cage with new disposable latex gloves. This animal was considered as representative for the exposed group in that cage as one sample unit. The Mackenzie toothbrush technique was used, brushing the entire animal's body during 1 min with a clean toothbrush with a flat surface (Scott et al., 2000). The toothbrush was placed into a paper bag that was transported to the laboratory where it was incubated the same week.

2.4. Parasite investigation

The presence of *C. parasitovorax* was investigated using a flea comb and a magnifying glass. The animals were combed during 1 min with a flee comb and the hairs and scales were examined under fluorescent light with a magnifying glass (Mellgren and Bergvall, 2008). In cases of doubt white paper was used as background.

2.5. Fungal culture

Sabouraud's dextrose agar plates containing cycloheximide 0.02% and depomycin 0.3% were inoculated by gently pressing the toothbrush into the agar. Some hairs left on the toothbrush were plucked off with sterile tweezers and pressed also on the agar. The plates were covered with plastic to prevent dehydration, incubated in the dark at 25° C for up to three weeks, and examined for fungal growth at Days 7, 14, and 21.

Suspicious *T. mentagrophytes* colonies were identified by colony characteristics as described in de Hoog et al., 2000. In case no fungal growth was seen within three weeks, the sample was considered as negative. The number of morphologically suspicious colonies per plate were also counted and photographed on Day 7. On Day 7 or 14, monocultures were made from morphologically suspicious colonies on malt extract agar (MEA) and incubated in the dark at 25° C for 1.5 − 3 weeks. All plates were stored at 7° C after the colonies were fully-grown.

2.5.1. Microscopic preparation and evaluation

From the MEA colonies, microscopic preparations were made by staining with one drop of lactophenol cotton blue and further examined based on the description(s) in de Hoog et al., 2000.

2.5.2. PCR

From every pet shop with suspected *T. mentagrophytes* colonies, at least one colony was identified using molecular techniques. In case two different morphological strains originated from one pet shop, both isolates where identified using molecular techniques. The Westerdijk Fungal Biodiversity Institute in Utrecht performed the DNA isolation and PCR. DNA was extracted from each colony using the MoBio − UltraClean™ Microbial DNA Isolation Kit. Fragments containing the Internal Transcribed Spacer 1 and 2 and the 5.8S gene (ITS) were amplified using the primers LS266 (GCATTCCCAAACAACTCGACTC) and V9G (TTACGTCCCTGCCCTTTGTA). The PCR fragments were sequenced with the ABI Prism® Big DyeTM Terminator v. 3.0 Ready Reaction Cycle Sequencing Kit. Samples were analysed on an ABI PRISM 3730xl Genetic Analyser and contigs were assembled using the forward and reverse sequences with the programme SeqMan from the LaserGene package. The sequences were compared on GenBank using BLAST and in a large fungal database of the Westerdijk Fungal Biodiversity Institute with sequences of most of the type strains. All strains mentioned in this article are named following the novel multi-

Table 2
Parameters of the investigated pet shops (in%).

	Rabbits	Guinea pigs
Number of animals sold / year		
< 20	10	13
21–50	35	35
51–100	34	33
> 100	21	20
Origin of the animals		
Wholesaler only	33	28
Other	67	71
Exchanging bowls between cages		
No	66	73
Yes	34	27
Mixing of the animals		
No	28	25
Yes	62	75

locus phylogenetic taxonomy for the dermatophytes (de Hoog et al., 2017).

2.6. Statistical evaluation

All collected data were added in excel and SPSS. An unpaired independent sample T-test was performed to compare the mean age of the animals and the chi-square test and Fisher's exact test was used to detect epidemiologic associations (sex, age, hair type), hygiene policy associations (exchange of bowls/food cups, coupling of animals, combining rabbits and guinea pigs) and store characteristics associations (cage occupation, turnover, and origin of the animals). In case of a significant correlation, a Cramer's V value was determined.

3. Results

3.1. Questionnaires

Wholesalers (animal traders) always supplied 33% (71/213) of the rabbits and 28% (51/179) of the guinea pigs. The remaining animals originated from other suppliers, mostly private breeders (Table 2). Prophylactic treatment was given in 25% and 52% of the shops to treat rabbits and guinea pigs, respectively, against mites like *Psoroptes cuniculi* (ear canker), *Cheyletiella parasitovorax* and *Trixacarus caviae* (sarcoptiform mange). In most cases a spot-on ivermectin formulation was used. No prophylactic treatment was used against fungal infection.

3.2. Rabbits

In total 213 rabbits (49% male) aged from 4 weeks to 2 years (median 16 weeks) were sampled (Table 3). None of the animals showed lesions during visual control. Based on culture colony morphology, 4.2% (9/213) of the samples were suspected of growth of *T. mentagrophytes*. The number of colonies counted on Day 7 ranged from 1 to 50. When examined microscopically, 1/9 culture was identified as *Acremonium*, the other 8 as *T. mentagrophytes*-like. The PCR results revealed that all were *T. benhamiae* of the *T. mentagrophytes* complex. Therefore, from 3.8% (8/213) of the rabbits, *T. mentagrophytes* was isolated (5/8 male) and the animals originated from 5.6% (5/90) of the pet shops. The median age was 12 weeks (range 6–20 weeks).

The median age of the non-infected animals was 16 weeks. *Cheyletiella* spp. was not found.

Statistical analysis did not indicate any specific risk factor concerning shop or animal parameters, and animal husbandry.

