Prophylactic Treatment of Flea-Infested Dogs with an Imidacloprid/Flumethrin Collar (Seresto® Bayer) to Preempt Infection with *Dipylidium caninum*

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Abstract

The objective of the study was to determine the sustained effectiveness of 10 % imidacloprid and 4.5 % flumethrin, incorporated in a slow-release matrix collar, in preventing *Dipylidium caninum* infection in dogs after repeated laboratory infestations with fleas infected with metacestodes of this tapeworm. Efficacy against infection with *D. caninum* was evaluated by infesting 16 dogs with cat fleas (*Ctenocephalides felis*) on study days 7, 14, 21, 28, 35 and 42, from batches suitably infected with *D. caninum* metacestodes. Prior to each post treatment infestation the *D. caninum* infection rate for the fleas was determined by microscopically examining 100 fleas for *D. caninum* cysticercoids. The *D. caninum* prevalence in the fleas used for infestations ranged from 23% to 52%. Medicated collars were fitted to 8 of the dogs on study day 0. The weight of the IVP collars varied between 35.48 g and 38.48 g (average 37.16 g), whilst animal weight varied between 12.20 kg and 17.98 kg (average 14.79 kg). Seven days later infestation of each of the 16 dogs with 250 fleas commenced. Infestations continued at weekly intervals until Day 42 with efficacy against fleas evaluated 24 hours after each infestation. From Days 21 to 74, infection of the dogs with *D. caninum* was verified (daily examination of faeces and cages for the presence of expelled proglottids). Calculation of prophylactic effectiveness of the collars in preventing infection with *D. caninum* was based on the difference in geometric mean number of scoleces between groups at necropsy on Day 75. Effective prevention of infection with *D. caninum* was found to be 96.6 %. Efficacy of the collars against fleas was ≥ 99.9 % for the duration of the assessment period.

Newly acquired infestations of fleas are rapidly eliminated by the insecticidal components of the medicated collars over a period of several months. In the event of fleas being infected with metacestodes, infection with *D. caninum* can be prevented in collared dogs, concurrently reducing the likelihood of transmission to humans.

Keywords: imidacloprid, flumethrin, collar, prophylaxis, flea, *Dipylidium caninum*, dog

Introduction

The cat flea, *Ctenocephalides felis,* infests both dogs and cats, is widespread throughout most regions of the world (Beaucournu and Ménier 1998; Ménier and Beaucournu 1999; Beck et al. 2006) and is considered to be one of the most important ectoparasites of dogs and cats (de Avelar et al. 2007). Infestation often results in itching and scratching, often progressing to hair loss and skin lesions caused by continuous grooming in more sensitive animals. The development of flea allergy dermatitis provoked by the saliva of feeding fleas has also been reported (Genchi et al. 2000). Consequently effective control of *C. felis* not only eliminates fleas, but also alleviates discomfort caused to its hosts. In addition, fleas are considered to be of considerable importance as vectors of pathogens in many parts of the world (Bitam et al. 2010). More specifically, *C. felis* plays host to a number of endosymbionts of veterinary and zoonotic importance, including three protozoan species and the metacestode stage of the dog and cat tapeworm, *Dipylidium caninum* (Pugh 1987; de Avelar et al. 2007).

Figure 1 Dissected *Ctenocephalides felis* showing the inner organs and metacestodes of *Dipylidium caninum* (arrows)

Dogs and cats acquire infection with *D. caninum* by ingesting infected fleas, most often during grooming. Infection of dogs and cats with *D. caninum* is a global phenomenon. The parasite is widely distributed in Italy (Oranto and Dantas-Torres 2010) and 38 of 63 adult dogs selected for an anthelmintic efficacy trial were infected (based on faecal examination for proglottids) (Genchi et al. 1990). *Dipylidium caninum* was also the most common helminth parasite in 156 dogs examined (either after treatment with arecoline hydrobromide or at necropsy) in Israel, with 97 of the dogs infected (Furth and

Figure. 2 SEM photograph showing a closer view of a metacestode of *Dipylidium caninum* (bar = 50 µm,box indicating the area shown in Fig. 3)

