Genetic epidemiology and pathology of raccoon-derived \textit{Sarcoptes} mites from urban areas of Germany

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Abstract. The raccoon, \textit{Procyon lotor} (Carnivora: Procyonidae), is an invasive species that is spreading throughout Europe, in which Germany represents its core area. Here, raccoons mostly live in rural regions, but some urban populations are already established, such as in the city of Kassel, or are starting to build up, such as in Berlin. The objective of this study was to investigate \textit{Sarcoptes} (Sarcoptiformes: Sarcoptidae) infections in raccoons in these two urban areas and to identify the putative origin of the parasite. Parasite morphology, and gross and histopathological examinations of diseased skin tissue were consistent with \textit{Sarcoptes scabiei} infection. Using nine microsatellite markers, we genotyped individual mites from five raccoons and compared them with \textit{Sarcoptes} mites derived from fox, wild boar and Northern chamois, originating from Italy and Switzerland. The raccoon-derived mites clustered together with the fox samples and were clearly differentiated from those of the wild boar and chamois samples, which suggests a fox origin for the raccoon mange infection. These results are evidence of the cross-transmission of \textit{S. scabiei} among wild carnivores. Although our results cannot elucidate whether raccoons became infected by frequent interaction with endemically or epidemically infected foxes or whether these cases resulted from occasional contacts among these animal species, they do nevertheless show that pathogens can be shared among urban populations of native and invasive carnivores.

Key words. \textit{Sarcoptes scabiei}, microsatellites, pathology, raccoon, wildlife.

Introduction

Sarcoptic mange is a contagious skin infection of domestic and wild animals caused by the mite \textit{Sarcoptes scabiei} (Fain, 1968; Pence & Ueckermann, 2002). The ectoparasite is considered as a single species divided into several varieties which show a certain degree of host specificity (Fain, 1978; Zahler \textit{et al.}, 1999; Bornstein \textit{et al.}, 2001; Pence & Ueckermann, 2002). However, the extent of host specificity is still widely discussed. Sarcoptic mange is reported in more than 100 mammal species worldwide.
we traced the origin of raccoon-derived mites col-
lected during previous studies, were jointly analysed. The DNA of each mite was extracted using the HotSHOT methodology (Bornstein et al., 2001) and occasionally the infection can reach epizootic proportions in some wildlife species such as Barbary sheep (Ammotragus lervia), Spanish ibex (Capra pyrenaica), Northern and Southern chamois (Rupicapra spp.) and red fox (Vulpes vulpes) in Europe (Pence & Ueckermann, 2002; Nimmervoll et al., 2013). Sarcoptes transmission may occur by direct or indirect contact because the mite can survive for a few days in dislodged skin crust under certain conditions (Arlian et al., 1988). Similarly to cross-transmission events between wild and domestic animal species (Arlian, 1989), zoonotic infections of *S. scabiei* in humans have been reported (Arlian, 1989). Human infection from an animal source is typically less severe and more short-lived (Arlian, 1989). Pet owners, personnel working with animals, veterinarians and wildlife biologists are most likely to become infected with animal-derived mites (Alasaad et al., 2013).

The disease is characterized by a severe dermatitis, usually accompanied by intense pruritus. Erythematous eruptions, papule formation, seborrhea and alopecia are often present (Bornstein et al., 2001). Histologically, orthokeratotic and parakeratotic hyperkeratosis, and epidermal hyperplasia with the formation of rete ridges are commonly described and are associated with dermal inflammatory cell infiltration by eosinophils, lymphocytes, mast cells and plasma cells (Nimmervoll et al., 2013).

Descriptive studies of *S. scabiei* epidemiology are regularly conducted in wild populations (Rossi et al., 2007), but the limitations of these classical epidemiological studies in revealing the host origin of an outbreak indicate the need for a population genetics-based approach (Alasaad et al., 2014).

The use of biomolecular investigations in epidemiology studies has contributed towards the gaining of new insights into transmission dynamics (Zahler et al., 1999; Walton et al., 2004; Rasero et al., 2010; Alasaad et al., 2011, 2014; Gakuya et al., 2011). Further, such investigations can improve surveillance studies and help to properly distinguish ‘genuine’ emerging and re-emerging cases of *Sarcoptes* mite infestation from pre-existing but neglected cases in the population (Oleaga et al., 2008).

