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The effect of nematode administration on canine atopic dermatitis

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ABSTRACT

Canine atopic dermatitis is a common disease and is considered as an animal model of the human disease. Immunomodulation by helminths is reported in several species. The aim of this study was to determine whether nematodes have an immunomodulatory effect on atopic dermatitis in dogs.

In the pilot study, 12 atopic dogs were infected with either embryonated eggs of *Trichuris vulpis* (500 and 2500 eggs in 3 dogs each) or L3 larvae of *Uncinaria stenocephala* (100, 500 and 2500 eggs in 2 dogs each), respectively, for 3 months. Pruritus was evaluated with visual analogue scales and clinical lesions with the canine atopic dermatitis extent and severity index (CADESI). Skin biopsies were obtained for histopathology at the beginning and end of the study. In the subsequent placebo-controlled, double-blinded, randomised study, 21 dogs received either 2500 embryonated *T. vulpis* eggs or placebo and were evaluated similarly. In addition, allergen-specific serum IgE concentrations were determined.

All dogs in the pilot study improved in their lesion scores, most in their pruritus scores. The cutaneous inflammatory infiltrate did not change significantly. In the subsequent randomised study, there was no significant difference between placebo and *Trichuris* administration in regard to pruritus or CADESI. IgE concentrations also did not change significantly. Infection with *T. vulpis* did not significantly change clinical signs of canine atopic dermatitis.

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1. Introduction

Canine atopic dermatitis (CAD) is one of the most common skin diseases in small animal practice (Scott and Paradis, 1990; Lund et al., 1999) and may be considered as an animal model of human atopic dermatitis (Nuttall et al., 2002). Atopic dermatitis (AD) is defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic features. Most commonly it is

associated with IgE antibodies to environmental allergens (Hill et al., 2001).

The diagnosis of canine atopic dermatitis is based on a compatible history and typical clinical signs (Zur et al., 2002). A set of criteria with high sensitivity and specificity has been established recently (Favrot et al., 2010). The successful management of CAD may involve a combination of different therapies such as glucocorticoids, antihistamines, cyclosporine, essential fatty acids, topical therapies, pentoxifylline and misoprostol (Olivry et al., 2010).

In humans, evidence for a protective effect by helminths has been reported in atopic diseases (Cooper et al., 2003, 2004). Atopic diseases show a significantly lower prevalence in developing countries than in industrialized

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countries. Helminth infections correlate negatively with atopy and the prevalence of allergic diseases (Carvalho et al., 2006). *Schistosoma mansoni* infection in asthmatic patients is associated with a milder course of asthma (Medeiros et al., 2003). The protective effect of helminth infections throughout immunomodulation is supported by various murine (Bashir et al., 2002; Lima et al., 2002; Mangan et al., 2004), porcine (Hurst et al., 2006) and bovine animal models (Graham et al., 2001). Parasitic infection and atopy both induce a type 2 immune response. However, atopy generally provokes an excessive type 2 response, whereas the production of IL-10 and TGF- β by regulatory T cells associated with helminth infestation to a degree protects the tissue from parasitic damage. These cytokines may be responsible for the protective effect against sensitisation and subsequent clinical signs of allergic disease (Carvalho et al., 2006). Currently, there is no published evidence of immunomodulation by helminths in dogs with atopic dermatitis.

The aim of this study was to determine, whether *Trichuris vulpis* or *Uncinaria stenocephala* induce an immune-modulating effect and thus a change in clinical signs, histopathological findings or allergen-specific IgE concentrations in dogs with atopic dermatitis.

2. Materials and methods

2.1. Study design

After an initial open pilot case study evaluating the effects of two different nematodes at different doses, a randomised, double-blinded, placebo-controlled study exclusively evaluating the effects of *T. vulpis* on clinical signs, IgE concentrations and histopathological findings of dogs with canine atopic dermatitis was conducted. To ensure blindness, the uninvolved study monitor assigned the dogs into groups. Neither the clinicians evaluating the dogs nor the owners were aware of treatment type. Randomisation occurred with the help of a randomisation table prior to both studies.