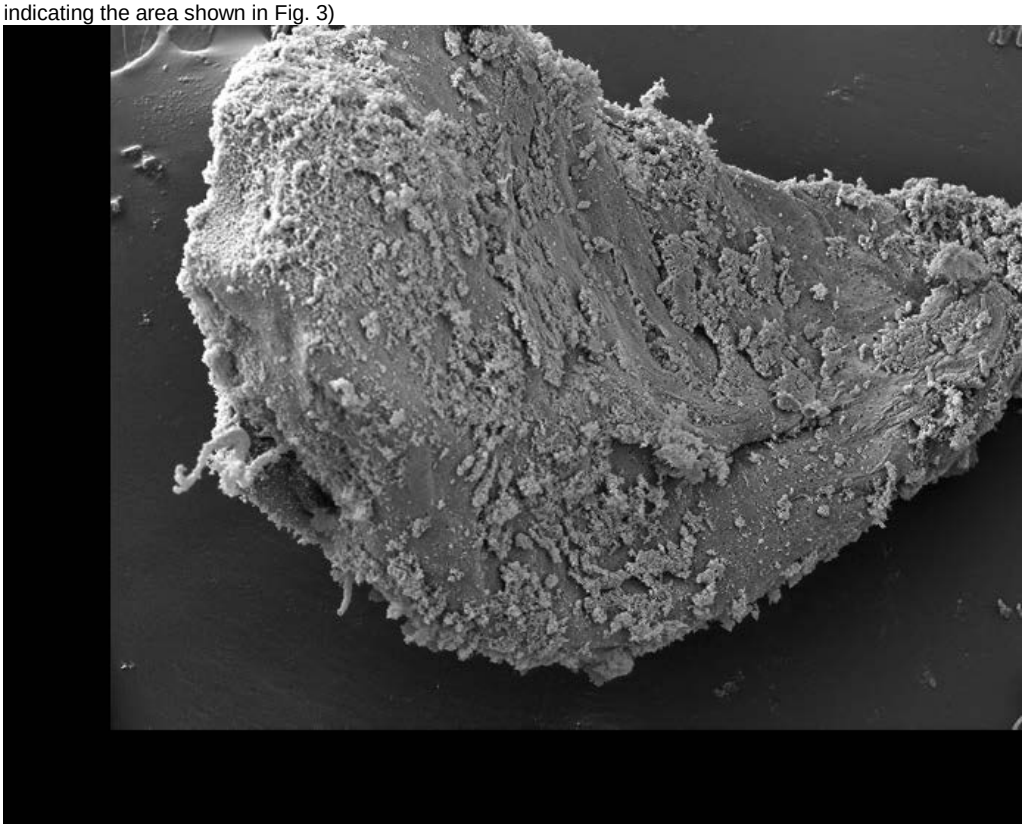
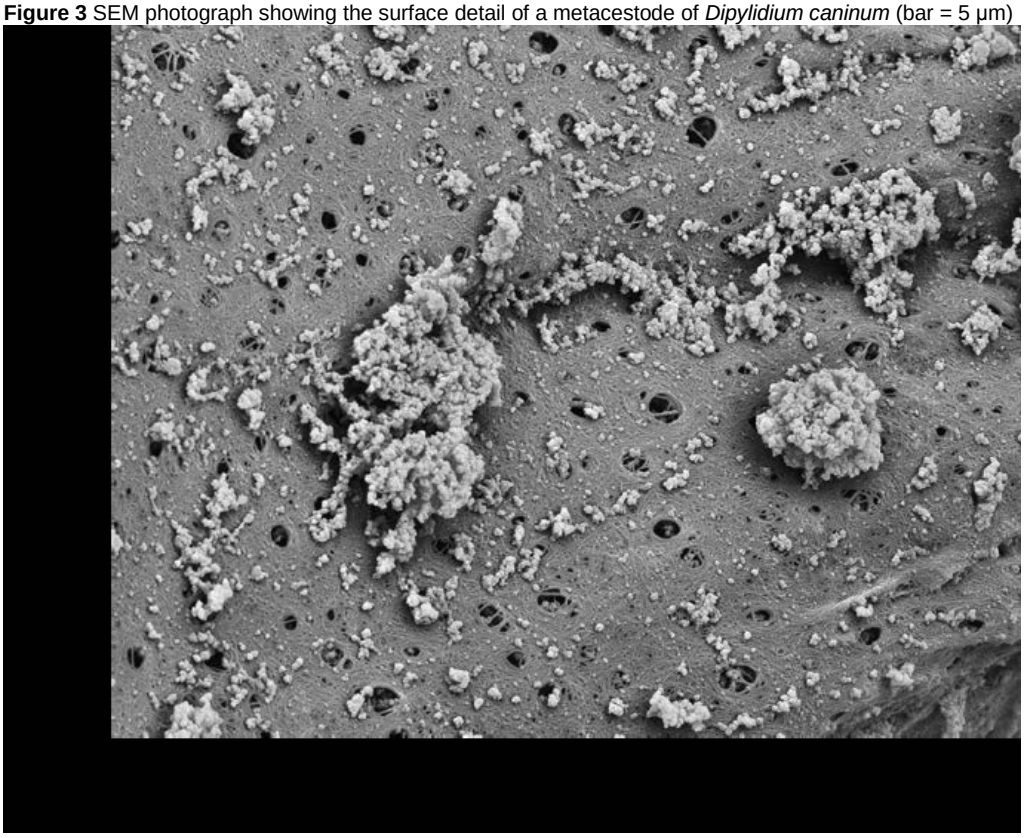