The raccoon, *Procyon lotor*, was introduced into Germany from 1934. Currently, two main populations are identified in the country: one is located in central Germany in the surroundings of the city of Kassel, and the other is situated in the northeast in the federal states of Brandenburg and Mecklenburg-Western Pomerania. Additionally to these two main stable populations, small groups of raccoons can be found throughout Germany. First reports on causes of raccoon mortality in sylvatic and rural populations have been published (Michler et al., 2009) and it was concluded that raccoons play no significant role in maintaining and spreading infections of major concern to humans or domestic animals in Germany. However, no similar information is available for urban raccoons.

To date, only one report has been published on sarcoptic mange in raccoons, specifically in three animals from North America (Fitzgerald et al., 2004). Using microsatellite analyses, we traced the origin of raccoon-derived *Sarcoptes* mites collected in two urban areas in Germany.

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**Materials and methods**

**Sample collection**

Between 2009 and 2013 a larger study on raccoons in Germany was conducted in 139 animals from urban areas and 97 raccoons from a national park. Skin lesions consistent with mange were detected in five of these raccoons. Two of these five were male raccoons collected in Kassel in the federal state of Hesse, central Germany (51°19′00″N, 09°30′00″E) during spring 2009. Both animals were killed by a hunter because of their poor condition and were sent to the Leibniz Institute for Zoo and Wildlife Disease for post-mortem investigations. Three additional raccoons were collected in the Berlin metropolitan area in northeast Germany (52°30′02″N, 13°23′56″E) between 2011 and 2013. One adult male and one juvenile female raccoon collected in Berlin were presented to the Small Animal Clinic of the Faculty of Veterinary Medicine, Free University Berlin, because of their striking skin lesions. After clinical examination, both animals were killed and submitted to the Landeslabor Berlin-Brandenburg (LLBB) for post-mortem examination. One female raccoon was killed in a traffic accident and sent to the LLBB for post-mortem evaluation. Further details on the individual raccoons are listed in Table 1.

**Necropsy and histology**

Macroscopic examination and a routine necropsy were performed on each carcass. Samples of affected skin, brain, heart, intestines, kidney, liver, lung, lymph nodes, reproductive organs, spleen and tongue were extracted for further examination. All tissues were fixed in 4% formalin, processed routinely and embedded in paraffin. Tissue slides were sectioned at 4 μm, stained with haematoxylin and eosin, and examined by light microscopy.

**Mite isolation and morphological identification**

The affected skin areas of each raccoon were scraped with a scalpel blade to obtain hairs and crusts for mite isolation. The skin scrapings were separately placed on glass slides with distilled water and examined under a light microscope. Mites detected on each animal were manually collected and stored in an Eppendorf tube filled with 70% ethanol for scanning electron microscopy examination. Species identification was based on typical morphological characters as described by Fain (1968).

**Molecular analyses**

Six representative adult mites were isolated from each of the five raccoons and stored at −80°C. Mites collected from red foxes in Italy and Switzerland (10 mites each), from Northern chamois (*Rupicapra rupicapra*, 10 mites) in northeast Italy, and from wild boar (*Sus scrofa*, nine mites) in northwest Italy, all gathered during previous studies, were jointly analysed. The DNA of each mite was extracted using the HotSHOT...
Table 1. Collection date, weight, sex and age of the raccoons examined.

<table>
<thead>
<tr>
<th>Raccoon ID</th>
<th>Collection date</th>
<th>Origin</th>
<th>Weight, kg</th>
<th>Age group</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID1</td>
<td>August 2011</td>
<td>Berlin</td>
<td>2.50</td>
<td>Juvenile</td>
<td>Female</td>
</tr>
<tr>
<td>ID2</td>
<td>February 2012</td>
<td>Berlin</td>
<td>3.99</td>
<td>Adult</td>
<td>Female</td>
</tr>
<tr>
<td>ID3</td>
<td>March 2013</td>
<td>Berlin</td>
<td>3.65</td>
<td>Adult</td>
<td>Male</td>
</tr>
<tr>
<td>ID4</td>
<td>March 2009</td>
<td>Kassel</td>
<td>4.11</td>
<td>Juvenile</td>
<td>Male</td>
</tr>
<tr>
<td>ID5</td>
<td>March 2009</td>
<td>Kassel</td>
<td>2.88</td>
<td>Juvenile</td>
<td>Male</td>
</tr>
</tbody>
</table>

