

Severe Trombiculiasis in Hunting Dogs Infested With *Neotrombicula inopinata* (Acari: Trombiculidae)

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Abstract

This study records the clinical findings in nine hunting dogs showing systemic illness associated with trombiculids and identifies the mite species involved. In fall, coinciding with the seasonality of mites, all dogs were infested with mites and had been in the risk area (Sierra Cebollera Natural Park, La Rioja, Spain) a few hours before the onset of symptoms. The symptoms included vomiting, anorexia, weakness and lethargy, diarrhea, and even stupor. The clinical picture was fast-acting and potentially fatal. The infestations varied from low to severe. Molecular analysis of mites that fed on the dogs confirmed that they were larvae of *Neotrombicula inopinata* (Oudemans, Acari, Trombiculidae). This is the first time that *N. inopinata* has been identified as feeding on dogs and implicated in canine systemic illness associated with trombiculids. In contrast to other chiggers, *N. inopinata* does not seem to cause dermatitis. Likewise, the clinical and epidemiological similarity between the clinical symptoms we describe herein and the occurrence of seasonal canine illness (SCI) led us to suspect that this illness may be caused by infestation with these mites. The condition could be the consequence of severe infestation from large numbers of feeding mites, especially *N. inopinata*. Whether or not the cases were due to a severe allergic host response to salivary proteins or the result of the transmission of a new or emerging trombiculid-borne pathogen is not known.

Key words: trombiculiasis, chigger mite, *Neotrombicula inopinata*, digestive affectation, seasonal canine illness

Trombiculidae are a family of mites with significance in the medical and veterinary fields. As nymphs and adults, they are found as free-living predators that feed on either plants or other arthropods, but as larvae, which are more commonly known as chiggers, they must feed on vertebrate hosts. Chiggers have a strong seasonal activity; in late summer and autumn, they climb up long grass so they may transfer to the host via direct contact (Wall and Shearer 2001). Chigger mites do not appear to have a preferred host and can therefore infest a number of vertebrates; however, their usual hosts tend to be wild animals, although agricultural domestic animals, household pets, and humans can also be infested (Le Net 2002, Seixas et al. 2006, Ramirez et al. 2009, Guaguère et al. 2011, Gomez-Puerta et al. 2012, Santibañez-Sáenz et al. 2014). In the first 24 h of attachment, chiggers take advantage of the host's hyperkeratotic skin cells to produce a feeding tube called a stylostome. This is then employed to feed in a highly efficient and concentrated manner on an extremely nutritive liquid comprising extraorally digested epidermal and occasionally dermal tissue. Therefore, feeding only takes place after the tube has been formed, which is presumed

to rely on a mutual interaction between larval secretions and the epidermal skin cells (Fischer and Walton 2014, Shatrov et al. 2014). The larvae feast on host fluid for 3–5 d before dropping off and continuing their life cycle as free-living mites (Beugnet and Bourdeau 1999).

Affected areas on the host's body are those that typically come into contact with plants or the ground—legs, feet, head (particularly the ears), and ventrum (appearing as orange-red spots)—and may correspond to the chigger's initial site of contact (Scott et al. 2001).

The fauna of Trombiculidae in Spain includes at least 20 species (Stekolnikov et al. 2014, 2016). La Rioja region in northern Spain records several cases of human and canine trombiculiasis each year during late summer to mid-autumn. This is the only area of Spain to have evidenced human trombiculiasis (Jiménez et al. 2001, Santibañez-Sáenz et al. 2014). All cases recorded in this region were observed in humans and dogs that had come into contact with vegetation in the Sierra de Cebollera Natural Park. The species involved in these examples was first identified as *Neotrombicula autumnalis*

(Oteo et al. 2006). However, recent studies carried out by morphological (Stekolnikov et al. 2014) and molecular identification (Santibañez-Sáenz 2015) suggest the causal agent is *Neotrombicula inopinata*.

