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Short communication

First report of *Neotrombicula inopinata* infestation in domestic cats from Portugal

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ABSTRACT

Trombiculids parasitize a wide variety of terrestrial vertebrates, including domestic animals, throughout the world. They are parasites only during their larval stages, causing several dermatological lesions on their hosts, such as acute dermatitis, erythema, excoriation, erosion, papules, crusts and alopecia on the ear margins, face, interdigital spaces and abdomen. *Neotrombicula* is one of the several genera in Trombiculidae family, which cause trombiculosis. The most common species implicated in clinical cases is *Neotrombicula autumnalis*. However, several reports have shown that *Neotrombicula inopinata* (Oudemans, 1909) can also play a role in trombiculosis. Here, we describe the first case of *N. inopinata* infestation in domestic cats from mainland Portugal. Since nucleic acids of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* have been found in *Neotrombicula autumnalis* and *Rickettsia* spp. in *Neotrombicula inopinata*, a correct taxonomical identification is essential to understand the role of these mite species as possible vectors of pathogens.

Trombiculids are also known as chigger mites or harvest mites and have the ability to parasitize a wide variety of terrestrial vertebrates throughout the world (Santibáñez, 2015). The majority of reported cases include cats, dogs and humans (Giannoulopoulos et al., 2012; Leone et al., 2013; Parcell et al., 2013; Tudor et al., 2015; Guarneri et al., 2017a; Cadiergues et al., 2018). Isolated cases have also been reported on deer, bears, wallabies and frogs (Santibáñez, 2015). Only the larval stages of these mites include parasitic behaviour and it is the six-legged larvae the responsible for the appearance of several clinical signs, such as acute dermatitis, on the affected hosts (Guarneri et al., 2005; Cadiergues et al., 2018). In cats erythema, excoriation, erosion, papules, crusts and alopecia, mostly on the ear margins, face, interdigital spaces and ventral abdomen, have been described (Schöler et al., 2006; Leone et al., 2013). Infested hosts can suffer from substantial pruritus, although some individuals may harbour high numbers of mites without any lesions or apparent discomfort (Schöler et al., 2006).

Neotrombicula Hirst, 1925 is one of several genera in the family Trombiculidae (phylum Arthropoda, class Arachnida, subclass Acari) (Tudor et al., 2015; Stekolnikov et al., 2016). The life cycle of *Neotrombicula* begins with the deposition of eggs in the soil. Within 10 days the hexapod larvae hatch, climbing to any available warmblood-host to feed for 2–10 days (Parcell et al., 2013; Guarneri et al., 2017a). During their blood meal, mites inject lytic enzymes in the upper layers of the skin through their chelicerae (Tudor et al., 2015). After their blood meal, larvae return to the soil, moulting to the next stages (three stages of eight-legged nymphs followed by adults) and feed on plant fluids or small insects (Guarneri et al., 2005). The adult female performs oviposition during spring and summer, with larvae being more abundant in late summer and autumn (Nuttall et al., 1998; Guarneri et al., 2005; Schöler et al., 2006; Leone et al., 2013).

Feline trombiculosis outbreaks caused by *Neotrombicula* larvae have been reported in Scotland (Nuttall et al., 1998), Italy (Leone et al., 2013), Spain (Martí-Ollé et al., 2011; Stekolnikov et al., 2014), Greece and Cyprus (Giannoulopoulos et al., 2012), Romania (Tudor et al., 2015) and France (Cadiergues et al., 2018). Some studies suggest that *Neotrombicula autumnalis* may have the potential to transmit, by transtadial and transovarian routes, *Borrelia burgdorferi*, the agent of Lyme disease in animals and humans (Kampen et al., 2004), and also to carry *Anaplasma phagocytophilum*, which causes zoonotic granulocytic anaplasmosis (Fernández-Soto et al., 2001). These agents have ticks as proved vectors and can cause considerable morbidity and mortality in the affected hosts (Fernández-Soto et al., 2001). In addition, amplicons

