CLINICAL AND HISTOPATHOLOGICAL FINDINGS IN GERMAN SHEPHERD DOGS WITH HOOKWORM DERMATITIS AND THE USAGE OF EPRINOMECTIN IN THE THERAPY

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Summary. Clinical and histopathological findings in a litter of German Shepherd dogs consisting of seven puppies with acute inflammation confined to the footpads were reported. Affected footpads were characteristically erythematous, swollen and painful. Lesions were present on all four feet in 3/7 cases and on the rear feet in 4/7 cases. None of the dogs had evidence of ectoparasitism. Fecal flotation, deep skin scrapings, bacterial and fungal cultures, and skin biopsies were performed in all dogs. Fecal flotation and histopathological examination revealed *Ancylostoma caninum* as the cause of pododermatitis. These lesions resolved in response to topical eprinomectin applications, suggesting that clinical signs were linked to hookworm pododermatitis.
Keywords: hookworm, dermatitis, dog, eprinomectin.

VOKIEČIŲ AVIGANIŲ KLINIKINIAI IR HISTOPATOLOGINIAI ANKILOSTOMŲ SUKELTO DERMATITO TYRIMAI IR GYDYMAS EPRINOMEKTINU

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Santrauka. Surinkti septynių vokiečių aviganių šuniukų, sergančių ūmiu letenų pagalvėlių uždegimu, klinikiniai ir histopatologiniai duomenys. Pažeistos letenos buvo eriteminės, patinusios ir skausmingos. Pažeidimai rasti ant visų keturių letenų trimis iš septynų atvejų; tik užpakalinių galūnių letenose – keturiais iš septynių atvejų. Nė vienas iš šunų neturėjo išorės parazitų požymių. Išmatų tyrimas flotacijos būdu, giluminių odos skutenų tyrimas, bakteriologiniai ir grybeliniai pasėliai, odos biopsija atlikta visiems šunims. Išmatų flotacija ir histopatologinis tyrimas parodė, kad pododermatitą sukėlė *Ancylostoma caninum*. Odos pakitimai pranyko naudojant eprinomektiną išoriškai. Galima daryti išvadą, kad klinikinius požymius sukėlė nematodų pododermatitas.
Raktažodžiai: nematodai, dermatitas, *Ancylostoma caninum,* šunys, eprinomektinas.

Introduction. Pododermatitis in dogs is associated with a variety of systemic and cutaneous diseases. The most common causes include: pemphigus foliaceus, hepato-cutaneous syndrome (Shaw, 1987), bacterial (Mason, 1991) and fungal infections (i.e. Malessezia sp.) (Naresh et al., 2002), babesiosis (Tarello, 2003), demodicosis (Deboer, 2003, Naresh et al., 2002), trauma (Anderson, 1998**)**, ectoparasitic infections (White, 1993), hookworm infections (Baker and Grimes, 1970, Buelke, 1971, Foil, 1995, Smith and Elliott, 1969), atopy (Foil, 1995) and foreign body penetrations (Shaw, 1987). A classification system has been established for pedal dermatoses, which include three categories: scaling, crusting and/or pruritic dermatoses; nodular, ulcerative and/or exudative dermatoses and claw and claw fold diseases (Foil, 1995). Hookworm dermatitis was described under scaling,crusting and/or pruritic pododermatoses by this classification (Foil, 1995).

Hookworm dermatitis is a relatively uncommon to rare cutaneous disorder due to the larval migration of hookworms such as *Ancylostoma caninum, Ancylostoma braziliense* or *Uncinaria stenocephala* (Baker, 1981, Baker and Grimes, 1970, Foil, 1995). The disease is presumed to be associated with poor hygiene where infected larva numbers are increased, and dermatitis occurs as a hypersensitivity reaction in association with migrating larvae (Baker, 1981). Third stage larvae penetrate the skin that is in contact with the ground (Baker, 1981, Baker and Grimes, 1970, Foil, 1995). Clinical signs associated with hookworm dermatitis involve erythematous papules, erythema, swelling, alopecia and lichenification. Typical clinical changes occur within the footpads, due to their natural contact within the ground, as pad margins become soft and separate from the underlying epidermal structure. The distal extremities and other relevant anatomical structures in contact with the soil are mostly affected (Baker, 1981, Baker and Grimes, 1970, Foil, 1995). Standard treatment applications for canine hookworm dermatitis include management of poor hygene, removal of feces and especially anthelmintic therapy (Scott et al., 2000). The present authors were unaware of finding detailed documented reports regarding clinicopathological features

of hookworm dermatitis and its relevant effective therapy protocole.