Figure 3 SEM photograph showing the surface detail of a metacestode of *Dipylidium caninum* (bar = 5 µm)



El-On 1990). Twelve of 15 dogs belonging to an aboriginal community on the South coast of New South Wales, Australia, were infected with the intensity of infection ranging between 1 and 65 tape worms (Jenkins and Andrew 1993). In an anthelmintic study conducted on dogs in Texas, USA, 18 noticeably flea-infested dogs were also all infected with *D. caninum* based on faecal examination (Craig et al. 1991). At necropsy, scoleces (numbers ranging from 2 to 44) were recovered from all but one of the 8 untreated control animals (Craig et al. 1991). Examination of 55 stray cats in Iraq yielded a *D. caninum* infection prevalence of 64 % (Al-Obaidi 2012). In Gauteng Province, South Africa, 27 of 69 dogs (belonging to a resource-limited community) were found to be infected at necropsy. The number of scoleces recovered varied between 1 and 288 (Minnaar and Krecek 2001). Boreham and Boreham (1990) reviewed some of the literature prior to 1990 and list Australia, India, Iraq, Jordan, Malaysia, Morocco, Nigeria, Pakistan, the USA and Zambia as countries in which infection with *D. caninum* has been encountered in dogs. The near global occurrence of *D. caninum* infection can be considered an indication of the extent of the problem.

It is generally accepted that infection with *D. caninum* produces few if any clinical signs in dogs (Boreham and Boreham 1990). These may include mild gastrointestinal signs and scratching (Mani and Maguire 2009). However, infection of pets is usually a cause of considerable distress and often embarrassment to their owners (e.g., when a dog drags its anus over an indoor carpet). More importantly, humans may also become infected with this cestode (Chappell et al. 1990; Raitiere 1992). Infections are most often diagnosed in small children as they are generally in closer contact with family

pets (and hence the fleas that infest them) (Chappell et al. 1990; Raitiere 1992; Jithendran and Bhat 2001) and because of their propensity for pica (Mani and Maguire 2009), but adults can also be infected (Adam et al. 2012). A stool passed by a 6-month-old infant contained 13 intact tapeworms and several short strands of proglottids on the day after treatment (Chappell et al. 1990).

The most obvious recourse to reduce the risk of infection with *D. caninum* in the human environment is certainly regular deworming of dogs with a praziquantel containing drug. However, the required frequency of treatment may be underestimated by the dog owner, especially as it can be re-infected at any time after successful anthelmintic treatment by the ingestion of an infected flea. Accordingly, simply deworming the infected family dog “more or less” frequently is not sufficient to provide reliable long-term protection against human exposure to infection.

Besides changes in human behaviour (Jithendran and Bhat 2001) to mitigate the risk of accidental ingestion of fleas, biological control measures such as nematophagous fungi that have an effect on *D. caninum* eggs in the environment (and would hence forestall infection of fleas) may be a preventative option (Araujo et al. 2009). Both options, however, pose logistical challenges and the last one is scarcely effective. According to Mani and Maguire (2009): “Precautionary measures are necessary to prevent zoonotic transmission of pathogens while keeping a pet. Routine and regular veterinary care of companion animal pets with appropriate preventative medicine is extremely important for prevention of transmission”. Chemical control thus appears to be an unavoidable option and when directed at breaking the *D.*

caninum life cycle, may be efficacious both against fleas and tapeworms. It is thus obvious that a formulation offering sustained efficacy against fleas on dogs may aid greatly in preventing the zoonotic transfer of parasites, including *D. caninum*.

Imidacloprid was introduced in 1996 and has since become one of the most successful and largest selling veterinary products for flea control (Schroeder et al. 2003). Due to the rapid mode of action of imidacloprid, it decreases flea feeding periods and hence reduce the risk of transmission of flea-derived diseases (Mehlhorn et al. 2001). It is often combined with actives such as permethrin (e.g. Mehlhorn et al. 2003) or Moxidectin (Mehlhorn et al. 2005; Schmahl et al. 2007) to also provide efficacy against ticks, mites and nematodes. As such products containing imodacloprid was shown to be effective against *C. felis* on hosts as diverse as dogs (Epe et al. 2003; Hellman et al. 2003), cats (Arther et al. 2003), mink (Larsen et al. 2005) and ferret (Wenzel et al. 2008). In all these instances an efficacy exceeding 90% was obtained for a period of at least up to 28 days post-treatment. These findings correspond well to that recorded against *Tunga penetrans* following treatment with an imidacloprid / permethrin combination (Klimpel et al. 2005).