2.2. Study objects

In the pilot study 12 dogs diagnosed with non-seasonal atopic dermatitis and owned by clients of the Centre for Clinical Veterinary Medicine, Ludwig Maximilian University, Munich, Germany were included. The diagnosis of canine atopic dermatitis was based on compatible history, clinical findings and the exclusion of possible differential diagnoses for pruritus (Willemse, 1986; DeBoer and Hillier, 2001). Glucocorticoids were not administered for at least 6 weeks prior to the study. Antimicrobial therapy was not permitted and no dog received anthelmintics during the trial. All dogs with a history of or clinical signs compatible with flea bite hypersensitivity received fipronil (Frontline® spot-on, Merial, Hallbergmoos, Germany) monthly. Non-steroidal antipruritic therapy was permitted if therapy had commenced more than 12 (fatty acids), 4 (antihistamines) and 2 weeks (shampoo therapy) prior to the study. Continuation of allergen-specific immunotherapy was also permitted as long as it had been given for at least 12 months

prior to the study. Modification of dose and frequency was not permitted during the trial. Before entering the study a faecal sample of each dog, collected by the owner 3 days prior to commencement of the study, was examined for endoparasites. Dogs with positive faecal samples were excluded. Dogs with concurrent diseases such as hyperadrenocorticism, hypothyroidism and diabetes mellitus and dogs with pyoderma or parasitic skin diseases were also excluded from the study. Twenty-two dogs were included in the placebo-controlled, double blinded trial. Inclusion criteria were those of the pilot study. The pilot study was approved by the responsible governance of Bavaria under the number 55.2-1-54-2531-1-08, the placebo-controlled trial under the number 55.2-1-54-2531-120-08.

2.3. Preparation and storage of helminths

For preparation of embryonated eggs of *T. vulpis*, patent infected dogs were treated with anthelmintics and female adult worms were isolated from the faeces. After extraction, the eggs were stored for 4–5 weeks at 26 °C and weekly oxygenated with a pipette. Successful embryonation was confirmed microscopically.

For preparation of L3 *U. stenocephala* larvae, faeces cultures (faeces, saw dust, and, if necessary, tap water) were incubated in a climate chamber for 1–2 weeks, before the larvae were isolated and stored in the refrigerator.

The embryonated eggs of *T. vulpis* and the L3 larvae of *U. stenocephala* were suspended in tap water. The infectious helminth stages were stored at 4 °C in a refrigerator. Additionally, the embryonated eggs of *T. vulpis* were weekly oxygenated with a syringe.

2.4. Treatment intervention

Three of the 12 dogs in the pilot study received 500 embryonated eggs of the canine whipworm *T. vulpis* once monthly for 3 months. Three dogs were administered 2500 embryonated eggs of the same species. Similar dosage was intended for infestation with *U. stenocephala* in the remaining six animals but due to development of diarrhoea in the first four treated dogs (500 and 2500 third stage larvae in two animals, respectively), the remaining two patients received a decreased dosage of 100 L3 *Uncinaria* larvae.

In the placebo-controlled study, 12 dogs received 2500 embryonated eggs of *T. vulpis* monthly for 3 months and 12 dogs received physiologic saline solution. Both the placebo (physiologic saline solution) and the worm egg suspension were slowly administered with 10.0 ml syringes orally. At the end of both studies, each dog was dewormed with febantel, pyrantel and praziquantel (Drontal® Plus, Bayer Vital GmbH, Leverkusen, Germany) or milbemycin oxime and praziquantel (Milbemax®, Novartis Animal Health GmbH, Munich, Germany) in cases of dogs with a food intolerance to beef.

2.5. Clinical evaluation

Lesion scores were determined using a validated lesional score, the canine atopic dermatitis extent and severity index (CADESI) (Olivry et al., 2007). The owner

assessed the pruritus using a visual analogue scale (Rybnycek et al., 2008). Dogs were clinically evaluated in the same fashion once monthly for the following 3 months. Any adverse effects such as diarrhoea and vomiting were recorded and treated as indicated.

2.6. Sample collection and processing

Faecal samples collected by the owner over three consecutive days prior to each visit were examined for helminths by a commercial parasite diagnostic system using 29.5% sodium nitrate (Parasiten-Diagnose-System, Jansen Animal Health, Neuss, Germany).