Plus Thermal SHOCK technique as previously described in Alasaad et al. (2008). Ten specific Sarcoptes mite microsatellites (Sarms 33–38, 40, 41, 44 and 45) were used in a 10x multiplex polymerase chain reaction as previously described by Alasaad et al. (2011) and Gakuya et al. (2011). Expected (Hₑ) and observed (Hₒ) heterozygosity, linkage disequilibria (LD) and Hardy–Weinberg equilibrium (HWE) were calculated using GENEPOP Version 3.4 (Raymond & Rousset, 1995). Deviations of HWE and LD tests were calculated using Fisher’s exact test and sequential Bonferroni corrections. The heterogeneity of genetic diversity among the different mite populations was estimated by the partition of variance components [analysis of molecular variance (AMOVA)] applying F₁T statistics using ARLEQUIN Version 3.11 (Excoffier, 2006). The relationship between mites was calculated using the Bayesian assignment test in the software STRUCTURE 2.3.4 (Pritchard et al., 2000). Twenty independent runs were run for K = 1–10. The Markov value for both burn-in and run lengths was 100,000. The most likely number of clusters was first determined by estimating the posterior probability for each K as recommended by the method of Evanno et al. (2005) using the website STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/structureHarvester) (Earl & vonHoldt, 2012). The degree of genetic relationship among populations was calculated using factorial component analysis (FCA) in GENETIX Version 4.05.2 (Belkhir, 1999).

Results

Macroscopic findings

During necropsy, raccoons ID1 and ID4 were found to be in good condition, whereas all other animals were in poor body condition. One of the Berlin raccoons (ID1) also had a fractured femur and a subcutaneous abscess at the left side of the neck. All animals had generally poor fur and displayed skin lesions of increasing severity. Small areas of alopecia and mild crusting starting from the dorsal neck and extending to the scapular region as far as the antebraclional regions of both front limbs were found in animal ID1. Raccoon ID2 exhibited patchy alopecia with mild crusting distributed along the thoracic dorsum and front limbs. Raccoon ID3 presented severe massive crusting and presumed subcutaneous abscesses along the dorsum and flanks. Alopecic areas were present at the front paws, caudal dorsum and tail. One male from Kassel (ID4) presented extensive areas of severe skin alterations with crusting and thickened skin extending along the caudal region of the dorsum towards both hind limbs and the base of the tail. Additionally, the skin of the ear pinna was severely thickened. Raccoon ID5, also from Kassel, showed severe proliferative dermatitis and alopecia over the entire abdominal and gluteal region, along the thighs and down to the knees.

Microscopic skin lesions

Histologically, the two males from Kassel presented severe (ID4) and moderate (ID5) parakeratotic hyperkeratosis of the skin, as well as generalized mild to moderate infiltration of mainly granulocytic inflammatory cells (ID4) or multiple small foci of inflammatory cells (ID5) associated with marked epidermal proliferation. The formation of rete ridges was observed in both male raccoons. There was moderate (ID5) or severe (ID4) distribution of intracorneal mites. Additionally, the pinna of one animal (ID4) had focal ulcerations of the epidermis with severe infiltration of mixed inflammatory cells. The raccoons from Berlin showed mild (ID1) and severe (ID3) parakeratotic and mild orthokeratotic hyperkeratosis (ID2) of the skin with mild intracorneal infestation of mites, mild (ID1, ID2) and severe (ID3) epidermal hyperplasia with rete ridges and mild multifocal superficial bacterial colonization; additionally, small subcorneal pustules filled with granulocytic cell debris and mild lichenoid infiltration of mixed inflammatory cells were found. Additional unrelated findings consisted of small chronic parasitic granuloma in the lymph nodes of one juvenile male (ID4), severe suppurative necrotic nephritis in the adult female (ID2) and mild interstitial pneumonia in the juvenile female (ID1).

Mite isolation and morphological examination

All skin scrapings revealed numerous eggs and roundish tortoise-shaped adult mites, nymphs and larvae. The adult female mites (n = 7) measured 203.1 × 309.9 μm, and the adult males (n = 2) measured 149.5 × 166.6 μm. Characteristically, thin ventral striations covered their idiosoma, triangular cuticular spines were present on the dorsal idiosoma and the anus was terminally located. Both anterior leg pairs carried bell-shaped suckers at the tip of unsegmented pedicels. The two posterior leg pairs did not extend beyond the margin of the body. Morphological features identified the mite species as S. scabiei.