The rapid and sustained efficacy of a slow-release matrix collar formulation of 10 % (m/m) imidacloprid and 4.5 % (m/m) flumethrin (Seresto®) against laboratory infections of dogs with the cat flea *C. felis* has recently been demonstrated by Stanneck et al. (2012). In addition, the efficacy of these collars in preventing infection with the cestode *D. caninum* in cats repeatedly infested with fleas infected with the metacestodes of the tapeworm has also been reported (Fourie et al. 2012). Control of the metacestode-infected fleas

resulted in a 99.7 % reduction in the number of scoleces recovered from collared cats compared to untreated cats (Fourie et al. 2012).

The aim of the present investigation was twofold. Firstly to assess the efficacy of the 10 % (m/m) imidacloprid/4.5 % (m/m) flumethrin collars against fleas that were infected with metacestodes of *D. caninum*, and concurrently evaluate the effectiveness of the formulation in preventing infection with the tapeworm in dogs.

Materials and methods

This parallel, group-designed, randomised, unicentre, controlled efficacy study was conducted in South Africa and involved two groups of dogs each comprising eight animals. Initially 20 mixed-breed domestic dogs ranging in age from sub-adults to adults were enrolled in the study. These dogs had not been treated with an acaricide, or insecticide, or a compound with an insect growth-regulating activity during the previous 12 weeks and were not infected with *D. caninum* (as confirmed by visual inspection of faeces and surrounds during acclimatisation). As an added precaution, all dogs were dewormed with Triworm-D® (praziquantel 50 mg; pyrantel pamoate 144 mg; febantel 150 mg; Cipla-Vet; South Africa) seven days prior to the commencement of the study.

The dogs were housed individually in pens for the duration of the investigation, and no contact between dogs was possible. The dog pens consisted of a 1.69 m x 0.7 m enclosed sleeping area and an outside run of 1.69 m x 3.0 m. A large roof covered all the pens and the dogs were therefore not exposed to rain, but were exposed to ambient temperature and sunlight. The pens had concrete floors to facilitate cleaning, and no bedding was

provided. The accommodation was in compliance with the South African National Standard (SANS 10386:2008. The care and use of animals for scientific purposes). The animals were fed once daily with commercially available dog pellets according to the manufacturer's recommendation. The pellets, and fresh, clean water were provided in stainless steel bowls and the water was replenished at least twice daily. The animals were maintained and handled with due regard for their welfare, and were acclimatised to the pen environment for 7 days prior to the commencement of the study.

On Day –6, all the dogs were infested with 100 fleas that were not infected with *D. caninum* and that originated from a laboratory-bred strain of *C. felis* (ClinVet European strain; routinely fed on dogs). The flea count of each animal 24 h after infestation was used for ranking and group allocation purposes. Three dogs with the lowest flea counts, and a dog that did not comply with the required study criteria were excluded from the remainder of the study. The remaining 16 dogs were ranked in descending order on their individual pre-treatment flea counts, and their IDs were used to break ties. They were then blocked into blocks of two animals each, and within each block, dogs were randomly allocated to two groups. The 16 animals included in the study weighed between 12.2 and 19.5 kg and their hair-lengths varied between 9.5 and 34.5 mm.

On Day 0, the medicated collars were fitted to the necks of the dogs allocated to the treatment group. Each collar was adjusted by means of the buckle to achieve a comfortable fit and any excess was cut off approximately 2 cm beyond the retaining loop. All collars were marked with the dog's ID number so that in case a collar was accidentally dislodged, it could easily be identified

and immediately re-applied. The weight of the IVP collars fitted in this manner, varied between 35.48 g and 38.48 g (average 37.16 g), whilst animal weight varied between 12.20 kg and 17.98 kg (average 14.79 kg). At pre-determined time intervals on the day that the collars were fitted all animals were carefully observed for adverse signs that could be ascribed to the collars or to the active ingredients that they contained. Thereafter they were observed at daily intervals for clinical signs that could be associated with the collars or for any other concurrent conditions.

