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SHORT COMMUNICATION

Do ectoparasites of the slow loris *Nycticebus pygmaeus*, pose a danger to humans?

Jolanta Behnke-Borowczyk[1] · **Dariusz J. Gwiazdowicz**[1]

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Abstract

Staff working with nocturnal mammals at Poznań Zoo, noticed erythematous bite marks on their hands and parts of their necks. No perpetrators were immediately obvious, but the bite marks were experienced mainly by persons caring for the slow loris *Nycticebus pygmaeus*. The purpose of this study was to collect ectoparasites from four *N. pygmaeus*, to identify the species involved and to ascertain whether they carry any pathogenic organisms that might pose a health risk to people who have been bitten. A total of 51 *Ornithonyssus bacoti* (Mesostigmata: Macronyssidae) mites were collected from the coats of four slow loris, 37 of which were used for molecular analysis to determine if the mites were carrying any disease-causing organisms. DNA was extracted and screened for candidate pathogens including *Babesia* spp. and *Rickettsia* spp., but none were identified. The authors suspect that because the zoo differs in its sanitary and veterinary conditions from those found in nature, the results obtained here may differ markedly from those existing in the natural environment. Although we cannot be certain at this stage that the mites did not carry other pathogens in addition to those that were detectable by the primers that were used, the erythematous reaction to bite marks likely reflects a response to secretions of the mites rather than to transmitted pathogens.

Keywords *Ornithonyssus bacoti* · Zoo · *Babesia* · *Rickettsia*

Introduction

Dust mites are a large taxon of arachnids among which there are numerous ectoparasitic species. In mammals, skin-dwelling species can cause ectodermal irritation leading to severe dermatitis and they can carry haemoparasites. For example, the presence of *Dermanyssus* spp. mites can lead to disease and loss of condition in humans. In birds, they can cause anaemia and egg degradation, resulting in economic losses, and deterioration of the welfare of birds.

Animals living in zoos, ranches, wildlife parks or farms can also be hosts for parasites. However, captive animals do not live in isolation. They belong to a complex ecosystem that also includes free-living animals, their ectoparasites, and pathogens carried by the ectoparasites. For a free-living vagile animal, the zoo's grounds represent an extension of their habitat, where food, water and space exist free from the pressure of predators. However, the migration of mites and the transmission of pathogens between captive and free-living animals in zoos is poorly understood. It is possible therefore, that free-living animal inhabitants in zoos, their ectoparsites and pathogens carried by both, pose some dangers to "captive and public health" animals and their keepers.

In April 2015, night-time workers at the Zoo in Poznań (Poland) noticed bite marks on their hands, but also occasionally on other parts of their bodies, e.g. on their necks. These were typically erythematous, exceeding one centimetre in diameter and often there were up to a dozen such bite marks per person. Although itching was experienced, no other ailments were evident. The direct cause of these symptoms was not immediately obvious, but they were experienced most often by people caring for the slow loris *Nycticebus pygmaeus* Bonhote, 1907. Consequently, the animals were isolated, and their hair carefully scrutinized for possible ectoparasites. Small mites (less than 1 mm in length) were discovered on hair samples. The purpose of this study was to identify the species of mites recovered from the coats of four *N. pygmaeus* and to determine whether they carried any pathogenic organism that could pose a threat to the health of individuals that have had contact with the slow loris.

✉ Jolanta Behnke-Borowczyk
jbehnke@up.poznan.pl

1 Department of Forest Pathology, Poznań University of Life Sciences, Wojska Polskiego 71c, 60-625 Poznań, Polska

Materials and methods

Ectoparasites were collected by brush from the coat of four *N. pygmaeus* and fixed in 70 % ethanol. Representative mites were cleared in hot 85 % lactic acid and mounted on slides for microscopic examination. The material was examined using the Zeiss Axioskop 2 microscope with differential interference contrast optics. Mites were identified using taxonomic keys by Micherdziński (1980). Mite specimens are deposited at the Collection in the Poznań University of Life Sciences. A total of 51 mites were collected, 37 of which were used for molecular analysis to determine if the mites carried disease-causing organisms. Isolation of DNA was performed using a Modified Sherlock AX (A&A Biotechnology, Poland) kit. Elongated lysis was used, which was carried out at 50 °C for 72 h with continuous shaking. Quantitative and qualitative evaluation of DNA extraction was carried out via the NanoDrop ®ND-1000 system (PeqLab Erlangen, Germany). The primers used were those applied for the detection of selected haemoparasites known to be transmitted by ectoparasitic arthropods: *Borrelia* spp., *Bartonella* spp., *Rickettsia* spp., *Hepatozoon* spp., *Trypanosoma* spp. The primers and cycling conditions used in this study have been described by Bajer et al. (2014), Regnery et al. (1991) and Roux et al. (2000). As positive controls, the DNA of haemoparasites detected in the blood of the bank vole *Myodes glareolus* (Schreber, 1780), the eastern spiny mouse *Acomys dimidiatus* (Cretzschmar, 1826), the golden spiny mouse *A. russatus* (Wagner, 1840) and the Wagner's dipodil *Dipodillus dasyurus* (Wagner, 1842) were used (Bajer et al. 2014; Al-Sarraf et al. 2016). PCR products were subjected to electrophoresis on a 1.5 % agarose gel, stained with Midori Green stain (Nippon Genetics). Marker Dramix (A&A Biotechnology, Poland) DNA was used as a marker.

