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Original Research

Diversity of Three Species of the Genus *Demodex* (Acari, Demodecidae) Parasitizing Dogs in Poland

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Abstract

The occurrence of hair follicle mites (Acari, Demodecidae) was investigated in dogs from Gdańsk Pomerania, Poland. The three species of demodectic mites (*Demodex canis*, *D. cornei*, and *D. injai*) specific to dogs not only differ morphologically, but are also found on different areas of the hosts' skin. Parasitic adaptations involve size and shape of the body, which is elongated and worm-like, providing a specific adaptation to life in hair follicles, in various glands and their ducts or epidermis. The differentiation in structure, biology, and microhabitats of these mites may be associated with the occurrence in dogs of forms of demodecosis with different symptoms and courses.

Keywords: *Demodex*, dog, demodecosis, parasites, infestation

Introduction

The *Demodex* parasites were the first of the Demodecidae family (Acari, Trombidiformes) discovered by scientists and to date have been thoroughly researched. The canine demodecosis they cause is the most frequently occurring skin diseases in veterinary medicine. Even with numerous communications and studies on canine demodicosis, its symptoms, and methods of treatments, knowledge of etiological factors of this type of parasitosis remains incomplete. For over a hundred years, any reported case has been tied to one species only: viz. *Demodex canis* [1]. Miscellaneous symptoms, the course of the disease, and problems with treatment have suggested the existence of a more complex etiology. Even though *D. canis* has been described in detail by many authors [1-5], only recently has a second species of *Demodex* mites been identified: *Demodex injai* [6]. A number of communications on infestations caused by the so-called short-bodied form, regarded by some authors as a separate species, *Demodex cornei* [7], described later in studies that applied scanning microscopes [8-10]. However, the descriptions of these species remain insufficient – *D. injai* has been described based on homogenous material from the U.S., while *D. cornei* descriptions contain incomplete measurements and are based on a small sample of specimens to boot. No description of the juvenile stage has been provided for this species.

The basic diagnostic methods used in detecting mites when demodicosis is suspected are based on microscopic analysis of skin scrapings. The disease is confirmed based on detection and identification of *Demodex* spp. It seems relevant, then, to find marker features in terms of morphological details or body proportions that would facilitate distinguishing species.

Experimental Procedures

The material comprised skin segments collected from 25 dogs, of which 20 had no symptoms of demodicosis and 5 had localized symptoms. The skin samples were taken from dead dogs in Gdańsk Pomerania between 2003 and

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2009. The skin samples were taken from the head (eyelid, cheek, nose, ear, upper lip), fore and hind groins, limbs (the knee region), belly, and genital-anal regions. They were preserved in 70% ethanol solution, then analyzed using the standard method of skin digestion [11]. The digested samples were examined under a phase-contrast microscope. All the mites found were mounted in Faure's medium.

Additionally, a retrospective study was carried out on mass microscopic sections made of skin scrapings from 100 dogs with symptoms of demodicosis dating back to the 1970s. 300 microscope slides of hair follicle mites present asymptomatically in 90 dogs were analyzed retrospectively. The slides were from our laboratory's collection and had been preserved in Canada balsam or polyvinyl lactophenol in 1973-77.

Results and Discussion

Occurrence and Infestations of Particular *Demodex* Mites

In the current study, *Demodex canis* has been found on 46% of the dogs. In the study by Stankiewicz et al. [12], based on a large sample (approximately 39,000 dogs in the Warszawa region), *D. canis* was found on 85% of hosts, but demodicosis was diagnosed only in 0.75% of dogs. The first observation conducted in the Pomerania region confirmed asymptomatic existence of this parasite on 38% of the 100 dogs examined at that time [13]. Subsequent studies describing infestation with *D. canis* in dogs from Gdańsk Pomerania also show a high level of extensiveness as well as the usually asymptomatic nature of the transmission [14, 15]. Thus, it is not surprising that in the current study the typical symptoms of canine demodicosis were found in only a few dogs as localized symptoms.

Demodex injai was found in three dogs. Skin lesions were visible on the whole body, on the head and limbs in particular. However, the frequency of occurrence of this parasite remains unknown, as it was found neither in the course of retrospective studies nor in earlier studies on dogs from the Pomerania region [14]. The first report of *D. injai* occurrence has been received only very recently [15]. Most likely this species displays low extensiveness of infestation.

Demodex cornei was found on 8% of dogs currently under study, but no symptoms of demodicosis were determined. In the retrospective analysis this species was found in 6% of the dog sample with demodicosis symptoms. It is difficult to establish which symptoms should be tied to its presence in the context of synhospital infestation with *D. canis,* which was a dominant species in this case. No data is available about its occurrence in other parts of Poland – it was first reported in Gdańsk [15].