Ctenocephalides felis, infected with a South African strain (isolated from resident ClinVet animals) of *D. caninum*, were used for all post-treatment infestations. Chervy (2002) defines a cysticercoid as a metacestode with a primary lacuna, retracted scolex with a cercomer or reduced cercomer. Pugh (1987) argued that *D. caninum* metacestodes cannot be considered cysticercoids as the primary lacuna develops but then disappears. He suggested the use of the term metacestode to ecompass all growth forms following the metamorphosis of *D. caninum* oncospheres and before the development of proglottids. We have accepted this reasoning and terminology and consequently refer to the developmental stages of *D. caninum* in the fleas as metacestodes. The fleas were infected with metacestodes by incubating thousands of their eggs, as well as the larvae that hatched, on flea-rearing medium mixed with proglottids and eggs of the tapeworm at temperatures varying between 24 °C and 28.5 °C. Prior to each post-treatment flea infestation of the dogs, the infection rate of *D. caninum* in the fleas was determined by microscopically dissecting 100 specimens and examining them for metacestodes. The prevalence of *D. caninum* metacestodes in the fleas

used for infestation varied between 23 % and 52 %. Each dog was infested with 250 of these fleas on the days indicated in Table 1. The fleas used for

Table. 1 Design of a study aimed at determining the effectiveness of imidacloprid/flumethrin collars in the prevention of *Dipylidium caninum* infection in dogs repeatedly infested with infected fleas

Study day	Activity
–7 to – 1	Acclimatisation to cage environment
– 6	Infestation with 100 non-infected fleas
– 5	Flea counts, 3 dogs with lowest counts excluded from remainder of study; 1 dog excluded as it did not comply with the inclusion criteria
– 2	Ranking and allocation to 2 groups of 8 dogs each
0	Imidacloprid/flumethrin collars fitted to treated group
7	Infestation with 250 infected fleas
8	Flea counts and re-infestation with the fleas that had been counted
14	Infestation with 250 infected fleas
15	Flea counts and re-infestation with the fleas that had been counted
21	Infestation with 250 infected fleas; daily examination of faeces for expelled proglottids commences
22	Flea counts and re-infestation with the fleas that had been counted
28	Infestation with 250 infected fleas
29	Flea counts and re-infestation with the fleas that had been counted
35	Infestation with 250 infected fleas
36	Flea counts and re-infestation with the fleas that had been counted
42	Infestation with 250 infected fleas
43	Flea counts
74	Daily examination of faeces for expelled proglottids ceases
75	Necropsy and collection and counting of scoleces

infestation were unfed and of mixed sex, and were not placed on or near the site of the fitted collar. Dogs were restrained by hand during infestation and during flea recovery.

A fine-toothed flea comb was used to recover fleas from the animal's hair coat and its skin surface. Combing was performed by several strokes of the comb over each body part of the dog, each time moving in the same direction and following the pattern of the hair coat. Movement, from one part of the animal's hair to the next, was via strokes overlapping each other, so that no area of the body or hair was missed. After completion of the combing of all body areas, the whole procedure was repeated so that all sites were combed a minimum of twice. If necessary, the combing was performed for a third time or more until no live fleas were found. Fleas collected in this manner were quickly counted and live fleas placed back on the dog from which they had come.

Counting of fleas was not blinded since the control dogs were not fitted with placebo collars, thus making blinding impossible.

The groups were compared on their flea counts by a one-way ANOVA (analysis of variance performed using the Proc GLM procedure in SAS Version 8, Release 8.02, released 2001 TS Level 02M0) with a treatment effect on the flea counts (count +1) after logarithmic transformation of the data.

Efficacy of the collars against *C. felis* was calculated as follows:

Efficacy (%) = 100 x (N1 – N2) / N1, where

N1 = geometric mean number of live fleas on dogs in the untreated control group

N2 = geometric mean number of live fleas on dogs in the treated group.

The dogs were observed daily from Day 21 to Day 74 in order to detect the presence of expelled proglottids (Table 1). This involved visual, macroscopic

examination of fresh faeces, the anal and perineal regions of the animals, their hair and their cages. If no proglottids were found, freshly excreted faeces were washed through steel-mesh sieves with an aperture size of 0.15 mm. The residues in the sieves were collected and suspended in a small amount of water, which was then examined macroscopically for the presence of proglottids. The proglottids or fragments of worms recovered were examined microscopically to ensure that identification was correct. All proglottids were preserved and retained until the completion of the study as a record of the diagnoses. Once a dog had shed proglottids on two separate occasions, no further faecal examinations or other examinations for proglottids were conducted on that dog.

All the dogs were euthanised on Day 75 by intravenous injection of Euthapent^{TM} (sodium pentobarbitone 200 mg/ml; Kyron laboratories) at an approximate dose of 1 ml/kg. Food was removed from the cages during the afternoon prior to euthanasia in order to reduce the volume of ingesta in the gastrointestinal tract at necropsy. At necropsy, a ligature was applied at the ileo-caecal junction between the small and large intestines and the digestive tract from the stomach to the rectum was removed from the abdominal cavity. The small intestine, including the stomach, and the large intestines were treated separately. They were carefully cut open and their contents flushed with water, and their mucosa thoroughly scraped. All material that had been flushed, washed or scraped from the small intestines and stomach were then washed over a sieve with 0.15 mm apertures. The contents of the large intestines and their mucosal scrapings were also washed over sieves with an aperture size of 0.15 mm. The residues in the sieves were collected and

preserved with formalin in labelled bottles. The bottles were coded for each dog in order to blind the counting of *D. caninum* scoleces.

