



# Focus on Parasitology

- 2nd Edition -

Contribution of Bayer Animal Health at the  
19<sup>th</sup> International Conference of the WAAVP

August 10 - 14, 2003 · New Orleans, USA



Bayer HealthCare



# **Focus on Parasitology**

**Contribution of Bayer Animal Health  
at the 19<sup>th</sup> International Conference  
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**August 10 – 14, 2003 – New Orleans, USA**

## **Bayer Symposium**

**Bayer Animal Health – Pets, Parasites and Product Solutions**

## **Scientific Programme**

## PREFACE

The 19<sup>th</sup> International Conference of the World Association for the Advancement of Veterinary Parasitology takes place in the city of New Orleans. As Chair of the local organizing committee, I am looking forward to a five-day conference with scientific stimulation and social interaction. Like most of biological science, the field of Veterinary Parasitology is undergoing major advances because of the growth in molecular genetics and cell biology. At the same time, many of our concerns and questions regarding our understanding of parasites, their host interactions and their control remain unchanged. The theme of the conference, **“Old Dreams – New Visions: Veterinary Parasitology in the 21<sup>st</sup> Century”**, was designed to stimulate discussion of the merging of new technologies with traditional studies to answer persistent as well as new questions. The program includes plenary lectures, symposia, round table discussions and oral as well as poster presentations. Organizing a world congress like this would not be possible without the support of the sponsors. Bayer Animal Health, a parasitology-focused company, is a major sponsor of this year’s WAAVP conference and will present papers of various parasitological topics. The presentations cover a wide variety of research fields in protozoology, helminthology and on ectoparasites. All presentations, whether oral or poster presentations, are combined in this supplement to ‘Parasitology Research’ titled “Focus on Parasitology”. The proceedings, together with this supplement, will remain as a reference document from this year’s WAAVP congress for years to come.

I would like to welcome you all to the WAAVP conference and the city of New Orleans, the city of plenty – Creole and Cajun cuisine, music like blues, jazz, rock and roll to name a few.

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## INTRODUCTION

# **Bayer Animal Health**

## **“Focus on Parasitology”**

### **– 2nd Edition –**

Bayer Animal Health business group is proud to participate – as in previous years – in the 19<sup>th</sup> International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP) 2003, in New Orleans, Louisiana, USA. Parasites continue to have an important impact on the well being of animals as well as human health and thus an effect on economic growth throughout the world. Diseases caused by parasites remain a major threat to farm, as well as pet animals and thus research and development of products in the field of veterinary parasitology is an important segment within the Animal Health industry. The total Animal Health market in the year 2002 was estimated at 14.0 billion Euro of which parasiticides were the largest market segment with 27% or 3.8 billion Euro. Bayer Animal Health business group is committed to supporting parasitology research to ensure constant improvement in existing therapeutics and the development of innovative remedies. In the ongoing battle against parasitic infections the veterinary profession, we believe, plays an important role in both the education and implementation of preventative care for producers as well as pet owners. Pet owners especially rely upon the expertise and advice given by veterinarians. Veterinarians are uniquely suited to the role of educating the public about the hazards of zoonotic diseases. The theme of this year’s WAAVP conference: “Old Dreams – New Visions: Veterinary Parasitology in the 21st century” expresses clearly where we stand with our discipline. We within Bayer Animal Health are proud to participate in this years meeting with a total of 25 presentations from all fields of parasitology: ectoparasites, helminths and protozoa, all combined in this brochure “Focus on Parasitology”, a supplement to Parasitology Research one of the leading international parasitology journals.

We hope you enjoy the conference, gain new scientific information, refresh old friendships, make new friends and enjoy the city of New Orleans.

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# Evaluation of Permethrin and Imidacloprid for Prevention of *Borrelia burgdorferi* Transmission from Blacklegged Ticks (*Ixodes scapularis*) to *Borrelia burgdorferi*-free Dogs

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Lyme borreliosis is a bacterial disease caused by the spirochete *Borrelia burgdorferi* and vectored by ticks of the *Ixodes ricinus* complex<sup>1</sup>. In the USA, the nymphal and adult stages of the deer tick, *Ixodes scapularis*, transmit the spirochete to dogs and humans. Larval ticks are infected via feeding on small rodents, most notably the white-footed mouse. The bacteria are then transmitted trans-stadially to nymphal and adult tick stages. Lyme borreliosis is generally confined to locations where the vector tick, the disease reservoir (the white-footed mouse) and the preferred host for adult *I. scapularis* ticks (the white tailed deer) are abundant. In such areas, prevalence of infection in dogs may range locally as high as 85%<sup>2</sup>.