Infested subjects suffer from the mechanical action of the mites, hypersensitivity, and/or secondary bacterial infections, which, in turn, produce varying degrees of local inflammation but do not usually affect the host's general condition. In fact, trombiculid mite larvae infestation usually produces dermatitis (Mullen and O'Connor 2002, Fischer and Walton 2014). Hosts may develop highly pruritic, erythematous nodules, crusted papules, and self-induced excoriations (Scott et al. 2001, Le Net et al. 2002, Font 2007, Guaguère et al. 2011, Tudor 2012).

In Europe, the Trombiculidae have not been reported to be vectors of diseases; however, they are carriers of *Borrelia* sp., *Anaplasma phagocytophilum*, and *Rickettsia* sp. (Fernández-Soto et al. 2001, Kampen et al. 2004, Literak et al. 2008, Mir'ková et al. 2015). Besides, trombiculids also have been implicated with the so-called seasonal canine illness (SCI), a potentially life-threatening disease described in eastern England (McGarry 2012).

Likewise, dogs affected by a systemic illness associated with trombiculids have been reported (Oteo et al. 2006, Santibañez-Sáenz et al. 2014, Santibañez-Sáenz 2015). This is a known process in the region (there have been anecdotal reports from veterinarians or physicians), but very little information regarding the clinical picture and etiological agent involved in the case of dogs has been published. The information about clinical picture of dogs was superficially treated because, in many cases, it is derived from studies carried out in man, in which the information related with dog is scarce. There is also no information on the etiological agent directly involved in these cases, since the identification has been carried out only in the mites collected from the vegetation (unfed mites).

This study records the clinical findings in a series of nine hunting dogs suspected of systemic illness associated with trombiculids and tries to identify the species involved.

Materials and Methods

The study was carried out between the months of October and December 2016.

Clinical Cases Follow-Up

During the fall of 2015, a case of systemic involvement associated with trombiculids was treated in a patient who had been in the risk zone a few hours before. Subsequently, prior to the summer–fall of 2016, we contacted different clinics that attended cases in the area and were asked to communicate the suspected cases. We performed a clinical follow-up on dogs suspected of suffering from trombiculid mite infestation. The criteria for including these patients for follow-up and study inclusion were clinical signs of systemic illness, presence of mites, and previous history of exposure in the risk area a few hours before. The cases were attended in four veterinary clinics in northern Spanish cities: Arnedo, Tudela, San Sebastián, and Vitoria. Data were collected through the authors' first-hand experience or by means of interviews with the referring clinicians. Detailed clinical histories were obtained for the dogs once they were admitted, along with the results of subsequent daily clinical examinations.

To evaluate parasite load, eight body zones were evaluated in each animal (over the legs, between the digits, around the eyes, ears, and lips, sternum, abdomen, and perineum) and for each zone three degrees of parasitic load were applied (1, 2, and 3). From the total

data and according to the percentage of mites covering their preferred areas of skin, the following scale was used: <30% = low; 30–60% = moderate; and >60% = severe.

Collection of Trombiculidae

To identify the species of trombiculid mites involved, specimens were collected directly from the vegetation in the study area comprising the Sierra de Cebollera Natural Park and its surroundings. The Sierra de Cebollera Natural Park has an area of 23,640 hectares and is located in the northwest of the Iberian Central System, northern Spain. Samples were taken specifically from four areas in the municipalities of San Pedro Manrique (42°05'59.68"N; 2°14'46.49"W; altitude 1,206 m) and Santa Cruz de Yangüas (42°04'03.91"N; 2°30'53.93"W; altitude 1,497 m). These sampling areas were selected based on records for cases of human and canine trombiculiasis. These areas had also been frequented by the dogs subject of our study.

To collect mites from vegetation, we employed the black-plate method, which is often used to obtain free trombiculid larvae from the ground. This process involves dragging a 20 × 40 cm black plastic plate over the ground. Then, a brush was used to transfer the chiggers collected on the plate into tubes filled with distilled water. Chiggers found on top of leaves and small dry branches were placed directly in tubes. In addition, any chiggers on affected dogs were removed using metal swabs.

The mites were preserved in vials containing 70% ethanol. Both fasting and fed mites were used in the identification process.