The effectiveness of the imidacloprid/flumethrin collars in the prevention of *D. caninum* infection was based on the difference between the geometric mean numbers of scoleces recovered from the control and treated groups of dogs. The geometric mean was calculated following logarithmic transformation. In cases where a scolex count was zero, all counts were modified by adding one (1) to each count prior to transformation. Thereafter one (1) was subtracted from the antilog value to meaningfully represent the geometric mean for each group. SAS Version 8 (Release 8.02, released 2001, TS Level 02M0) was used for all statistical analyses.

Prophylactic effectiveness against infection with *D. caninum* by infected fleas was calculated as follows:

Prophylactic effectiveness (%) = 100 x (N1 – N2) / N1 where

N1 = geometric mean number of *D. caninum* scoleces recovered from the untreated control group of dogs

N2 = geometric mean number of *D. caninum* scoleces recovered from the group of dogs fitted with medicated collars

Results

The geometric mean flea counts of the two groups of dogs on the various assessment days are summarised in Table 2. The mean flea counts of the untreated control group (from Day 8 to Day 43) varied between 139.9 and 226.5, indicating a robust flea challenge on all post-treatment assessment days. The geometric mean flea counts of the collared group of dogs differed statistically significantly ($p < 0.05$) from those of the untreated control group

Table. 2 Efficacy of an imidacloprid/flumethrin collar applied on study day 0 against *Ctenocephalides felis* on repeatedly infested dogs

Study day	Geometric mean number of fleas recovered		Efficacy (%)
	Untreated control dogs	Dogs fitted with collars*	
8	139.9	0.0	100
15	205.4	0.3	99.9
22	201.5	0.2	99.9
29	220.7	0.1	99.9
36	226.5	0.0	100
43	200.5	0.0	100

* The flea counts of the collared group of dogs differed statistically significantly ($p < 0.05$) from those of the untreated control group of dogs on all post-treatment assessment days

on all post-treatment assessment days. The efficacy of the collars against infestation with *C. felis* was ≥ 99.9 % for the 42-day duration of that part of the study devoted to fleas.

Based on the collection of expelled *D. caninum* proglottids, 87.5 % (7/8) of the dogs in the untreated control group and 25 % (2/8) of the dogs fitted with collars were infected with *D. caninum*. The time between fitting the collars and detection of *D. caninum* proglottids in the dogs' faeces or in their immediate surroundings is summarised in Table 3. All but one of the dogs in the

Table. 3 The presence of *Dipylidium caninum* proglottids in the faeces and surroundings of untreated dogs and of dogs fitted with imidacloprid/flumethrin collars

Untreated control dogs		**Dogs fitted with collars**	
Dog ID	**Days proglottids detected**	**Dog ID**	**Days proglottids detected**
420	34 and 35	7F8	None
059	37 and 38	937	None
E9A	43 and 44	219	None
A2D	None	B54	24 and 39
177	38 and 39	E80	None
D08	37 and 39	3EA	25 and 40
71E	37 and 39	931	None
150	41 and 48	539	None
Infection (%)	**87.5**	**Infection (%)**	**25.0**

untreated control group and two dogs in the collared group shed proglottids and consequently were considered to be infected with *D. caninum*. The first proglottids to be detected were present in the faeces on Days 24 and 25 of two dogs in the treated group. The majority of untreated dogs had started

shedding proglottids by Day 38, but one dog only started shedding proglottids on Day 41 and another on Day 43 after the collars had been fitted.

Table. 4 *Dipylidium caninum* scoleces recovered from untreated control dogs and from dogs fitted with imidacloprid/flumethrin collars

Untreated control dogs		**Dogs fitted with medicated collars**	
Dog ID	**Number of scoleces**	**Dog ID**	**Number of scoleces**
420	126	7F8	0
059	2	937	0
E9A	1	219	0
A2D	3	B54	1
177	86	E80	0
D08	1	3EA	1
71E	5	931	0
150	0	539	0
Total	224	Total	2
Geometric mean	5.5	Geometric mean	0.2
		Prophylaxis (%)	**96.6**

The numbers of *D. caninum* scoleces recovered from the intestinal tracts of the dogs at necropsy are summarised in Table 4. Scoleces were recovered from all but one of the untreated control dogs and numbers varied between 1 and 126. No proglottids had been recorded for dog A2D in the untreated control group prior to euthanasia, yet it was found to be positive at necropsy and harboured 3 scoleces. On the other hand dog 150 shed proglottids on Days 41 and 48, but harboured no scoleces at necropsy. Two of the collared dogs were infected and each harboured 1 scolex. Each of the latter dogs had shed proglottids during the period prior to euthanasia.