The aims of this report were to describe the clinical and histopathological findings of hookworm dermatitis in a litter of German shepherd dogs and to report the efficacy of eprinomectin therapy in these patients. To the authors's knowledge the efficacy of eprinomectin for the treatment of hookworm dermatitis in dogs have not been evaluated.

Material and methods. Case Description

History and clinical features. Seven, two month old, German shepherd dog litter mates, five males and two females, were initially presented for skin changes and pruritus confined to the footpads. Signs were noticed by the owner four days before presentation. The puppies were licking at their feet and had problem walking. Decreased appetite and diarrhea were also reported by the owner. There was no previous history of drug administration or vaccination and no exposure to chemicals or other irritants was noted by the owner. All puppies were kept in the same box and under poor sanitary conditions. They were allowed to roam outside on a dirty area soiled with urine and feces.

Diagnostic tests performed and findings. Complete blood cell count (CBC) and serum biochemistry profile were performed in all cases. CBC and serum biochemistry analysis were also performed in seven, two-month-old healthy dogs (four males and three females) with no pedal disease for comparison. Blood samples were collected from the cephalic vein and placed in serum separator and EDTA treated glass tubes from all affected and healthy dogs. Serum biochemical analyses included urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma glutamyl transferase.

Other tests performed included skin scrapings (7/7 dogs), cytology (2/7), bacterial and fungal cultures (4/7); direct fecal examination and Fulleborn's fecal flotation (7/7), and skin biopsy (7/7).

Excision biopsies of lesional skin were obtained from two feet of each dog using local anesthesia and the biopsy sites were allowed to heal as second intention. The samples were fixed in 10% neutral buffered formalin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

Therapy applications. All puppies were treated with a pour-on formulation of eprinomectin (Eprinex® Pour-on, Merial, Manukau City, New Zealand) weekly for three treatments, at the manufacturer's recommended dose for cattle of 0.5 mg kg^{-1} of body weight. Eprinomectin was administered directly to the skin at the base of the neck. In an attempt to prevent possible secondary bacterial infection of affected areas, soaking of the feet in a 4% chlorhexidine shampoo for 15 minutes two times daily for five consecutive days was recommended. In addition, the owner was instructed to improve sanitation of the box where the puppies were kept and the outdoors area that they had access.

Results. Clinical findings. An erythematous and crusting dermatitis with swelling confined to the footpads in contact with the ground was the typical clinical feature in all seven puppies. Erosive to ulcerative lesions at the center of the footpads was noted in three dogs (Fig. 1-2). All four feet were affected in three of the seven puppies and only the rear feet in four cases. No pustules or purulent discharges were evident. All dogs showed discomfort on pedal examination and were observed licking at their feet during examination. No other dermatologic signs were evident in any of the dogs. The puppies were anaemic on physical examination and the tail, rear legs and especially the footpads were covered with fecal material supporting the history of diarrhea.

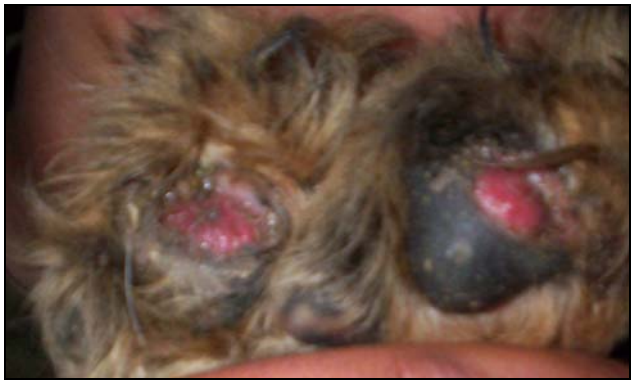

Fig. 1. **Hookworm lesions confined to footpad in a German shepherd puppy.** Marked erythema, sweling and alopecia were evident