Identification

Morphological identification

Morphological identification was only carried out on unfed individuals collected from the vegetation. The morphological analysis was carried out in 40 specimens. To this end, the mites were clarified and mounted on a slide in Hoyer's medium (45°C/5 d), then sealed using nail polish. The specimens were examined with a light microscope and identified according to the taxonomic keys published by Krantz and Walter (2009) and Stekolnikov et al. (2014) using 25 characters. Measurements were expressed in microns.

Molecular identification (qPCR)

A Maxwell 16 Lev Blood DNA Kit (Promega, Madison, WI) was used according to the manufacturer's instructions to purify DNA from the collected specimens, with the exception of the following modification: after adding lysis buffer and before digestion with protein K for 1 h at 70°C, the sample was homogenized in an Eppendorf tube using an electric pestle. The extracted DNA was then used to conduct a qPCR analysis of the cytochrome oxidase subunit 1 (COI) gene GenBank: KR337639.1. *Neotrombicula inopinata* COI gene was used for designing two sets of primers. The aim of qPCR in our study is to confirm the already morphologically determined the parasite, with a detailed measurement and observation of the specimen (unfed mites) and extrapolated the results for the samples obtained from parasitized dogs. It is well accepted the use of COI gene for species classification (Wang et al. 2018). The Blastn of the partial sequence of *N. inopinata* COI gene revealed to have significant variation with other similar genes from other species. Real-time PCR amplification was carried out using the GoTaq qPCR Master Mix (Promega) reagent system and the primers were prepared using IDT's integrated tool (qPCR assay design) with forward primer Nino F: 5'-TGGTTATTAGTCCCTTCTAT-3' and reverse primer Nino R: 5'-AAAGAGGAGGGTAGATTG-3'. The qPCR assays were carried out with a CFX Connect Real-Time PCR

Detection System (Bio-Rad Laboratories, Hercules, CA) according to a protocol involving 5 min at 94°C for GoTaq enzyme activation, followed by 44 cycles of 10 s at 94°C and 40 s at 55°C for annealing, elongation, and fluorescence data acquisition. We measured the melting curve between 65 and 90°C at a temperature transition rate of 0.5°C/s after the last amplification cycle. All amplification reactions were performed in triplicate.

Molecular identification was carried out on specimens feeding on the dogs and unfed specimens collected from the vegetation. As for the number of samples per dog used for molecular study, at least two samples per animal in triplicates. From the mites collected from the vegetation, two samples in triplicate were made for each zone where the affected dogs had been.

Results

Clinical History

Nine cases of dogs with a clinical picture indicative of trombiculiasis were reported. Table 1 shows clinical signs and examination findings. All cases occurred from mid-October to mid-November 2016, and the dogs—three females and six males—had visited the risk area in the previous 24–60 h before the appearance of clinical signs. The animals were aged between 2 and 8 yr and belonged to four different hunting breeds. The dogs belonged to different owners, and they resided in cities located between 16 and 139 km away from the risk zone. They lived in kennels and were fed mostly commercial food. Two animals (dogs 1 and 2) were treated a week before against ectoparasites with imidacloprid and permethrin and three animals (dogs 6, 7, and 8) were treated with organophosphates just before entering the risk zone. The animal's medical history did not include any relevant data, with the exception of a dog which had had a similar incident the previous year but had already recovered.

Clinical signs

All dogs were infested by chiggers, which had formed several dozen orange clusters, each containing 50–100 mites. These aggregations were widely distributed on the face, mainly around the ears, eyes, and lips, over the legs, sternum, abdomen, perineum, and between the digits. The parasite load varied between low to severe depending on each animal.