Before and after the pilot study a 4 mm skin punch biopsy was obtained from lesional skin on the ventral abdominal or inguinal skin of all dogs under local anesthesia with 2% lidocaine hydrochloride. The specimen was placed in formalin and routinely processed for histology. Haematoxylin and Eosin, Giemsa and Luna stains were performed. In the placebo-controlled study, only four owners gave their consent to biopsies. Sections were analysed using a translucent upright microscope (BX51, Olympus Imaging Europa GmbH, Hamburg, Germany), a digital camera and additional imaging software (AnalysisFIVE, Olympus Imaging Europa GmbH, Hamburg, Germany). Each section was evaluated in a blinded fashion. The surface area of the evaluated superficial and deep dermis was measured and inflammatory cells were counted individually. Their numbers were expressed in cells/mm².

Ten millilitres of blood were obtained from all dogs included in the placebo-controlled study by jugular venipuncture, centrifuged with 2664 × g and the serum was removed, frozen and stored at −20.0 °C. Allergen-specific IgE was determined by a commercial ELISA based on the Fcε receptor (Heska Laboratories, Fribourg, Switzerland).

2.7. Statistics

Pruritus and lesional scores and the determined cell numbers before and 3 months after therapy were compared with Kruskal–Wallis and Dunn post test. Pruritus and lesional scores and the cutaneous cellular infiltrate (mast cells, eosinophils and neutrophils) were compared with a Wilcoxon matched-pairs signed-ranks test. Based on the standard deviation and improvement of CADESI scores in the pilot study, it was determined that to detect a difference half as large as was seen in the pilot study with a power of 80%, the number of animals needed in each group was 12. In the placebo-controlled study, a per protocol analysis (including only dogs that completed the study) and an intention to treat analysis of all dogs included with the last value carried forward was conducted. In all dogs of the treatment group and in the three least improved dogs in the placebo group, serum allergen-specific IgE concentrations before and after treatment were compared with a Wilcoxon matched-pairs signed-ranks test. The correlations between IgE concentrations and CADESI and pruritus scores were evaluated with a Spearman test. Graphpad Prism 5.0 and

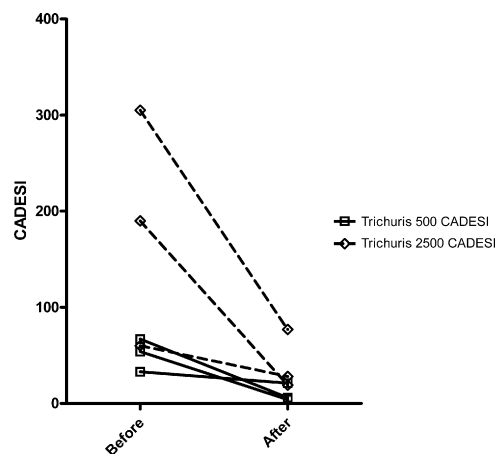


Fig. 1. CADESI scores of atopic dogs before and after administration of *Trichuris vulpis* embryonated eggs.

Instat 3.06 (Graphpad Software, San Diego, USA) were used and a $P < 0.05$ was considered significant.

3. Results

3.1. Study objects

12 dogs were included in the pilot study and 21 dogs in the placebo-controlled trial. The dogs' mean age in the pilot study was 5.8 years (range 2–13 years). Eight of the dogs were male (two of them desexed) and four were female (three of them spayed). The dogs belonged to nine different breeds. One of the dogs had to be excluded from the study after the second visit because of severe side effects (diarrhoea and vomitus).

In the placebo-controlled study, the mean age was 6.5 years (range 1–14 years), 12 of the dogs were male and 9 female. One dog in the treatment group had to be excluded due to gastrointestinal problems, five more dogs had mild intermittent diarrhoea not necessitating exclusion. Diarrhoea was only seen in one dog in the placebo group. Six dogs did not complete the study because of a dramatic deterioration in skin lesions (5) or pruritus (1). Three of the lesional dogs were in the helminth group and the other two in the placebo group, the dog with increased pruritus was in the placebo group.

3.2. Clinical evaluation

Irrespective of the type of infection, a reduction in pruritus and CADESI scores was seen in the pilot study (Table 1). Grouping the scores of all animals, this improvement was statistically significant (Wilcoxon matched-pairs signed-ranks test, $P = 0.0005$).

Dogs that were administered *T. vulpis* improved significantly in their CADESI scores (Wilcoxon matched-pairs signed-ranks test, $P = 0.0313$, Fig. 1). However, the decrease in pruritus was not statistically significant (Wilcoxon matched-pairs signed-ranks test, $P = 0.1563$,

Table 1

Canine atopic dermatitis extent and severity index (CADESI) and pruritus scores of atopic dogs before and after helminth administration in the open pilot study.