Canine demodicosis is indeed the most frequently diagnosed skin disease in veterinary medicine and *Demodex* spp., responsible for its symptoms, constitutes a typical canine parasite fauna [15, 16]. However, infestation is usually asymptomatic. Skin symptoms on the host usually appear due to excessive multiplication of one of the *Demodex* species, less frequently as a result of synhospital infestation [12-14]. The disease usually entails a decrease in the host's immunity [17, 18]. The symptoms of demodicosis have been dealt with extensively in the literature, and many studies describe the development and course of the disease, various symptoms and variants, histopathological changes, diagnostic methods, and treatment, but lesions linked to canine demodicosis were always attributed to *D. canis* [3, 19-25]. However, today we know that in many cases the etiological factors might not have been correctly established and the cause of the infestation in question might, in fact, lie in one of the three species of the genus *Demodex* or synhospital infestation. Descriptions of infestation brought about by other species have only recently been reported, so data on infestation with *D. cornei* comes from China, Japan, Greece, and Poland, and with *D. injai* from the U.S., Spain, and Poland [6, 8, 10, 15, 26, 27].

Taxonomical Characteristics of *Demodex* spp. in Dog

Demodecid mites are small, tiny, usually with elongate bodies with four pairs of short, stump-like, forward-pointing legs displaced toward the front; stiletto-like chelicerae; three-segmented palpi; a massive hip element; and 4-5 rod-shaped bristles on the distal segment. The body is separated into three distinct tagmas. The small gnathosoma has a trapezoidal or rectangular shape; the podosoma supports the much-reduced legs with a pair of forked claws, which usually protrude only slightly beyond the line of the podosoma; on the ventral side of most adult mites the epimeral plates are readily discernible. The opisthosoma is usually elongated with cuticular striations [3, 5, 28, 29] (Fig. 1).

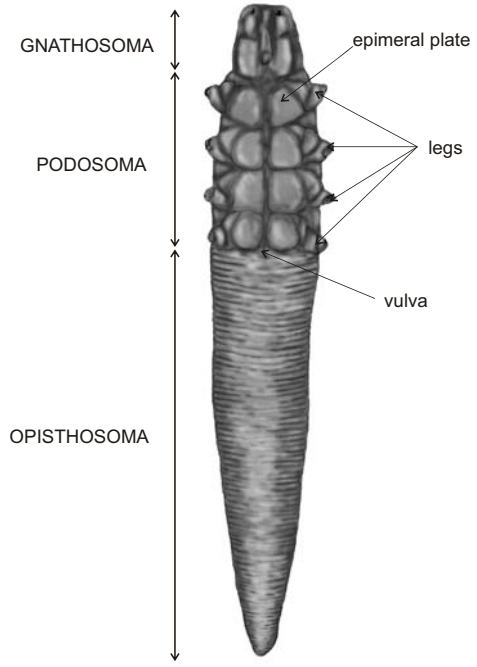

Fig. 1. Morphology of *Demodex canis* (female, ventral view).

Table 1. Comparison of characteristic of *Demodex* spp. living on a domestic dog.

	D. canis		*D. cornei*		*D. injai*	
	male	female	male	female	male	female
Length of gnathosoma	26.9	25.2	19.9	19.1	22.3	23.1
	[21-31]	[18-30]	[15-26]	[15-28]	[21-25]	[21-26]
	SD 2.5	SD 3.1	SD 2.6	SD 3.9	SD 1.2	SD 1.7
Width of gnathosoma	23.7	21.9	21.4	20.9	28.8	28.6
	[19-30]	[16-29]	[18-26]	[16-28]	[25-32]	[25-32]
	SD 3.7	SD 3.4	SD 2.4	SD 4.5	SD 2.3	SD 2.2
Length of podosoma	63.8	63.4	52.1	53.9	87.3	82.3
	[50-81]	[58-75]	[39-65]	[40-66]	[82-94]	[66-88]
	SD 9.2	SD 3.4	SD 9.2	SD 7.6	SD 4.0	SD 5.4
Width of podosoma	38.8	37.2	35.9	37.6	43.5	44.4
	[33-46]	[28-41]	[29-40]	[28-39]	[35-47]	[40-49]
	SD 3.7	SD 2.5	SD 3.2	SD 4.6	SD 3.5	SD 3.9
Length of opisthosoma	116.5	133.1	46.2	65.4	264.7	224.3
	[74-166]	[100-167]	[36-54]	[47-99]	[203-340]	[161-281]
	SD 24.3	SD 17.6	SD 4.2	SD 12.6	SD 57.0	SD 49.1
Width of opisthosoma	33.3	34.6	35.3	33.2	35.6	36.7
	[28-40]	[25-42]	[23-40]	[27-40]	[31-40]	[33-41]
	SD 3.9	SD 3.6	SD 4.5	SD 4.2	SD 3.7	SD 3.2
Total length of body	195.2	226.0	120.8	139.4	371.8	330.9
	[146-251]	[177-265]	[93-136]	[120-165]	[309-455]	[245-396]
	SD 33.2	SD 20.1	SD 13.2	SD 17.9	SD 58.4	SD 45.5
Ratio of length to width	4.5-5.5	5.9-6.4	3.2-3.5	3.6 -4.2	8.5-9.5	6.5 : 7.6
Ratio of length opisthosoma to body length [%]	51	59	39	43	71	68
	[50-59]	[56-63]	[38-40]	[39-50]	[66-74]	[66-70]
Microhabitat	hair follicles		epidermis		sebaceous glands	