Signs developed 24–60 h after walking through the infested area in Sierra de Cebollera Natural Park. The animals were found to be lethargic and anorexic, and presented weakness that ranged from moderate to severe; three of them (dogs 1, 8, and 9) presented a state of semiconsciousness with prostration, that could be associated with hypoperfusion (capillary refill time > 2", mucous pallor, weak tarsal pulse, and mean arterial pressure <70 mmHg). Dog number 9 died within 24 h of exposure showing severe parasite load and bilious vomiting of hyperacute presentation. The animal was not subject to a postmortem examination. Vomiting was common in all dogs, and three of them also had diarrhea. Dogs 2, 3, 5, and 8 also showed signs of abdominal pain. The attending veterinarians also observed mild to severe dehydration secondary to the digestive signs and one subject exhibited dyspnea (dog number 7).

Regarding skin involvement, the animals did not manifest significant skin lesions or pruritus; only two dogs had localized erythema in the areas of parasite infestation (most notably dog number 8). None of the animals presented neurological signs or abnormal body temperature. There was no evidence suggesting consumption of any substances that might produce these clinical signs.

Response to treatment and patient progress

The patients received immediate intravenous fluid therapy, along with antiemetics and corticosteroids at antiallergic doses. They were also treated against ectoparasites with pyrethroids by means of baths and/or local application. Four of the eight surviving dogs required hospitalization (dogs 1, 2, 5, and 8) and three of them evolved favorably in 48–72 h with the treatment, which was continued until the clinical signs remitted and each patient recovered their appetite. However, one patient (dog number 5) did not respond to the treatment of clinical signs and X-rays revealed abnormalities at the intestinal level (generalized enteritis). An exploratory laparotomy was performed, and the surgeons observed necrotic enteritis affecting a large section of the intestine, so they proceeded with an enterectomy. The enterectomy, of approximately 8 cm, was performed in the duodenum. Following the usual postoperative treatment, the animal showed a favorable evolution. The remaining four patients did not require hospitalization and continued with the treatment of corticosteroids, antiemetics, and rehydration therapy until they recovered their appetite. The recovery period varied between 3 and 10 d and showed no correlation with the initial severity. Thereafter, none of the dogs presented any sequela after a follow-up of 5–7 d.

Table 1. Clinical data from a series of nine cases of suspected trombiculiasis in hunting dogs

Dog	Sex	Age (years)	Condition	Digestive symptoms		Pain symptoms	Respiratory symptoms	Dermatological symptoms		Parasitic load ^a
				Vomiting	Diarrhea			Pruritus	Others	
1	♀	2	Semiconsciousness	Yes	No	Yes	No	No		Severe
2	♀	4	Weakness	Yes	No	Yes	No	No		Severe
3	♀	5	Weakness	Yes	No	No	No	No	Erythema	Low
4	♂	4	Weakness	Yes	No	No	No	No		Low
5	♂	6	Weakness	Yes	No	Yes	No	No		Low
6	♂	6	Weakness	Yes	Yes	No	No	No		Low
7	♂	8	Weakness	Yes	Yes	No	Yes	No		Low
8	♂	6	Semiconsciousness	Yes	Yes	Yes	No	No	Erythema	Moderate
9 ^b	♂	3	Semiconsciousness	Yes				No		Severe

♀ = female; ♂ = male.

^aAccording to the percentage of mites that cover the areas of preference: low: <30%, moderate: 30–60%, and severe: >60%.

^bThe animal died in 24 h following a picture of hyperacute vomiting.

Identification

The morphological study could only be carried out on unfed specimens due to the conformational change the mites undergo after feeding. The results are presented in Table 2. When comparing our data against those of Stekolnikov et al. (2014), measurements suggest the mite could be the species *N. inopinata*. In addition, qPCR analysis detected the presence of the COI gene and confirmed that *N. inopinata* was present in the samples collected from both fed and unfed specimens.

Discussion

This is the first complete description of a clinical picture associated with chigger mites and detrimental to the dog's overall health reported in Europe. Similarly, until now this association was only based on epidemiological evidence, but this work identifies the chigger species from specimens found feeding on dogs affected by a systemic illness associated with trombiculids.