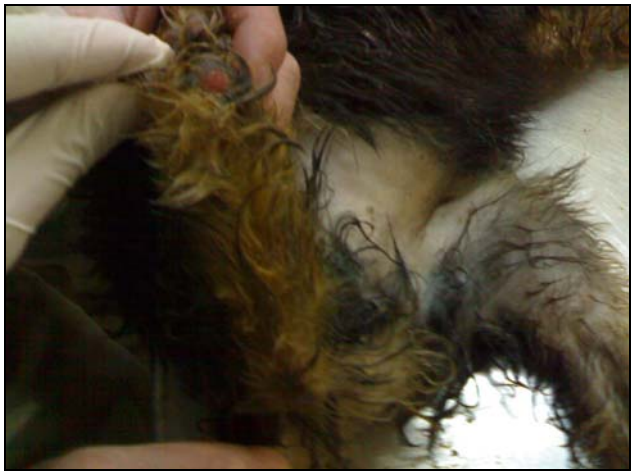

Fig. 2. **Erythematous and alopecic footpad lesion on the rear feet**

The differential diagnoses included contact dermatitis, parasitic dermatitis and auto-immune diseases.

Clinical pathology. Affected puppies showed no significant abnormalities in the biochemical parameters examined (data not shown). However, CBC showed mild to moderate increases in the mean white blood cell (three dogs), lymphocyte (seven dogs) and monocyte (three dogs) counts and a mild decrease in neutrophil (two dogs) counts (Table 1). Mean values of erythrocyte count, packed cell volume, haemoglobin and mean corpuscular volume were low in dogs with pododermatitis compared to the healthy puppies and to the reference range (Table 1). Platelet counts were within the normal range.

Table 1. **Hematological variables in German shepherd puppies with hookworm dermatititis and age-matched control control group** (G II) **and their statistical significances**

Parameters	Dogs with pododermatitis	Age-matched control	Reference range
RBC (n x 10^6/µl)	4,68±0,231*	6,31±0,141	5.5-8.5
WBC (n x 10^3/µl)	16,03±0.978*	10,99±1,102	6-17
NE (n x 10^3/µl)	3,79±0,796	5,73±1,033	3-11.4
LYM (n x 10^3/µl)	9,63±1,115*	2,74±0,280	1-4.8
MO (n x 10^3/µl)	2,34±0,191*	0,85±0,216	0.15-1.35
Hb (g/dl)	9,94±0,249**	15,80±2,010	12-18
MCV (fL)	68,32±0,676**	78,60±3,369	60-77
MCHC (g/dl)	31,62±2,119	34,91±1,177	32-36
PCV (%)	32,1± 4,2*	49,6±6,4	37-55

Blood values in dogs with pododermatitis (n=7) and age matched control group (n=7). Results are expressed as means ± Standard errors; *p<0.01, **: p<0.05; RBC:red blood cell, WBC:white blood cell, NE:neutrophil, LYM:lymphocyte, MO:monocyte, Hb: haemoglobin, MCV:mean corpuscular volume, MCHC:mean corpuscular haemoglobine concentration, PCV:packed cell volume

Deep skin scrapings were negative for ectoparasites including pelodera larvae in all cases. Cytology only revealed few neutrophils and bacterial and fungal cultures were negative in all four dogs tested. In all seven dogs the fecal tests revealed *A. caninum* eggs.

Histopathological analysis. The epidermis showed acanthosis and parakeratotic hyperkeratosis (4/7 dogs). The superficial and deep dermis of all samples showed a perivascular and interstitial infiltrate of mononuclear cells, lymphocytes and eosinophils. Dermal oedema (5/7 dogs) and collagen tissue hyalinization (3/7 dogs) were also present. Spongiosis and spongiotic vacuoles, and neutrophil and eosinophils were present in superficial epidermis (4/7 dogs). In two of seven dogs, rounded structures with a pink cuticle and purple center, representing the segment of a nematode larva were imbedded in the superficial layers of the epidermis (2/7 dogs) (Fig. 3 a-b). Calcium deposits was recorded in the superficial dermis in association with the cellular infiltrates (6/7 dogs).