With high seroprevalence of canine Lyme borreliosis in certain areas, and the significant public health aspects of this disease, tick control on dogs exposed to tick-infested habitats is now widely regarded as paramount. There are two aspects of tick control that are the most important. As Lyme borreliosis, and a wide variety of other tick-borne diseases, are transmitted via the tick bite, prevention of tick attachment and feeding must be seen as the first obligation of any tick control agent. Failing that (and no compound may be expected to be 100% effective in this at any given time) a tick control product must be able to kill the tick before it has the opportunity to transmit any pathogens.

While various tick control compounds have demonstrated utility in the killing of ticks, little has been written about the ability of these compounds to prevent transmission of tick-borne disease to susceptible dogs. Elfassy, et al<sup>1</sup>, demonstrated that amitraz-impregnated collars could successfully prevent transmission of *B. burgdorferi* from adult *Ixodes scapularis* (deer) ticks to dogs 7 days after treatment. Similar efficacy has been seen for fipronil spray at days 7 and 33 post-treatment<sup>3</sup>. However, to date, no reports have emerged for any of the popular topical “spot-on” tick control products. As these spot-on products occupy the overwhelming majority of the tick control market, this lack of data is a significant void in our understanding of the utility of these products.

K9 Advantix is an effective tick control agent first registered in November 2002 in the USA. It is a spot on product containing

8.8% (w/w) imidacloprid and 44% (w/w) permethrin. It is labeled to repel and kill four species of ticks, including *Ixodes scapularis*, for up to four weeks. It is also labeled to repel and kill mosquitoes and kill flea adults and larvae. Methfessel and Turburg have demonstrated *in vitro* that permethrin and imidacloprid enhance one another’s activity against the parasites’ nervous system via separate and complementary activity along the axon and post-synaptic membrane, respectively<sup>4</sup>. Thus, this new combination of two proven compounds provides a useful tool for tick control.

While various reports have focused upon the clinical and laboratory efficacy of K9 Advantix<sup>5,6</sup>, this investigation was designed to assess the ability of the product to prevent tick attachment and feeding of ticks known to be infected with *B. burgdorferi* as measured by seroconversion in treated and untreated dogs.

Adult beagle dogs confirmed to be free from *B. burgdorferi* exposure via IFA testing, were randomly assigned to two groups (treated and non-treated control) of 8 animals each and housed separately in a BL-2 facility. Field caught adult *Ixodes scapularis* were used as source of *B. burgdorferi* organisms. On day 0,

**Figure 1** Two ticks attached with hair loss and redness





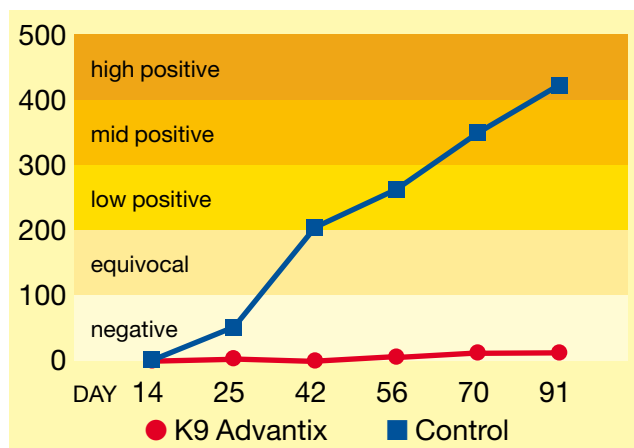
**Figure 2** Tick stages: In their natural habitat, on a dog and engorged females

Group 1 received a single application of a spot on product containing imidacloprid and permethrin (K9 Advantix™) according to the label dose. Group 2 served as untreated controls and received no treatment. On day 7, all dogs were infested with 100 *I. scapularis* adults with an average *B. burgdorferi* infectivity rate of 57.6%. On day 14 all ticks were removed from the dogs and all dogs in both groups received a dose of K9 Advantix to ensure any remaining ticks were dead. (This was to limit potential exposure of personnel to *B. burgdorferi* over the remainder of the study). At this time, four tick attachment sites from each of two dogs in the control (untreated) group were identified and marked. Biopsies were obtained from these sites at day 91 and submitted for PCR analysis.