The clinical cases occurred in October and November, coinciding with the seasonality of chigger mites (Guarneri et al. 2005, Oteo et al. 2006, Santibañez-Sáez et al. 2014). The dogs were infested within a well-defined geographical area. The origin of the infestation could be the same, as all the dogs had been in the natural park between 24 and 60 h before the clinical signs developed. The morphological and molecular analysis of the mites collected from the vegetation in these zones confirmed the presence of *N. inopinata*. This is the second time the trombiculid species *N. inopinata* has been documented in Spain (Stekolnikov et al. 2014).

Although we were able to infer the causative agent of trombiculiasis based on the questing chiggers collected from vegetation in the study area, the removal and identification of engorged

trombiculid mites with fully formed stylostomes from domestic animals is still essential in order to define the etiological agent of trombiculiasis (Stekolnikov et al. 2014). Accumulations of orange ectoparasites were observed on the skin of all affected animals. The molecular analysis of fed mites confirmed they were *N. inopinata* larvae. This is the first report of *N. inopinata* feeding on dogs and is associated with a canine clinical picture of systemic illness associated with trombiculids.

In the present work, we have described trombiculiasis with extradermal involvement. Chigger infestations are known to cause dermatitis in both humans and dogs, but in previous publications infestation was not detrimental to the host animal's health (Le Net et al. 2002, Seixas et al. 2006, Tudor 2012, Fischer and Walton 2014). However, in this study, the main clinical signs associated with trombiculid infestation are vomiting, anorexia, weakness, and lethargy, generally accompanied by diarrhea and even stupor. The signs first appeared 1–3 d after the subjects were present in the risk area. The digestive signs cause severe dehydration and in turn pronounced weakness. This condition develops quickly and is potentially fatal, the clinical picture often becoming severe within a matter of hours. However, if antiparasitic medication is established and clinical signs are treated in time, the animals seem to overcome the condition.

The parasite load detected in the animals varied from low to severe and generally in dogs with higher loads the severity of the process was greater. Nevertheless, this was not confirmed in all cases as dog number 5 had one of the worst clinical conditions but only presented a low parasite load. Since the parasite load was evaluated when the animal arrived at the clinic (approximately 72 h after being in the risk zone), then the initial infestation may have been greater as some mites could have already completed feeding and dropped off the host. A severe allergic host response to the mites' salivary

Table 2. Measurements (range and mean) of specimens collected from vegetation

Variable	Examined specimens	
	Range	Mean
Distance between anterolateral scutal setae	69.5–81.38	75.44
Distance between posterolateral scutal setae	92.82–95.55	94.185
Distance between sensillary bases	31–34.52	32.76
Distance from the level of sensillary bases to extreme anterior margin of scutum	29.03–31.03	30.03
Distance from the level of sensillary bases to extreme posterior margin of scutum	30.3–32.4	31.35
Length of scutum	57.33–65.52	61.43
Distance from the level of posterolateral scutal setae to extreme posterior margin of scutum	27.3–30.03	28.67
Distance between antero- and posterolateral scutal setae on one side	31–34.52	32.76
Length of anteromedian scutal setae	40.95–46.41	43.68
Length of anterolateral scutal setae	43.68–45.14	44.41
Length of posterolateral scutal setae	54.6–62.79	58.69
Length of sensilla	65–66.2	65.6
Length of humeral setae	54.6–60.06	57.33
Length of dorsal setae	54.6–57.33	55.96
Length of ventral setae	40.95–46.41	43.68
Length of leg I (including coxa) (pa)	312–332	322
Length of leg II (including coxa) (pm)	287–295.2	291.1
Length of leg III (including coxa) (pp)	307–331	319
Sum of leg lengths (pa + pm + pp)	894.4–873.6	884
Number of dorsal idiosomal setae (including humeral) (DS)	35–41	38
Number of ventral idiosomal setae (excluding coxal and sternal) (VS)	24–30	27
Number of idiosomal setae (DS + VS)	59–66	62.5
Length of leg tarsus III (TaIII)	79–84.8	81.9
Distance between tarsal base and mastitarsal setae of leg III (dmt)	13.65–16.38	15.02
Ratio dmt/taIII	0.12–0.16	0.14

proteins might also be the cause of the clinical signs. It is suspected for some cases that dogs may exhibit intraspecies variability to toward chigger infestation (Seixas et al. 2006).