Treatment and follow-up. All seven puppies experienced clinical remission over a 2-3 week period and no side effects were noted during eprinomectin treatment. All dogs were examined weekly and remained clinically healthy throughout the 20-week follow-up period. Fecal tests repeated in all dogs on days 14, 28 and 90 after the first eprinomectin treatment were all negative. Complete blood counts performed on days 30 and 60 after initial presentation for all seven puppies were within reference ranges (data not shown).

Discussion. Cutaneous larva migrans, or creeping eruption, is a dermatitis in humans most commonly caused by infectious larvae of the nematodes *A. caninum* and *A. braziliense* (Alonso, 2000; Davies et al., 1993; Lucchina and Wilson, 1999; Van Gasselt and Van de Sandt, 2000; Wear et al., 2000). Hookworm dermatitis in dogs and cats is caused by the larvae of *Uncinaria stenocephala, A.braziliense* or *A. caninum* (Bowman, 1992). *Uncinaria stenocephala* generates a significant dermatitis via skin penetration, however, rarely completes the life cycle via this route (Bowman, 1992). In contrast, larva of *A. caninum* completes its life cycle by penetration through skin (Scott et al., 2000). The larvae of *A. caninum* encyst and remain in the skin for a short duration before migration via subcutaneous tissues for weeks to months before completing the life cycle (Markell et al., 1999). Cutaneous lesions have been reported to be more common with *U. stenocephala*, however, dogs with ancylostomiasis may also have skin lesions (Scott et al., 2000). The puppies in this report developed moderate to severe pododermatitis caused by *A. caninum* larvae.

In natural and experimental *U. stenocephala* infections similar clinical and histopathological lesions were recognized (Mathews, 1981). Third stage larvae enter the dog's skin in contact with the ground and penetrate parallel to the skin surface. Following penetration through the epidermis, the dermis causes little obstacle to larvae migrating (Mathews, 1981).

The dogs in this report were kept in crowded conditions and allowed to roam on an area with poor sanitation. On physical examination the tail, rear legs and especially footpads of the puppies were covered with feces-contaminated ground where they laid. Fecal examinations showed that all puppies were infected with *A. caninum* and the direct contact of their footpads with the larvae in contaminated soil led to development of hookworm dermatitis. The footpad skin has a thick epidermis and stratum corneum that can hinder larvae penetration; however, the puppies were kept in a moist environment soiled with urine and feces which likely contributed to maceration of the stratum corneum facilitating larvae penetration. The lesions were confined to the footpads in the present cases, and other body parts were not affected. This may be in part explained by the frequent contact of the footpads with the ground and the early diagnosis. Temperature above 15°C is necessary for the development of *A. caninum* larvae in the environment (Bowman, 2009). The dogs were residing in a region of Turkey (Marmara), where the temperature is equal to or above 15°C throughout the year.

Diagnosis of hookworm dermatitis is usually based on the patient's history and the characteristic clinical appearance of the lesions. Typically larvae are not recognized in

most tissue samples because they remain in the skin for a short duration (Little et al., 1983, Scott et al., 2000, Wear et al., 2000), however, when present they appear as round bodies with an outer pink cuticle and centrally located purplish structures, representing the larvae intestine (Balfour et al., 2002). Identification of the parasite species may not be possible in tissue sections (Little et al., 1983, Wear et al., 2000). Nematode larvae, with the aforementioned features were present histologically in skin samples from two puppies. This may be due to the early diagnosis and the large numbers of nematode larvae present in the environment.