Approximately 10 mls of whole blood were drawn for ELISA testing for *B. burgdorferi* antibodies on Days 14, 25, 42, 56, 70, and 91. Whole blood was allowed to clot then centrifuged and the serum separated for testing. Kinetics ELISA testing of all samples was performed at the Animal Health Diagnostic Laboratory, Cornell University. Additionally, IFA testing was performed at Auburn University on the samples collected on days 14, 25, and 42 to provide an early screen confirming that the untreated control dogs were successfully infected.

By day 42, all untreated control dogs had seroconverted via IFA testing and all K9 Advantix-treated dogs remained negative. One control dog remained of equivocal infection status and another remained negative via ELISA through day 56 but both strongly converted by day 70. There was complete agreement between IFA and ELISA on the status of all treated dogs and all remained *B. burgdorferi* negative at all sample points. The mean ELISA titers of treated and untreated dogs are shown in figure 3. In addition, skin punch biopsies obtained from untreated control dogs were positive for *B. burgdorferi* DNA.

The results of this study indicate that K9 Advantix is effective 7 days after treatment in the prevention of Lyme borreliosis transmission from infected *Ixodes scapularis* ticks to dogs. This report is the first evidence that such interruption of transmission is possible with a topical spot on tick control agent. ●



**Figure 3** Arithmetic mean antibody titers via kinetics ELISA

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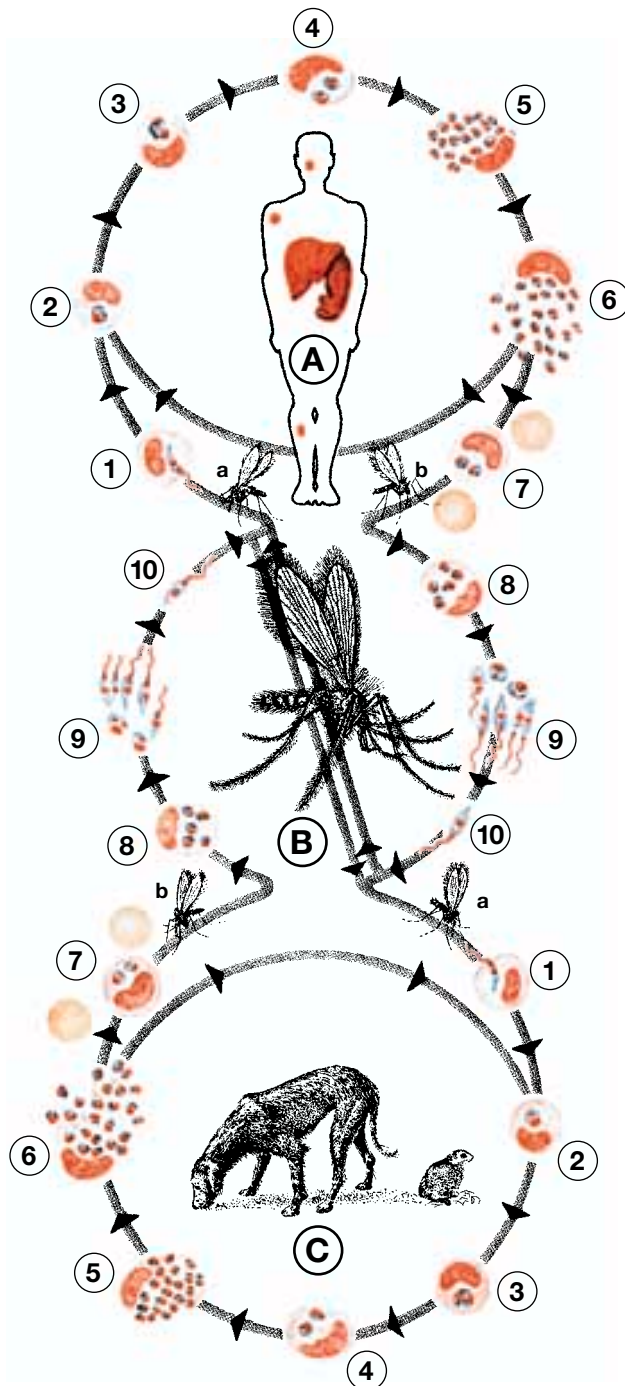