The geometric mean number of scoleces recovered from the negative control group of dogs (5.5) differed statistically significantly ($p < 0.05$) from that of the

collared group of dogs (0.2). Based on the geometric mean number of scoleces recovered, the collars were 96.6 % effective in preventing infection with *D. caninum* in the dogs.

Discussion

The efficacy of 99.9 % to 100 % of the imidacloprid/flumethrin collar (Seresto®) against repeated infestations with *C. felis* during the first 42 days of the study confirms the results of earlier investigations on the efficacy of the collars against fleas on dogs (Stanneck et al. 2012). The geometric mean number of 139.9 fleas counted on the untreated dogs on Day 8 of the investigation is probably the most reliable indication of the number of *C. felis* that became established after each infestation with 250 fleas. All subsequent counts exceeded 200 fleas, with the arithmetic mean number collected on Day 36 exceeding 225, 5 more fleas than used for infestation. These high burdens probably result from the fact that once fleas had been counted they were replaced on the dog from which they had been collected. This increase in flea numbers on the dogs differs from the flea counts of cats in a similar study in which the greatest mean number of fleas was collected on Day 8 and lesser numbers thereafter (Fourie et al. 2012). The lack of an increase in flea numbers on the cats was ascribed to the extraordinary grooming efficiency of these animals as reported by Hinkle et al. (1998).

Differences in faecal worm egg counts between treated and control groups of animals may in some instances prove to be valid for the determination of anthelmintic efficacy against nematodes, yet they are more often considered as supportive of results obtained during necropsy. No such quantitative methods (with regard to faecal egg count evaluation) exist for efficacy studies

against cestodes. This is particularly true for *D. caninum* as the shedding of proglottids is neither quantitative nor consistent. This phenomenon is evident for one dog in the control group in which there was a break of 7 days between the first and the next appearance of proglottids and two dogs in the treated group where this gap was 14 days. Furthermore, one of the dogs in the control group shed no proglottids, but harboured 3 scoleces at necropsy. These scoleces, however, appeared to have resulted from a recent infection as each had developed only a few segments beyond its neck.

The shedding of proglottids by two dogs in the treated group, 24 and 25 days after the commencement of the study represents prepatent periods of 17 and 18 days after infestation with the first batch of infected fleas on Day 7. These prepatent periods are a day or two shorter than the 19 days observed in a cat similarly infected in an earlier study (Fourie et al. 2012), and a few days less than the three weeks quoted by numerous authors without stating their source. The short prepatent periods in these two dogs suggests that metacestodes in the fleas with which they were infested had completed their development to infectivity off-host, and that the infected fleas were ingested shortly after release onto the dogs. Proglottids were detected in one of the dogs in the untreated group on Days 41 and 48, but this dog appears to have lost its infection as no scoleces were recovered at necropsy 27 days later. A similar instance of self-cure has been recorded in a naturally infected dog, selected for inclusion in an anthelmintic trial on the presence of proglottids in its faeces, yet at necropsy it was found to harbour no *D. caninum* (Craig et al. 1991).

There is a 10-fold difference in the geometric mean number of scoleces collected from the 8 untreated dogs in this study and from 8 untreated cats in a similar study (Fourie et al. 2012). A geometric mean of 5.5 scoleces was recovered from the dogs compared to 58.3 from the cats. Infection in only 2 of the dogs, one harbouring 126 scoleces and the other 86 scoleces, fell within the range of 19 to 349 scoleces recovered from individual cats (Fourie et al. 2012). It is difficult to explain the disparity in scolex numbers between the dogs and cats. It could be due to spontaneous loss of infection as appears to have occurred in the dog that shed proglottids, but harboured no scoleces at necropsy. It could possibly also be due to an immune reaction to the large number of metacestodes with which the dogs were infected over a period of 6 weeks resulting in self-cure.