Molecular analyses

Of 30 processed mites, only one from a Kassel raccoon and six from all Berlin raccoons were suitable for microsatellite
Sarcoptes infections were investigated in urban raccoons from Germany. The epidemiology and transmission dynamics of *S. scabiei* are still not well understood. Humans have been proposed to represent the original primary host of the mite (Fain, 1978) and it is thought that the mite subsequently spread to domestic animals and further to wildlife species. In free-ranging raccoons, sarcoptic mange has been reported only in Michigan, U.S.A. (Fitzgerald et al., 2004), whereas in Germany there are anecdotal reports of sightings of raccoons bearing overt skin lesions (Michler et al., 2009). In Europe, outbreaks of sarcoptic mange are commonly noted in sylvatic (Nimmervoll et al., 2013) and urban (Börner et al., 2009) foxes and the disease is endemically present in many regions in this species (Börner et al., 2009; Rasero et al., 2010; Nimmervoll et al., 2013).

Traditional epidemiological studies must be enhanced with biomolecular approaches in order to increase current understanding of Sarcoptes epidemiology (Alasaad et al., 2014). Genetic studies to differentiate *S. scabiei* are scarce and attempts...
using internal transcribed spacer 2 (ITS2) rDNA have proved unsuccessful (Alasaad et al., 2009).

The present study used microsatellite markers, which in the past have efficiently differentiated mite populations from different (non-raccoon) host species and geographical regions, eventually showing a lack of genetic flow among populations and a low degree of heterozygosity among individuals within populations (Walton et al., 2004; Rasero et al., 2010; Alasaad et al., 2011; Gakuya et al., 2011). Using microsatellite markers, Walton et al. (2004) showed genetic differentiation among mites from humans and dogs in Australia. Similarly, Rasero et al. (2010) and Alasaad et al. (2011) described genetic differentiation between Sarcoptes mite populations from different host taxa among wild animals in Europe. These authors reported a lack of genetic flow among populations from carnivorous, omnivorous and herbivorous hosts, a phenomenon known as the ‘host taxon law’ (Rasero et al., 2010), which explains why mange-free populations of herbivores such as the Alpine ibex (Capra ibex) and Northern chamois in the Western Alps can share habitat with endemically or epidemically infected foxes (Rasero et al., 2010).

In the current study, the Bayesian assignment test and FCA results showed an obvious differentiation between raccoon-derived and wild boar- and chamois-derived mites, all of which clustered separately. By contrast, mites from raccoons clustered together with Swiss and Italian fox-derived mites, suggesting that they belong to the carnivorous host taxon and that sarcoptic mange infection in raccoons is likely to have originated from infected foxes. However, additional biomolecular studies on mites derived from sympatric foxes and other carnivores, including dogs, would be desirable to rule out other putative sources of raccoon infection within the carnivorous taxon. Cross-transmission of S. scabiei mites belonging to the same host taxon (e.g. var. vilipes or var. canis) can occur between foxes and domestic canids (Rasero et al., 2010), and transmission from dogs or foxes to humans has been reported (Folz, 1984).

With respect to the German raccoon population, rare anecdotal sightings of rural raccoons with mange-like skin lesions have been reported, but the aetiology of these lesions has not been established. Moreover, raccoons in rural or sylvatic populations possibly interact mainly amongst themselves and do not interact much with other carnivore species. Therefore, interspecies transmission of infectious pathogens such as Sarcoptes seems to occur only rarely in this ample environment.

By contrast, cityscapes are characterized by habitat defragmentation and the reduction of green spaces. These restrictions force wildlife species to share most of their habitats and result in increasing rates of contact. Thus, the transmission of infectious pathogens such as Sarcoptes between raccoons and foxes may be more easily facilitated. As they also share areas such as parks with domestic pets, raccoons may represent a potential source of infectious disease for domestic animals and possibly humans.

However, it could be speculated that despite sharing the same habitat, direct or indirect contact between foxes and raccoons may occur at a low rate so that cross-infection is infrequent and overt clinical disease in raccoons is relatively rare. Moreover, as affected raccoons can be detected more easily in cities than in rural areas, we cannot rule out the possibility that mange cases in raccoons may be subject to reporting bias. To ameliorate this lack of epidemiological knowledge, wildlife professionals and veterinary authorities should be encouraged to record mange-infected raccoons, foxes and other carnivores, despite the fact that such findings are often felt to be of minor significance.

In conclusion, the current study describes the cross-transmission of S. scabiei mites among urban wild carnivores, as well as the presence of sarcoptic mange within raccoon populations outside their native range. However, it remains uncertain whether transmission was derived through continuous interaction or sporadic direct or indirect contact. The results of this study increase current knowledge about S. scabiei epidemiology in free-ranging hosts, showing that the host taxon phenomenon also applies in the urban wildlife context. Additional studies to elucidate whether Sarcoptes infection in raccoons in Germany represents an emerging disease or a neglected and unreported condition are necessary.

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Author’s declaration of interests

No competing interests have been declared.

References


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