It is worth noting that dog number 8, which was severely affected, had already suffered a similar but milder episode a year earlier. Unfortunately, we do not have details about clinical signs and parasite load during this episode.

Only a few accounts of trombiculiasis as the causative agent of a systemic syndrome have been described in dogs. Some studies, which mainly focused on human trombiculiasis, have provided brief descriptions of digestive and neurological signs in dogs (Oteo et al. 2006, Santibañez-Sáenz et al. 2014, Santibañez-Sáenz 2015). We did not observe ataxia or other neurological signs, although anecdotal reports by veterinarians have suggested neurological involvement.

Trombiculid mite larvae infestations are known for their irritating bites in humans and domestic animals (Mullen and O'Connor 2002, Fischer and Walton 2014). Most cases are associated with pruritis derived from an allergic reaction to the mite's salivary secretions in both humans (Jiménez et al. 2001, Oteo et al. 2006, Santibañez-Sáenz et al. 2014) and dogs (Seixas et al. 2006, Tudor 2012). In other chigger infestations, such as straelensiosis, pruritus does not manifest as a clinical sign (Le Net 2002, Ramírez et al. 2009). However, unlike other chiggers, *N. inopinata* does not seem to cause dermatitis. It is worth highlighting that none of the nine cases reported here, presented any itchiness, while only two dogs had slight erythema in the areas of parasite fixation.

Although clear conclusions cannot be drawn due to the small number of animals, there does not appear to be any predisposition in terms of breed, age, or gender, in contrast to observations in trombiculid infestations made by other authors (Le Net et al. 2002).

The chronology and synchronicity of events strongly suggest that the condition was a consequence of severe infestation and exposure to salivary secretions from a large number of feeding mites.

There are no reports of Trombiculidae acting as disease vectors in Europe. Studies carried out in Germany, the Czech Republic, Spain, and Slovakia have reported that larval mites could be carriers of *Borrelia* sp., *A. phagocytophilum*, and *Rickettsia* sp. (Fernández-Soto et al. 2001, Kampen et al. 2004, Literak et al. 2008, Mit'ková et al. 2015). Mites can harbor these pathogens but there is no evidence that they can transmit them. Nevertheless, even though chigger mites could transmit these pathogens, the clinical picture they cause in dogs does not coincide with the one observed in our study. Normally, these processes cause fever and apathy (Greene 2006) but not a picture of digestive disorders of acute presentation like the one presented in this study.

However, we did observe many parallels with SCI in terms of both the epidemiology and clinical picture. Except for trembling and pyrexia, several of the clinical signs and all the epidemiological findings shown by our subjects mimicked those reported for SCI. In eastern England, animals are usually affected between August and November after exercising outdoors. The clinical signs first appear 1–3 d after the dogs are walked in a woodland area. SCI generally produces vomiting, diarrhea, and lethargy; in addition, individuals may develop abdominal pain, anorexia, trembling, and pyrexia. Given that the underlying etiology remains unknown, veterinarians can only make a presumptive diagnosis of SCI based on nonspecific clinical signs. Exotic woodland plants, fungi (Hall and Barton 2013), poisons, plant toxins, bracken spores, blue-green algae, Protozoa, and the harvest mite *N. autumnalis* (McGarry 2012), among others, have all been suggested as potential causative agents. Harvest mites are believed to be involved in the onset of SCI as they have frequently been observed on dogs affected by the condition (McGarry 2012).

However, to date no evidence has been published to suggest that British harvest mites carry or transmit any known diseases, so no clear connection has been established at this stage. The clinical and epidemiological similarity between the picture described in this article and that of SCI leads us to suspect that it could be the same process. As far as we know, this is the first time that any potential cases of SCI have been cited outside the United Kingdom.