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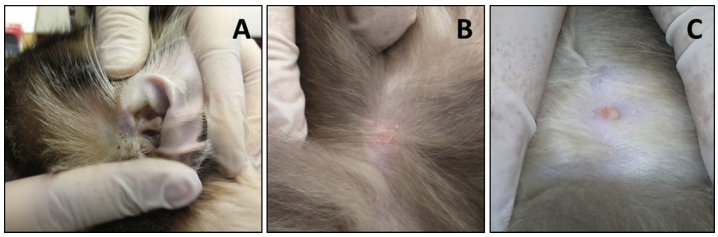

Fig. 1. Dermatological lesions observed in the ear (A), the neck (B) and ventral abdomen (C) of the youngest cat.

compatible with *Rickettsia* spp. infection were detected by molecular techniques in *Neotrombicula inopinata* unfed larval stages collected over vegetation (Santibáñez et al., 2015).

Neotrombicula autumnalis (Shaw, 1790) (syn. *Trombicula autumnalis*) is frequently suspected in clinical cases of human and animal trombiculosis (Schöler et al., 2006; Stekolnikov et al., 2016), although other species, such as *N. inopinata* (Oudemans, 1909), can also be the main cause of this disease (Mąkol et al., 2010). Since they are closed related, *N. inopinata* can be confounded with *N. autumnalis* (Stekolnikov et al., 2014). *Neotrombicula inopinata* has been reported in several European countries (Santibáñez et al., 2015) and may infest humans (Stekolnikov et al., 2014). Thus, adequate taxonomic identification of collected specimens is essential to understand the epidemiology of trombiculosis (Stekolnikov et al., 2014, 2016).

On the 25th of October 2017, two domestic male cats, aged 9 months (4.1 kg body weight) and 6 years (4.3 kg body weight), both neutered, were presented at the Veterinary Teaching Hospital, University of Lisbon, Portugal, with a history of recent skin lesions in the head, ear, neck and abdomen, appearing the week prior to consult, according to the owner (Fig. 1). The cats had outdoor access in a rural environment and were cohabitants. The clinical signs appeared first in the youngest patient, who spent most time outdoors, whereas the oldest one showed similar clinical signs about 4 weeks later.

The cats presented a normal general physical examination. The dermatological evaluation presented small crusty pruritic lesions in the head and neck of both patients. Orange larval mites were detected macroscopically in the head and neck crusts and a large number of mites were observed in the ear canal during direct visual inspection and also at otoscopic examination. Dermatophyte culture from skin lesions samples was negative.

Specimens were collected with swabs and mounted between slide and cover using lactophenol (20% phenol; 20% lactic acid; 40% glycerine; 20% distilled water). The slides were dried in an incubator at 37 °C for 3–4 days. For taxonomic identification, several morphological features were analysed and different identification keys were used (Stekolnikov et al., 2014, 2016; Stekolnikov, 2018). The observed specimens had the characteristic traits of the genus *Neotrombicula*, including palpal three-pronged claw (Fig. 2A), nude galeal setae, subpentagonal or subhexagonal scutum, wider than longer, with rounded posterior margins (Fig. 2B). *Neotrombicula inopinata* differs from *N. autumnalis* due to the presence of eight or more setae in the first two rows of dorsal idiosomal setae, sometimes with six or seven setae in one of these rows, while *N. autumnalis* has always six setae in both rows (Stekolnikov et al., 2014). In collected specimens, dorsal idiosomal setae were arranged as (8–9)-(8–9)-(7–8)-6-4-2 (Fig. 2C). After these observations, the specimens were identified as *N. inopinata*.

In each cat, the initial treatment included a spot-on solution of fipronil 75 mg, (*S*)-metopren 90 mg, eprinomectin 3.6 mg and praziquantel 75 mg (Broadline®, Boehringer Ingelheim) application to be repeated 1 month later. Due to tutor compliance challenges, it was not possible to implement the 3 days fipronil spray treatment as recommended in the European Scientific Counsel Companion Animal Parasites guidelines (ESCCAP (European Scientific Counsel Companion Animal Parasites), 2018). Therefore, the fact that the youngest cat spent most time outdoors made it unrealistic to accomplish a daily treatment.