Table 3
Parameters of the investigated animals (%)

	Rabbits	Guinea pigs
Sex		
Male	49	61
Female	51	39
Age (weeks)		
0–13	57	58
14–26	35	32
> 26	8	10
Other animals present		
None	54	37
Rabbits	37	28
Guinea pigs	9	35
Hair type		
Short	-	86
Medium	-	2
Long	-	12

3.3. Guinea pigs

In total, 179 guinea pigs (62% male) aged from 4 weeks to 2 years (median 12 weeks) were sampled (Table 3). None of the animals showed lesions during visual control. Growth of *T. mentagrophytes,* based on culture colony morphology, was suspected for 16.8% (30/179) of the fur samples originating from 27.3% (24/88) of the pet shops. The PCR results revealed that 21 of the 24 positive samples were *T. benhamiae* (88%), two were identified as being positive for *T. mentagrophytes* (8%) and one as *Chrysosporium keratinophilum* (4%). The median age was 14 weeks and median age of the non-infected animals was 18.8 weeks (not significant). *Cheyletiella* spp. was not found.

No significant correlations could be established between infection with *T. mentagrophytes* and animal or shop parameters.

4. Discussion

The results of this study indicate that up to 27% of Dutch pet shops are selling *T. mentagrophytes*-positive rabbits and/or guinea pigs, and the latter species was found to be more infected (16.8%) than the former (3.8%). None of the animals showed clinical signs and were, therefore, asymptomatic carriers. One guinea pig contained a *Chrysosporium keratinophilum.* This species is most commonly found in keratin-rich, dead materials such as feathers, skin scales, hair, and hooves (de Hoog et al., 2000; Campbell et al., 2013; Pin et al., 2011). It is not identified as pathogenic, but a regular contaminant of cutaneous specimens (Sigler, 2003). Consistent with other studies, *A. benhamiae* was most frequently isolated. In contrast to other studies where significantly younger animals were infected (D'Ovidio and Santoro, 2015; Kraemer et al., 2012; Vangeel et al., 2000), we did not find an age predilection. This may be explained by the overall younger ages of our sampled animals. In a comparable German study *T. mentagrophytes* was isolated from 8.5% of 164 healthy guinea pigs and from 7.7% of 26 guinea pigs with skin lesions. In contrast, no sample was positive for dermatophytes of 140 healthy rabbits and of 17 rabbits with skin disease (Kraemer et al., 2012). In an Italian study dermatophytosis prevalences of exotic pets with skin lesions were reported as 3.3% in rabbits (15/455), 24.7% in guinea pigs (23/93), 4.7% in ferrets (3/64), 30.1% in Chinchillas (4/13), and 9% in rats (1/11) (D'Ovidio and Santoro, 2015).

There are several reports of infections among pet shop employees or new owners of infected animals (Halsby et al., 2014). Recently, a significant increasing prevalence of positive guinea pig fungal cultures was reported by the Utrecht University Diagnostic Lab in the Netherlands. The average percentage of positive samples increased from 50%

(2011–2015) to 69% in 2015. The prevalence of positive rabbits was 13.2%. From all 4040 tested samples of dogs, cats, guinea pigs, and rabbits between 2010 and 2015, 11% of the owners reported dermatophytosis signs in family members. This percentage was the highest in guinea pig owners (20%). It was concluded that especially guinea pigs are asymptomatic carriers that pose a higher zoonotic risk than dogs, cats, and rabbits (Uiterwijk et al., 2016).

Animals infected with dermatophytes in pet shops are undesired. Not only because of zoonotic exposure for staff and customers, but also for the risk of spreading the infection to other animals. For this reason, preventive measures may be taken. After arrival in pet shop, quarantining of these animals in clean environments as well as fungal culturing are options to achieve dermatophyte-free animals (Miller and Hurley, 2009). A more pragmatic option may be a prophylactic washing of the animals with enilconazole.

Newly developed PCR-tests have reduced the time of culture results from weeks to just a few days and make this more feasible nowadays. When dermatophytes are cultured, the infected animals should not be sold to the public, but treated. Used cages need to be cleaned and disinfected with either enilconazole (1:100), sodium hypochlorite (1:32 or 1:100), accelerated hydrogen peroxide (1:16), or 2% potassium peroxymonosulphate (Moriello, 2015). Regular hygiene measures should be considered when handling animals. Using a single animal caretaker minimizes the exposure of pathogens to other staff and animals. Their hands should be washed and disinfected after working with the animals and clothes should be changed. Cages should be disinfected after cleaning and exchange between cages of bowls, food cups or other material should be prevented. Mixing of animals, especially rabbits and guinea pigs, in the same cage should be avoided.

Finally, staff and customers need to be informed about zoonotic infection risks. Pet shop owners have a responsibility to provide education to the public and for the prevention of the spread of dermatophytes. In a recent survey, however, it was found that 77% of the pet shop employees did not know the word ‘zoonosis’, and 27% did not follow any education for the job (Van Dam et al., 2016). For that reason it is advised to start the education at the pet shop level.

From none of the investigated rabbits and guinea pigs, *Cheyletiella* spp. could be isolated. This may be because up to 50% of the pet shops give prophylactic ivermectin treatments to the animals at arrival, but also because this parasite is not often found. In an Italian study of 455 pet shop rabbits, 3.3% was found to be infected with *C. parasitovorax*. In the same study, none of the 93 guinea pigs was infected (D’Ovidio and Santoro, 2014).

The risk of getting a *Cheyletiella* infection from pet shop animals in The Netherlands, either for the present staff, purchasing customers, or other animals, is therefore considered minimal.

Conflict of interest statement

none

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