# Repellent Efficacy of a Combination Containing Imidacloprid and Permethrin against Sand Flies (*Phlebotomus papatasi*) on Dogs

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Infections in dogs with the protozoan parasite *Leishmania* are widespread in Mediterranean countries. Furthermore, canine leishmaniasis has a worldwide distribution and can be found in Asia, Africa and America. First reports on canine leishmaniasis were recently published also from the US, it remains unclear until now whether the disease was imported from abroad, or has already established within the country. Leishmaniasis are vector-borne diseases: the promastigote stage of the parasite is transmitted to the host during the blood feeding of an insect vector, the sand fly. The causative agents of canine leishmaniasis are *Leishmania infantum* in the Mediterranean Basin and the Middle East and *L. chagasi* in South and Central America. Initially, *L. chagasi* was considered to be distinct from *L. infantum*, however, recent molecular studies indicate that they are indistinguishable. Today we believe that the parasite reached the New World in dogs transported from Europe to the Americans by the colonists. *L. infantum/chagasi* is also an important human health problem and dogs serve as the main reservoir animal. Several studies showed that the prevalence of human leishmaniasis could be significantly decreased with control of leishmaniasis in dogs. Prevention of dogs from sand fly bites and thus reducing the risk of infection with *L. infantum* is therefore our veterinary obligation.

**Fig. 1** Life cycle of *Leishmania*

**A** Development in Man: 1 Flagellated *L. donovani* parasites carried by *Phlebotomus* species (promastigote stage) enter macrophages (Giemsa stain); 2–6 Intracellular development in macrophages and later in endothelial cells; 7 Macrophages in peripheral blood containing amastigote stages

**B** Development in the sand fly vector: 8 Amastigote stages, within host cells, in the fly's midgut; 9 Growth and multiplication of the promastigote stage; 10 Flagellate stage (metacyclic promastigote form) from fly's proboscis

**C** Development as in man A in animal reservoir (dogs, small rodents etc.)

Transmission by the sand fly can occur as follows:

From Man to Man      **A→B→A**  
 From Animal to Animal **C→B→C**  
 From Animal to Man   **C→B→A**  
 and vice versa       **A→B→C**

Taken from: PIEKARSKI G. (1989) Medical Parasitology; Springer – Berlin, Heidelberg, New York

## Biology of *Leishmania* and their vectors

While there is no morphological differentiation between the *Leishmania* species, molecular techniques implemented today distinguish the previously described species as the *L. donovani* complex (three species: *L. donovani*, *L. infantum* and *L. chagasi*), the *L. mexicana* complex (three main species: *L. mexicana*, *L. amazonensis* and *L. venezuelensis*), further *L. tropica*, *L. major*, *L. aethiopica* and the subgenus *Viannia* with four main species (*L. braziliensis*, *L. guyanensis*, *L. panamensis* and *L. peruviana*).

Within humans and dogs *Leishmania* multiply within a parasitophorous vacuole of macrophages as amastigotes (forms without flagella). Finally, these macrophages rupture and amastigotes enter other phagocytic cells, mainly of the reticulo-endothelial systems in liver, spleen, bone marrow and lymph nodes. Sand flies feeding on infected host ingest a blood meal with infected monocytic cells. In the midgut of the sand fly the amastigotes transform into a flagellated promastigotes, multiply and then, during the second blood feeding, they are transmitted to the next vertebrate host. Development to the infectious promastigote occurs under favorable tropical conditions within 5 – 8 days. The incubation period in dogs is several weeks to months.

Sand flies belong to the insect order Diptera, suborder Nematocera. The Old World sand flies belong to genus *Phlebotomus*, the New World ones to the genus *Lutzomyia*. Both these genera are important vectors of *Leishmania* parasites. The biology of the adult sand fly is that of a typical bloodsucking Nematocera: both sexes feed on sugar solutions but females need a blood meal to produce eggs. Larvae hatching from the eggs develop through 4 larval instars. They are terrestrial and live in soil rich in organic substrates where they also pupate. The adult females seek hosts for a blood meal in a clear circadian activity. They are nocturnal blood feeders, resting over the hot day away from sunlight in relatively cool and humid places, like cellars, stables, crevices or rodent holes. Once landed on the potential host, the dog or other mammal, they hop over the coat aiming for less hairy place; in the head region they like to bite around the muzzle, eye or mouth. Similarly to other bloodsucking insects, the sand fly saliva contains anticoagulants, vasodilatory peptides and enzymes with antiinflammatory, antihemostatic and anaesthetic properties. These components of saliva are important for transmission and serve as enhancing factors of the parasite infection. Sand flies are fast feeders; once they created a small pool of blood in epidermis of the host they rapidly fill their midgut with blood. *Leishmania* is well adapted to this feeding habit, entering the host via the proboscis with the saliva injected into the host.