	CADESI before	CADESI after	Pruritus before	Pruritus after
Mean	93	25	4.7	2.7
Confidence interval	44–143	13–37	3.2–6.2	1.2–4.2
P value ^a	0.0005		0.0425	

^a A Wilcoxon matched pairs signed rank test was used.

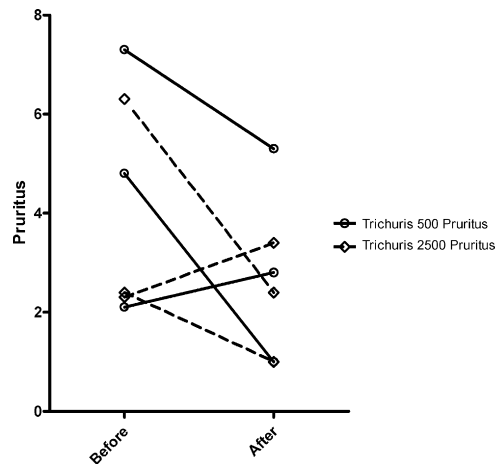


Fig. 2. Pruritus scores of atopic dogs before and after administration of *Trichuris vulpis* embryonated eggs.

Fig. 2). Similarly, the *Uncinaria* group showed a significant reduction in CADESI scores (Wilcoxon matched-pairs signed-ranks test, $P=0.0313$), the pruritus reduction was not significant (Wilcoxon matched-pairs signed-ranks test, $P=0.2188$).

In the placebo-controlled study, there was no significant difference between treatment groups in CADESI and pruritus scores using the intention-to-treat analysis ($P=0.5507$ and $P=0.4510$). Similarly there was no difference in CADESI (Table 3) and pruritus scores (Table 4) within the groups. Dogs in the placebo group deteriorated in regard to pruritus and CADESI, although that change did not reach significance. Similar results were obtained with the per protocol analysis. In the treatment group, 4/11 dogs improved in their lesion score by more than 50%, in contrast to the placebo group, where 1/10 improved by more than 50%. Pruritus scores improved by more than 50% in 3/11 dogs treated with *Trichuris* eggs, none of the dogs in the placebo group improved more than 50%.

Table 2

Inflammatory cells in the dermis of atopic dogs before and after helminth administration.

	Mean cell numbers/nm ² (confidence interval)	
	Before	After
Lymphocytes	0.213 (0.134–0.293)	0.176 (0.124–0.228)
Mast cells	0.195 (0.048–0.341)	0.138 (0.066–0.209)
Eosinophils	0.017 (0–0.042)	0.007 (0–0.014)
Neutrophils	0.012 (0.003–0.022)	0.017 (0.005–0.03)

3.3. Histopathological examination

The observed inflammatory infiltrate consisted of mononuclear cells (lymphocytes, plasma cell, macrophages, mast cells), neutrophils and occasional eosinophils. Cell counts for lymphocytes, mast cells, macrophages and neutrophils were obtained from H&E, Giemsa and Luna stained sections. In the pilot study a decrease in eosinophil, mast cell and macrophage numbers was observed, albeit not significant (Wilcoxon matched-pairs signed-ranks test, $P=0.8438$, $P=0.5186$, and $P=0.4238$, respectively, Table 2). Due to the small number of dogs for which owners had given permission to biopsy the skin a statistical analysis was not performed for cell numbers in the placebo-controlled study.

3.4. Allergen-specific IgE concentrations

IgE was determined in three dogs of each group, the three most improved dogs in the treatment group and the three least improved dogs in the placebo group. There was no difference in the IgE concentration before and after placebo administration. However, there was a significant increase in allergen-specific IgE in those three dogs, which had improved the most with helminth administration. Subsequently allergen-specific IgE was determined in all 12 dogs treated with *T. vulpis* eggs. There was no significant change in IgE concentrations ($P=0.1193$). A correlation between IgE concentrations and CADESI scores was not present ($P=0.7194$), nor was there a correlation between IgE concentrations and pruritus scores ($P=0.3490$). Similarly, there was no correlation between the difference in IgE concentrations before and after treatment and the difference in CADESI scores ($r=-0.079$, $P=0.818$) or pruritus scores ($r=-0.578$, $P=0.133$).