Pugh (1987) has demonstrated that at temperatures below 30 °C metacestodes are unable to complete their development to infectivity in adult fleas unless the fleas are placed on a mammalian host for a few days. The host's surface temperature, and not blood meals taken by the flea, then enables the metacestodes to mature and become infective for the definitive host (Pugh 1987). The latter observation is of particular significance in the present study during which the fleas in the pool used for infestation were maintained at temperatures ranging from 24 °C to 28.5 °C. This is in agreement with maximum temperature ranges encountered in microhabitats in many households and locations around homes, where temperatures ranging between 13 and 27°C favour the survival of flea larvae (Guardis et al. 1992). At this temperature range (i.e., 24 °C to 28.5 °C) many of the metacestodes in the approximately 14-day old groups of fleas used for infestation would not

have developed to infectivity. These metacestodes would thus have required that their flea hosts infested a mammalian host for a few days before they became infective. It was precisely for this reason that once fleas had been counted they were released back onto the same dog from which they had come, so as to ensure that any metacestodes with which they were infected could develop to infectivity and thus more closely resemble conditions pertaining in the field. Apart from temperature, the diet available to flea larvae (yeast content) may also have a slight influence on metacestode development (Benesh 2010), potentially resulting in additional variability in parasite development under field conditions.

Within a home environment the temperature may fluctuate considerably, but the average daily temperature is unlikely to exceed 25 °C, as demonstrated by Guardis et al. (1992). In sheds, stables, kennels, yards, outside rooms or verandahs frequented by dogs the average daily temperature is liable to be even lower. Under these circumstances infected fleas would have to spend a few days on a dog before the metacestodes become infective. It is during this pre-infective period on the host that infected fleas must be killed to prevent infection of the host animal with *D. caninum*. Studies prior to the present one have shown that the imidacloprid/flumethrin collars killed > 99 % of fleas that access a dog within 24 h after the collars had been applied (Stanneck et al. 2012). Furthermore, the collars also proved to be > 94 % effective within 24 h of subsequent infestations with *C. felis* over a period of 8 months (Stanneck et al. 2012). It is thus the rapidity with which the active ingredients of the collars kill fleas that prevents them spending sufficient time on the host for the

metacestodes with which they may be infected to develop to maturity and capable of infecting a host.

Dog owners who detect fleas on their animals, and who at the same time notice single or chains of proglottids in their dogs' faeces or on their hair coats, are likely to seek advice from their local veterinarians. The animals should then be treated for tapeworms and the owners informed that fleas must be controlled on the dogs in the future. Should imidacloprid/flumethrin collars be used for flea control the prolonged period of protection they afford against flea infestation will play an integral role in the prevention of re-infection with *D. caninum.* The logic behind this approach is that provided temperature and moister are adequate, *D. caninum* proglottids that had been shed several weeks or even months previously, remain viable (Craig et al. 1991), and consequently flea larvae, that were already present in the dogs' environment before treatment, are still likely to become infected. The sustained efficacy of the collars will then eliminate the resultant infected adult fleas that access the dogs long after the collars have been fitted.

The imidacloprid/flumethrin collars are not only effective against adult fleas, but also effective against flea larvae in the dogs' immediate surroundings (Stanneck et al. 2012). Thus if larvae are eliminated in the collared dog's favourite sleeping, resting or loafing places in the home or beyond its confines, the numbers available to ingest *D. caninum* eggs will be drastically reduced. This in turn will result in a significant decline in the number of infected adult fleas, of which the vast majority will in turn be killed on the dog.

In the context of preventing dipylidiasis, another flea-transmitted helminth is worth mentioning. Fleas are also the recognised vectors of the filarial

nematode, *Acanthocheilonema reconditum*, which has a worldwide distribution and is the cause of canine subcutaneous filariosis (Hinkle et al. 1998). The rapidity with which the imidacloprid/flumethrin collars eliminate fleas and their sustained efficacy make them an excellent candidate for the prevention of this condition.

Conclusions

The rapidity with which the insecticidal components of 10 % imidacloprid/4.5 % flumethrin collars eliminate newly acquired infestations of fleas and the collars' sustained high level of efficacy imply that should fleas that access dogs be infected with the metacestodes of *D. caninum*, infection of the dogs can be prevented by application of the collars. Because of their sustained release kinetics and hence efficacy against fleas over a period of 8 months the collars can also be regarded as a means of protecting humans from *D. caninum* infection in preference to regular short-term treatment applications by the animal owner otherwise needed for rigorous flea management.

Ethical standards

All institutional and national guidelines for the care and use of laboratory and study animals were followed.

Conflict of interest

This clinical study was completely funded by Bayer Animal Health GmbH, Monheim, Germany, of which D. Stanneck (Germany) is an employee.

ClinVet, of which J.J. Fourie and D. Crafford are employees, is an independent, South African, Contract Research Organisation contracted to manage the conduct of the study. I. G. Horak is a long-term, contract employee of Clinvet and an Extraordinary Professor at the Universities of the Free State and Pretoria. All authors voluntarily publish this article and have no personal interest in these studies other than publishing the scientific findings.

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