## Clinical Canine leishmaniasis

Clinical features of canine leishmaniasis vary widely, while skin lesions are the most usual manifestation. The incubation period is 4 weeks to several years. The pathomechanism of the infection is a combination of chronic inflammation of skin, liver, GI-tract, kidneys, eyes and bones and a immune mediated polyarthritis, glomerulonephritis, arthritis and uveitis. In addition, dogs presented to the clinic often have concomitant infections, due to immunosuppression thus complicating the diagnosis. The skin lesions are alopecia with intense, dry desquamation, usually on the head. In most cases weight loss is common. Circulating immunocomplexes causing glomerulonephritis, proteinuria and subsequently renal failure is a common cause of death in affected animals. Besides the dogs with visible clinical signs of the disease, asymptomatic carriers are frequently reported. While in endemic areas most dogs have contact with the parasite, the prevalence of the disease is usually up to 20%. The reason why some dogs develop the disease and others are resistant is not fully understood yet. Diagnosis of canine leishmaniasis is complicated as most tests available are not sensitive or specific enough. Several treatment regimes are recommended but a once dog got ill the parasites will never been completely eliminated. Prevention of sand fly bites is therefore the most important way to stop the circulation of canine leishmaniasis.

## Human Leishmaniasis

In humans the protozoan parasite of the genus *Leishmania* causes cutaneous (CL), mucocutaneous (MCL) and visceral (VL) leishmaniasis. The WHO reported that worldwide 350 million



**Fig. 2** Sand fly female (size 2.5 mm)





humans in 88 countries are at risk and 12 million people are affected by leishmaniasis, with about 1.5 – 2.0 Million new cases of CL and 0.5 Million new cases of VL cases annually. More than 90% of the VL cases are reported from Bangladesh, Brazil, India, Nepal and Sudan. Visceral leishmaniasis, also known as ‘Kala-azar’ is caused by *L. infantum* in the Old World, *L. chagasi* in the New World, and by *L. donovani* in Africa and Asia. Coinfection in humans with immunodeficiency syndroms like AIDS is common. After incubation period (usually 2-8 months) patients develop pyrexia, wasting and hepatosplenomegaly; especially children are at risk. There is a long list of clinical findings, with fever, discomfort from an enlarged spleen, abdominal swelling, weight loss, cough and diarrhea being the most prominent ones. While untreated the mortality is about 90%. After successful recovery from VL due to *L. donovani* infection, patients may develop so-called ‘post kala azar dermal leishmaniasis’.

The mucocutaneous form (Espundia) occurs in some cases of *L. braziliensis* infection in South America. Disease is fully manifested months or years after the cutaneous sores have healed. Papules and ulcerative lesions occur on the nose, mouth and larynx and finally may destroy the whole face.

In the Old World, *L. major* and *L. tropica* are the causative pathogens of the cutaneous form (oriental sore), while in the New World this form is caused mostly by the parasites of *L. mexicana* and *L. brasiliensis* complexes. Primary skin lesions occur at the site of sand fly bite, often at unprotected body regions like arms, legs and in the face. Most common type of lesion is a chronic ulcer with an indurated margin. The majority of these lesions are self-healing in several months leaving a scar.

### Efficacy of the Imidacloprid/Permethrin combination

The objective of the blinded, negative controlled laboratory GCP-study was the evaluation of the repellent and insecticidal efficacy of a combination containing Imidacloprid 10% (w/v) and Permethrin 50% (w/v) in a spot-on formulation against the sand fly species (*Phlebotomus papatasi*) on dogs. The repellency efficacy criterion was based on the feeding rate of sand fly females in the treated dogs compared to the untreated control ones. The insecticidal efficacy criterion was based on the survival rate of sand fly females in the treated group compared to the untreated control group.