3.5. Faecal samples

Monthly faecal samples of each dog were examined for either *T. vulpis* or *U. stenocephala* using normal flotation with a commercial kit. *U. stenocephala* eggs were present in the faecal specimens of all dogs administered *U. stenocephala*. Eggs of *T. vulpis* were not detected in any of the samples in both studies.

4. Discussion

In a smaller pilot study, administration of the helminths *T. vulpis* and *U. stenocephala* in different doses to dogs with non-seasonal atopic dermatitis led to a significant decrease of the dogs' clinical scores and pruritus. However, in

Table 3

Canine atopic dermatitis extent and severity index (CADESI) of atopic dogs before and after administration of *Trichuris vulpis* eggs or placebo.

	CADESI before treatment	CADESI after treatment	CADESI before placebo	CADESI after placebo
Mean	40	26	12	28
Confidence interval	4–76	9–42	2–22	3–52
P value ^a	0.8203		0.1094	

^a A Wilcoxon matched pairs signed rank test was used.

the subsequent placebo-controlled, double-blinded study evaluating the effect of *T. vulpis* eggs on canine atopic dermatitis, no statistically significant difference was detected.

The International Study of Asthma and Allergies in Childhood (ISAAC) revealed a striking difference in the prevalence of atopic disorders between developing and industrialized countries (Commitee, 1998). Helminth infection was negatively associated with atopy and the prevalence of allergic diseases. Many studies grant helminths a protective effect against allergies (Hagel et al., 1993; Lynch et al., 1993; van den Biggelaar et al., 2000; Cooper et al., 2003, 2004). Immediate skin test reactivity and allergen-specific IgE to common aeroallergens was inversely correlated to *Schistosoma* (*S.*) *mansoni* infections (Araujo et al., 2000). Children chronically infected with helminths are at increased risk to develop atopic reactivity after anthelmintic treatment (van den Biggelaar et al., 2004). However, a higher risk of allergic diseases with helminth infections was also reported in some studies (Lynch et al., 1997; Palmer et al., 2002). Different population age, parasite burden, chronicity and helminth species may be responsible for these contrary results (Carvalho et al., 2006). Based on the above findings, parasites may be a potential therapy for immune-mediated diseases. In one small case series, most patients with Crohn's disease and all patients with ulcerative colitis achieved remission within 12 weeks after a single oral dose of 2500 *Trichuris suis* eggs, although the effect was temporary (Summers et al., 2003). *T. suis* does not develop in the human intestinal tract and the clinical improvement occurred within 12 weeks, so it seems likely that the simple passage of the helminth eggs through the intestinal tract was responsible for the changes. This was supporting the use of *Trichuris* eggs in the pilot study and (as the results were promising) the double-blinded trial. The gastrointestinal signs seen in some dogs during the studies support the assumption that administration of the eggs had a clinical effect on the host.

Fujiwara et al. induced a Th2 immune response in dogs vaccinated with irradiated *Ancylostoma caninum* larvae. Serum of treated dogs resulted in decreased penetration of larvae in a tissue penetration assay (Fujiwara et al., 2006). In the initial pilot study, we detected a decrease in clinical signs of canine atopic dermatitis 3 months

after administration of *T. vulpis* embryonated eggs and *U. stenocephala* third stage larvae. A higher immunomodulation was expected using dog-specific worms, and serious adverse effects were not anticipated with these species. *U. stenocephala* lives in the small intestine, does not feed on blood and rarely infects household pets. *T. vulpis* lives in the caecum. Infections are often asymptomatic, diarrhoea can occur. Laboratory dogs at the Veterinary Faculty in Hannover did not show adverse effects when infected with helminths of similar type and dose as used in this study. However, in the pilot study, gastrointestinal adverse effects were noted in all four dogs receiving either 500 or 2500 *Uncinaria* larvae which led to a dose decrease to 100 larvae in the last two dogs. This emphasizes the fact that household pets may not always react the same ways as dogs kept under laboratory conditions. Cutaneous eruptions with *U. stenocephala* have been reported in the warmer climates of Africa and South-East Asia (Blackwell and Vega-Lopez, 2001; Rivera-Roig et al., 2008). Infection with *T. vulpis* has been reported in a woman with unspecific gastrointestinal signs (Dunn et al., 2002). Embryonation of the eggs and therefore development of infectious stages occurs slowly and may last weeks to months, eggs of both parasites are initially not infectious. Therefore, a transmission to humans or other pets in the household was considered unlikely. Nevertheless, good hygiene, including removal of the faeces after deposition, was recommended to minimize the risk of infection.