Adaptations for parasitism are in this case applicable to both the size and the shape of the body. Due to the adaptation for living in the host's tissue, the Demodecidae suffered an extreme reduction in number of morphological elements [30], e.g. they have only a few, strongly reduced bristles, which makes species identification difficult. In view of the reduction of a number of morphological features, there are not many characteristics that can be used for diagnostic purposes in demodecid taxonomy. The following are usually taken into account: the shape of the setae (supracoxal spines and subgnathosomal setae); the number, position and shape of the pedipalp claws/setae; the position and length of the aedeagus (male) and genital opening in the female; the presence of the opisthosomal organ; and the presence of epimeral plates and their shape or the shape of the posterior section of the opisthosoma [28, 29, 31, 32].

Demodex canis is an elongated species, the opisthosoma becoming slender and sharp toward the terminal end. Opisthosoma long and slender, gradually tapering to the posterior end. Capitulum (gnathosoma) of moderate width, being widest at the base, where it is slightly wider than long; the two spines on its dorsal surface (supracoxal setae) are very short and pointed. Epimeral plates are trapezoidal in shape. Male – size very variable, specimens of this sex being sometimes much shorter than females (Figs. 1, 2, Table 1).

Demodex cornei have a short, wide body; compared with *D. canis* and *D. injai*, it has a stumpy body shape. The dorsal surface of the podosoma is flat, while shallow transverse grooves are observed over the entire opisthosoma. The supracoxal spines of the dorsal gnathosoma have centrally orientated spines. The epimeral plates are almost rectangular.

Fig. 2. *Demodex canis* – male and female.

Fig. 3. *Demodex cornei* – male (ventral and lateral view) and female.

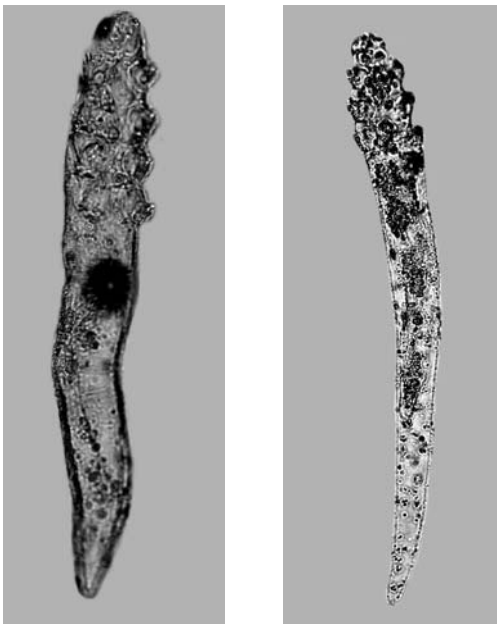

Fig. 4. *Demodex injai* – female and nymph

There is a band-like plate dividing the lower edge of the fourth coxisternal plate and the opisthosoma (Fig. 3, Table 1).

Demodex injai is a long-bodied species more than twice the length *D. canis*. Each supracoxal spine is a conical peg somewhat recessed into the gnathosomal cuticle. An opishosomal organ (proctodeum) is currently known as a narrow tube (Fig. 4, Table 1).

Additionally, the three *Demodex* species show not only morphological differences but they also settle in different habitats on the canine skin. *D. canis* lives in the hair follicles, while *D. cornei* is always found in the corneal layer of the epidermis and *D. injai* is linked to glands and their outgoing ducts [6, 9, 15]. In Demodecidae, the shape, dimensions and proportions of the body constitute a specific adaptation to the occupied microhabitat, which was confirmed here – *D. canis* resembles other species linked to hair follicles, e.g. *Demodex folliculorum* in humans. The long-bodied *D. injai*, on the other hand, is linked to species with similar locations in ungulates or bats. The small size and proportions of *D. cornei* resemble analogical species in the feline epidermis or the golden hamster [29, 32-35]. Topical and host specificity is most likely a typical feature of the evolutionary development of Demodecidae which, within particular host species, adapted to various types of dermal microhabitats, thus creating specific adaptation to life, nutrition, location, and transmission. This led to the emergence, within particular host taxons, of synhospital demodectic species of different topography and specificity toward dermal tissues/elements. Analogical sets/groups of synhospital species were described on rats (four species), cattle (three species), horse, sheep, and cats (two species in each) [28, 32].

Undoubtedly, species with different localtion and, consequently, with different adaptive strategies can cause infestation of different courses and varied symptoms. This is why, when diagnosing canine demodicosis, the correct identification of the demodectic species, which is the etiological factor, is of utmost importance. However, features used in the taxonomy of Demodecidae are not very helpful in veterinary diagnostics. The taxonomy of these miniature mites is based on the analysis of bristles that are a few micrometers long or other elements hard to discern and identify. The current morphometric analysis of *Demodex* spp. infesting dogs is based on a study of a representative sample of specimens (at least 100 specimens of a given sex for each species) and reveals significant diversity in terms of size and body proportions (Table 1). It seems that these features might prove useful in veterinary diagnostics.

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