Although there is very limited literature regarding the risk of trombiculiasis in the study area, the local population is aware of the problem. In fact, the regional government has published brochures to increase public awareness of the risk of trombiculiasis in the area and the measures to implement to reduce this risk for both humans and their pets. The guidelines recommend the use of repellent and the local application of an ectoparasite treatment, and preferably avoiding the infested areas during the larval season. However, it seems that ectoparasite treatment does not prevent infestation by *N. inopinata*, given that five of the patients had been treated before entering the area. There is a lack of data on the treatment of trombiculid infestations in dogs. Smal et al. (2004) reported that infestation persisted after 8 d despite the application of a topical permethrin-pyriproxyfen antiparasitic treatment.

In conclusion, the chronology and synchronicity of events suggest that the condition we described in this article is the consequence of infestation from feeding mites, specifically *N. inopinata*. Whether the cases were due to a severe allergic host response to the mites' salivary proteins or the transmission of a new or emerging trombiculid-borne pathogen remains unknown. There is a pressing need for further study to unveil the cause.

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References Cited

- Beugnet, F., and P. Bourdeau. 1999. Les ectoparasites du chat. *Prat. Med. Chir. Anim.* 34: 427–435.
- Fernández-Soto, P., R. Pérez-Sánchez, and A. Encinas-Grandes. 2001. Molecular detection of *Ehrlichia phagocytophila* genogroup organisms in larvae of *Neotrombicula autumnalis* (Acari: Trombiculidae) captured in Spain. *J. Parasitol.* 87: 1482–1483.
- Fischer, K., and S. Walton. 2014. Parasitic mites of medical and veterinary importance—is there a common research agenda? *Int. J. Parasitol.* 44: 955–967.
- Font, A. 2007. Straelensiosis in a dog in Spain. *Vet. Dermatol.* 18: 67–68.
- Gomez-Puerta, L. A., J. Olazabal, M. T. Lopez-Urbina, and A. E. Gonzalez. 2012. Trombiculiasis caused by chigger mites *Eutrombicula* (Acari: Trombiculidae) in Peruvian alpacas. *Vet. Parasitol.* 190: 294–296.
- Greene, C. E. (ed.). 2006. Infectious diseases of the dog and cat, 3rd ed. Elsevier, St. Louis, MO.
- Guaguère, E., F. Degorce-Rubiales, and D. Connefroy. 2011. La straelensiose du chien: une dermatose parasitaire de description récente en France. *Prat. Med. Chir. Anim.* 46: 3–8.
- Guarneri, E., A. Pugliese, E. Giudice, C. Guarneri, S. Giannetto, and B. Guarneri. 2005. Trombiculiasis: clinical contribution. *Eur. J. Dermatol.* 15: 495–496.
- Hall, J., and L. Barton. 2013. Mushroom toxicosis in dogs in general practice causing gastroenteritis, ptyalism and elevated serum lipase activity. *J. Small Anim. Pract.* 54: 275–279.
- Jiménez, S., I. Gastón, A. Pérez, and J. A. Oteo. 2001. A seasonal outbreak of human parasitosis by chiggers in La Rioja (Northern Spain). *J. Clin. Microbiol.* 7(s1): 348.