After the pilot study revealed surprisingly good results, a double-blinded, placebo-controlled trial was initiated. Exclusive treatment with *T. vulpis* eggs was chosen as treatment with *U. stenocephala* had led to adverse effects. In this study, no difference between placebo or *Trichuris* administration was detected. Similarly, although 3 and 4 of 12 dogs treated with *Trichuris* eggs improved by more than 50% in pruritus and CADESI scores, respectively, the improvement in the treatment group was not statistically significant. As expected, in the placebo group only one dog improved regarding CADESI and none regarding pruritus. The difference between the two studies emphasizes the importance of randomised, placebo-controlled trials in the evaluation of therapeutic interventions in veterinary medicine.

Table 4

Pruritus scores of atopic dogs before and after administration of *Trichuris vulpis* eggs or placebo.

	Pruritus before treatment	Pruritus after treatment	Pruritus before placebo	Pruritus after placebo
Mean	4.5	4.5	3.3	4.8
Confidence interval	3.5–5.5	2.5–6.5	1.2–5.3	2.3–7.3
P value ^a	0.8457		0.0977	

^a A Wilcoxon matched pairs signed rank test was used.

In contrast to the pilot study, the mean CADESI score was lower and most dogs had only mild atopic dermatitis. However, two of the four dogs improving by more than 50% were severely affected. Possibly, administration of *T. vulpis* eggs has a higher success rate in severely affected dogs. In a recent publication, it was recommended to evaluate patients with severe disease separately from those with mild clinical signs due to possible differences in success rates (Olivry et al., 2008). In future studies it could be considered to include exclusively dogs with severe disease or alternatively to increase the numbers of dogs included to allow separate statistical evaluation of dogs with mild versus severe atopic dermatitis. Longer duration of the study would increase the likelihood of adult worms developing in the intestinal tract and it is possible, that adult *T. vulpis* have a more pronounced effect on the immune system. In addition, only two different helminths were evaluated in this study and other helminth species may be more suited for influencing the canine immune system. It was reported previously that different parasite burden, chronicity and helminth species may be responsible for differing results in various studies (Carvalho et al., 2006). Immunomodulation of the host by antigens derived from helminths is complex and in addition to the above-mentioned factors, it is very likely that various host species react differently to individual helminth antigens, which emphasizes the need for further studies in this area.

Mast cell and lymphocyte numbers vary with body site and are generally increased in atopic skin (Wilkie et al., 1990). Eosinophils, which are rarely found in normal canine skin, are increased in atopic dermatitis, although their numbers are low (Hill and Olivry, 2001). Neutrophils are only present in low numbers in the cellular infiltrate of canine atopic dermatitis (Olivry et al., 1997; Wilkie et al., 1990). Summarising our results, the inflammatory cellular infiltrate in this trial mainly consisted of lymphocytes and mast cells with low numbers of eosinophils and neutrophils. These findings are consistent with previous studies (Olivry et al., 1997). Although there was a decrease in inflammatory cell numbers/mm², this was not significant. Differences in chronicity and severity of the biopsied lesions may be responsible for this.

U. stenocephala was constantly recovered in the faeces of atopic dogs which had received this endoparasite in the pilot study. *T. vulpis* were not detected in any of the monthly faecal samples during the study. This nematode reaches sexual maturity within 70–90 days in the host. The present study was terminated 3 months after administration of helminths, possibly before faecal stages were produced. Furthermore, false negative faecal examination for *T. vulpis* is not infrequent even with heavy infections (Jacobs, 1984). In addition, prevalence of an infection with *T. vulpis* is significantly higher in dogs up to 1 year (Barutzki and Schaper, 2003), possibly because of dogs developing immunity as it has been shown for *T. suis* in pigs (Pedersen and Saeed, 2001). As centrifugation has been shown to increase the sensitivity of faecal examinations (Blagburn and Butler, 2006), it is possible that results were false-negative due to the use of a commercial flotation assay without centrifugation.

5. Conclusion

In summary we could show clinical improvement of canine atopic dermatitis after administration of helminth eggs and larvae in a pilot study but failed to confirm these encouraging results in the subsequent placebo-controlled, double-blinded study. Based on these results, further studies should possibly consider using different worm species and including dogs with clinically moderate to severe atopic dermatitis.

Conflict of interest

None of the authors reported a conflict of interest.

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