Otological treatment was performed by filling the ear canal with a 10% (v/v) ivermectin auricular gel (Otimectin®, Esteve) once a week for 3 weeks. Ivermectin was chosen for two main reasons: (a) the massive infestation of the ears; and (b) no other specific treatment for this parasite was available as acaricide topical treatment. Skin lesions were smaller in both patients at the 2-month follow-up evaluation, yet mites still appeared at macro and microscopical assessments. Hence, a treatment with a spot-on of fluralaner at 112.5 mg/tube (Bravecto®, MSD) per cat was applied. Swab controls were performed every 2 weeks, and the first negative swab was obtained on the 3th of March 2018 (i.e. 18 weeks after treatment onset).

The two cats had free outdoor access. Because of their exploratory instinct, cats tend to be carriers of various parasites, including trombiculids. When returning to their domestic environment, these animals can transmit these mites to other animals with which they cohabit and also to their owners (Guarneri et al., 2005, 2017a;2017b; Parcell et al., 2013). Trombiculid mites cause pruritus, skin lesions and may be potential vectors of pathogens (Santibáñez et al., 2015; Tudor et al., 2015).

In this clinical case, the infestation was eliminated 4 months after the initial treatment. The available antiparasitic products are not registered specifically for trombiculid mites and active substances must be repurposed. Furthermore, correct diagnosis of these parasites is important for epidemiological studies. However, in most of the scientific studies concerning trombiculosis, specimens are not always classified correctly down to a species level, a circumstance that may be reflected on the fact that *N. autumnalis* is generally incriminated as the main agent of this disease. Taxonomic classification has also been hampered by the lack of identification keys for trombiculid mites. In this way, further work should be carried out in order to standardize the classification of these parasitic agents, complemented with molecular biology analysis whenever possible.

The occurrence of trombiculosis in domestic animals and humans is important since *Neotrombicula* spp. may be vectors of different pathogenic agents. Veterinarians, as well as human dermatologists, must be

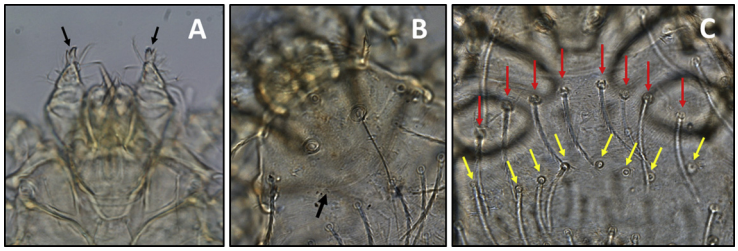

Fig. 2. *Neotrombicula inopinata*. (A) Palpal three-pronged claws (black arrows), compatible with the genus *Neotrombicula*. (B) Subpentagonal scutum wider than longer, with rounded posterior margin (black arrow). (C) Dorsal idiosomal setae arrangement, with eight setae on the first two rows (red and yellow arrows, respectively), a unique morphological characteristic of *N. inopinata* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

aware of these less known mites, considering they cause dermatological lesions to affected hosts, and must explain the importance of this infestation to the owners of domestic animals. Another important issue to solve is the almost non-existent identification keys for different genera and species within the family Trombiculidae, so a better knowledge concerning trombiculosis epidemiology is achieved. Further studies of drug efficacy must also be performed.

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Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

David W. Ramilo: conceptualization, investigation, methodology, writing – original draft preparation; writing – reviewing and editing. Carla Monteiro: conceptualization, methodology, writing – original draft preparation; writing – reviewing and editing. Marrion Carreira: methodology. Isabel Pereira da Fonseca: resources, supervision, writing – reviewing and editing. Luís Cardoso: supervision, writing – reviewing and editing.

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