- Kampen, H., A. Schöler, M. Metzen, R. Oehme, K. Hartelt, P. Kimmig, and W. A. Maier. 2004. *Neotrombicula autumnalis* (Acari, Trombiculidae) as a vector for *Borrelia burgdorferi* sensu lato? Exp. Appl. Acarol. 33: 93–102.
- Krantz, G. W., and D. E. Walter (eds.). 2009. A manual of acarology, 3rd ed. Texas Tech University Press, Lubbock, TX.
- Le Net, J. L., A. Fain, C. George, S. Rousselle, V. Théau, and L. Longeart. 2002. Straelensiosis in dogs: a newly described nodular dermatitis induced by *Straelensia cynotis*. Vet. Rec. 150: 205–209.
- Literak, I., A. A. Stekolnikov, O. Sychra, L. Dubska, and V. Taragelova. 2008. Larvae of chigger mites *Neotrombicula* spp. (Acari: Trombiculidae) exhibited *Borrelia* but no *Anaplasma* infections: a field study including birds from the Czech Carpathians as hosts of chiggers. Exp. Appl. Acarol. 44: 307–314.
- McGarry, J. W. 2012. Canine trombiculiasis and seasonal canine illness. Vet. Rec. 20: 406.
- Mit'ková, K., L. Berthová, S. Kalúz, M. Kazimírová, L. Burdová, and E. Kocianová. 2015. First detections of *Rickettsia helvetica* and *R. monacensis* in ectoparasitic mites (Laelapidae and Trombiculidae) infesting rodents in south-western Slovakia. Parasitol. Res. 114: 2465–2472.
- Mullen, G. R., and B. M. O'Connor. 2002. Mites (Acari), pp. 449–516. In G. Mullen and L. Durden (eds.), Medical and veterinary entomology. Academic Press, Amsterdam, The Netherlands.
- Oteo, J. A., A. Portillo, S. Santibáñez, J. R. Blanco, L. Pérez-Martínez, and V. Ibarra. 2006. Cluster of cases of human *Rickettsia felis* infection from Southern Europe (Spain) diagnosed by PCR. J. Clin. Microbiol. 44: 2669–2671.
- Ramírez, G. A., J. Altamira, B. García, M. Fernández, and M. Vilafranca. 2009. Clinical, histopathological and epidemiological study of canine straelensiosis in the Iberian Peninsula (2003–2007). Vet. Dermatol. 20: 35–41.
- Santibáñez-Sáenz, P. 2015. Trombicúlidos y trombiculiasis en La Rioja. Tesis doctoral. Universidad de La Rioja, Logroño, Spain.
- Santibáñez-Sáenz, P., A. Palomar-Urbina, E. Imaña-Rodríguez, and J. Oteo-Revuelta. 2014. Dermatitis pruriginosa tras paseo por la montaña. Enferm. Infecc. Micr. Cl. 32: 610–611.
- Scott, D. W., W. H. Miller, and C. E. Griffin (eds.). 2001. Muller and Kirk's small animal dermatology, 6th ed. Saunders, Philadelphia, PA.
- Seixas, F., P. J. Travassos, M. L. Pinto, J. Correia, and M. A. Pires. 2006. Dermatitis in a dog induced by *Straelensia cynotis*: a case report and review of the literature. Vet. Dermatol. 17: 81–84.
- Shatrov, A. B., M. Takahashi, S. Noda, and H. Misumi. 2014. Stylostome organization in feeding *Leptotrombidium* larvae (Acariformes: Trombiculidae). Exp. Appl. Acarol. 64: 33–47.
- Smal, D., P. Jasmin, and P. Mercier. 2004. Treatment of *Neotrombicula autumnalis* dermatitis in dogs using two topical permethrin-pyriproxyfen combinations. J. Small Anim. Pract. 45: 98–103.
- Stekolnikov, A. A., P. Santibáñez, A. M. Palomar, and J. A. Oteo. 2014. *Neotrombicula inopinata* (Acari: Trombiculidae) - a possible causative agent of trombiculiasis in Europe. Parasit. Vectors. 7: 90.
- Stekolnikov, A. A., H. Waap, J. Gomes, and T. Antunes. 2016. Chigger mites of the genus *Ericotrombidium* (Acariformes: Trombiculidae) attacking pets in Europe. Vet. Parasitol. 221: 60–63.
- Tudor, P. 2012. Trombiculidae harvest mites (*Neotrombicula autumnalis*) infestation in dog in winter season—a case report. Sci. Works Ser. C Vet. Med. 58: 306–311.
- Wall, R., and D. Shearer. 2001. Veterinary ectoparasites: biology, pathology and control, 2nd ed. Blackwell Science Ltd, Oxford, United Kingdom.
- Wang, Z. L., X. Q. Yang, T. Z. Wang, and X. Yu. 2018. Assessing the effectiveness of mitochondrial COI and 16S rRNA genes for DNA barcoding of farmland spiders in China. Mitochondrial DNA A. DNA Mapp. Seq. Anal. 29: 695–702.