Results and discussion

All mites were identified as the tropical rat mite *Ornithonyssus bacoti* (Hirst, 1913). The molecular analysis of 37 female *O. bacoti* failed to reveal any evidence of the presence of potentially pathogenic microorganisms.

The tropical rat mite *O. bacoti* is known to be active at night and to seek dark hiding places during the daytime. Preferred hosts are wild rodents such as the rats *Rattus norvegicus* Berkenhout, 1769 and *R. rattus* (Linnaeus, 1758), house mice *Mus musculus* Linnaeus, 1758, jirds *Meriones unguiculatus* (Milne-Edwards, 1867), meadow voles *Microtus pennsylvanicus* (Ord, 1815), white-footed mice *Peromyscus leucopus* (Rafinesque, 1818), cats and other wild and domestic carnivores, some birds, opossums, and humans (Soliman et al. 2001; Reeves et al. 2007; Beck and Fölster-Holst 2009; Kowal et al. 2014). All developmental stages of *O. bacoti* exclusively consume the blood of their hosts during daytime and then leave their hosts and return to the nest area and hide in cracks and crevices in the immediate vicinity. Previous reports have shown that *O. bacoti* recovered from wild hosts are vectors of nematodes *Coxiella burnetii* (Derrick, 1939) Philip, 1948 (Q-fever causative agent), hantavirus *Borrelia* spp., *Bartonella* spp., and *Rickettsia* spp. as reviewed by Yunker (1964), Traub et al. (1978), Renz and Wenk (1981) and Walter and Shaw (2005). Under laboratory conditions, it has been observed that *O. bacoti* can be a vector of *Rickettsia akari* Huebner et al., 1946 (causing rickettsial pox), *Francisella pestis* (McCoy and Chapin, 1912) Dorofe'ev, 1947 (causative agent of plague), *Coxsackie virus*, *Francisella tularensis* (McCoy and Chapin, 1912) Dorofe'ev, 1947 (causing tularemia) and *Trypanosoma cruzi* Chagas, 1909 (causing Chagas disease). Reeves et al. (2007) reported a prevalence of 6.7 % of *Rickettsia* spp. in O. *bacoti* in Egypt, and identified two genotypes, indicating divergence in the *Rickettsia* species.

Research on diseases transmitted by ectoparasitic arachnids (for example ticks) has been reported previously by Stoebel et al. (2003), Širmarová et al. (2014), Ticha et al. (2016), Gonzalez et al. (2017), Hrnková et al. (2021). These studies focused mainly on the negative effects of the ectoparasites on their animal hosts. Nelder et al. (2009) reported on ectoparasite populations and the pathogens they transmitted in zoos in North Carolina in 2004–2007, and detected six taxa of pathogens including: *Anaplasma phagocytophilum* (Foggie, 1949) Dumler et al., 2001 in the tick *Ixodes dentatus* Marx, 1899 from an eastern cottontail rabbit, *Bartonella clarridgeiae* Lawson and Collins, 1996 in the cat flea *Ctenocephalides felis* (Bouché, 1835) from a Virginia opossum, *Bartonella* sp. in the squirrel flea *Orchopeas howardi* (Baker, 1895) from an eastern grey squirrel, *Bartonella* sp. T7498 in the sucking louse *Neohaematopinus sciuri* Jancke, 1932 from a squirrel, *Rickettsia* sp. Rf2125 in *C. felis* from a zookeeper and a grizzly bear, and Rickettsiales Ib, 2006 in *Ixodes brunneus* Koch, 1844 from an American crow. To-date little is still known about how pathogens such as *Anaplasma phagocytophilum* (the causative agent of human granulocytic anaplasmosis) and *Bartonella clarridgeiae* (the causative agent of cat scratch-like disease) may affect humans. However, it is clear that ectoparasites and their pathogens, especially those originating from free-roaming animals, present a potential threat to captive animals and humans.

None of the studies reported to-date have indicated any detrimental effects of ectoparasites on people working at zoos as keepers of nocturnal mammals. Although no pathogens have been identified in the mites by the range of primers that we used, the possibility that other microorganisms were harboured by the examined mites cannot be excluded. The slow loris in our study have lived at Poznań Zoo for a number of years and it is highly probable that over the prolonged period of their captivity at the zoo, they have recovered from any infections originally brought with them from the wild. Consequently, the mites they still harbour may have been devoid of pathogenic microorganisms.

Indeed, the failure of our staff to report any further consequences apart from itching and temporary erythema suggests that no organisms infectious to humans were harboured. It is therefore highly likely that the reactions to the bite marks reported by the staff were reactions to the secretions of the mites, released in attempts to feed on human skin. The frequent proximity of the keepers and their charges at the zoo creates an environment in which transfer of mites from the slow loris to humans is highly feasible and in the absence of a natural host, these mites may occasionally feed on humans, causing allergic reaction.

Ethical approval The authors declare that no institutional or national guidelines for the care and use of animals need to be followed in this study. All appropriate permissions from museums authorities for the use of specimens included in the study have been obtained.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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