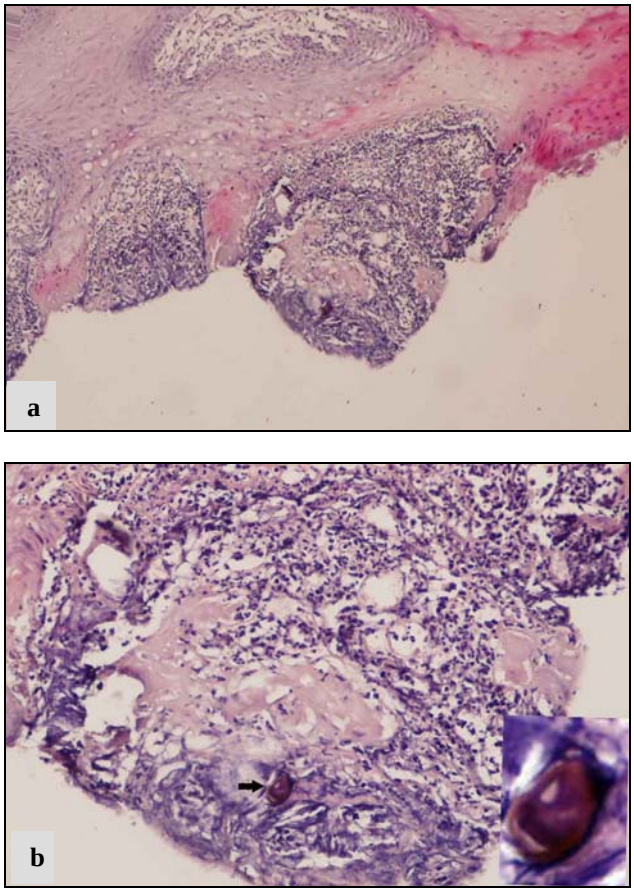

Fig. 3. **a – Histopathological features of a footpad lesion from a puppy with hookworm dermatitis.** Marked parakeratotic hyperkaratosis, multifocal, dense inflammatory infiltrate in the superficial epidermal layers, epidermal necrosis, marked spongiosis and spongiotic vesicles. H&EX100; **b – Higher magnification of previous figure.** Cross section of nematode larva (arrow) in the superficial epidermis surrounded by inflammatory cells. H&EX400. Inset: cross section of nematode larva characterized by thick wall and purple center. H&EX1000.

Hookworm larvae must be differentiated from *Pelodera* larvae, demodex mites, *Strongyloides* larvae and schistosomal agents (Scott et al., 2000). In the present report, the history, clinical signs, fecal examination findings and the histological features classical of *Ancylostoma* larva supported the diagnosis of hookworm dermatitis.

All puppies were anaemic on physical examination and hematological findings indicated a microcytic, hypochromic anemia typical of chronic intestinal parasitosis. All hematological abnormalities returned to normal once the hookworm infestation resolved, supporting an association.

In humans, cutaneous larva migrans can be treated with cryotherapy and systemic drugs such as thiabendazole, albendazole, and ivermectin (Rizzitelli, 1997). A recent study (Bouchard et al., 2000) reported cure rates of 77% and 96% for single and additional doses of ivermectin, respectively. Standard treatment recommendations for canine hookworm dermatitis include management of poor housing conditions, removal of feces and anthelmintic therapy (Scott et al., 2000). The owner of the present cases was instructed to improve sanitary conditions by maintaining adequate hygiene and removing the feces from the puppies' environment.

Eprinomectin, approved for use as a topical endectocide for beef and dairy cattle, is a powerful antihelminthic against nematode infections (Cramer et al., 2000, Pitt et al., 1997, Shoop et al., 2001). Toxicity studies in dogs have shown that eprinomectin administered orally at doses higher than 2 mg kg^{-1} body weight per day can cause mydriasis, salivation, emesis, decreased appetite, weight loss, decreased activity, ataxia, and even death (Kloss and Bagden, 1996, Kloss et al., 1996). At the oral dosage of 1.6 mg kg^{-1} body weight administered daily, no treatment-related clinical signs or mortality were observed, but decreased food consumption and body-weight gain were evident (Kloss et al., 1996).

Conclusions

Given the efficacy of eprinomectin against nematode infections, we tried this treatment modality in the affected puppies and no side effects were noted at the manufacturer's recommended dose for cattle of 0.5 mg kg^{-1}.

This report shows that hookworm dermatitis can be limited to the footpads of affected dogs and topical eprinomectin in conjunction with hygiene measurements can be considered a treatment option for these cases. To the authors knowledge hookworm dermatitis has not been previously reported in Turkey.

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