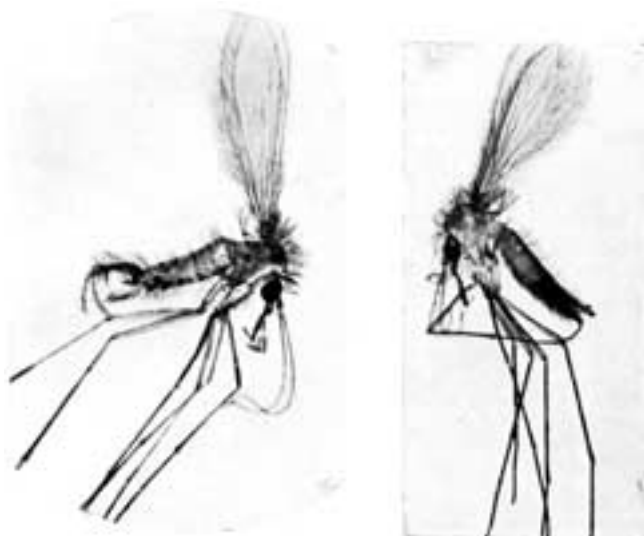
Twelve laboratory-bred beagle dogs were allocated to two groups of six dogs each. On day 0 the animals in the treated group received 0.1 ml/kg bodyweight Imidacloprid 10%/Permethrin 50% spot-on and the untreated control dogs received a placebo treatment. Dogs were sedated and exposed to laboratory-bred *Phlebotomus papatasi*. This species is not a specific vector of *Leishmania infantum* but is a very common Old World sand fly frequently biting man and dogs in Mediterranean area. The dogs

**Fig. 3** Dogs with skin lesions



were exposed to the sand flies in fine gauze cubic net-cages (1.2 m x 1.2 m square bottom and 1.8 m height) for about 1.5 hours on a weekly base, the study duration was four weeks. About 200 females plus some males were used as infestation load. The room temperature during the exposure periods ranged from 22 – 25°C, the humidity ranged from 50 – 60% rh. Dead flies were counted immediately after the exposure and 20 hours later. Then, live flies were collected, frozen and counted for survival rate determination. The feeding rate was determined by microscopical examination, female with any blood in the gut was assigned as the blood-fed.

The survival rate of the sand flies in the control group ranged between 96% and 93% during the whole study that indicates favorable climatic conditions in the experimental room. The product showed a repellent efficacy of 94.6% (day 1), 93.3% (day 8), 80.0% (day 15), 72.8% (day 22) and 55.9% (day 29). Due to the high repellent effect, the insecticidal efficacy was rather low with 60.0% (day 1), 46.2% (day 8), 42.6% (day 15), 35.2% (day 22) and 29.3% (day 29). The general and dermal tolerance of the product was very good and no adverse reactions were observed in any of the treated dogs during the study. The study clearly demonstrated the high repellent potential of the imidacloprid/permethrin combination, thus protecting dogs from sand fly bites. This may have important consequences in protection of dogs against *Leishmania* infection. ●



**Fig. 4** *Phlebotomus papatasi* male (left) and female

Taken from Mühlens P, Nauck E. & Vogel H. (1942), *Krankheiten und Hygiene der warmen Länder*. Georg Thieme, Leipzig

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## Relevant web sites

CDC: [www.cdc.gov/ncidod/dpd/parasites/leishmania](http://www.cdc.gov/ncidod/dpd/parasites/leishmania)

WHO: [www.who.int/health-topics/leishmaniasis.htm](http://www.who.int/health-topics/leishmaniasis.htm)



# Evaluation of the Efficacy of an Imidacloprid 10% / Moxidectin 1% Spot-on against *Otodectes cynotis* in Cats

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## OBJECTIVE

The aim of this study was to determine whether a single or two treatments, four weeks apart, with a novel, topically applied, formulation of Imidacloprid 10% plus Moxidectin 1%, applied at a dose of 0.1 ml/kg body mass, would be effective in the treatment of ear mite, *Otodectes cynotis*, infestations in cats.

## STUDY DESIGN AND METHODS

This study was performed in compliance with VICH GL9 “Good Clinical Practice, June 2000” at ClinVet International (Pty) Ltd, Bloemfontein, Republic of South Africa. Thirty cats naturally infested with *O. cynotis* were allocated to three groups of 10 cats each by randomisation through minimization with Day -1 body weight as the primary criterion. Group 1 served as a negative placebo treated (Days 0 and +28) control. Group 2 received a single treatment with the Imidacloprid/Moxidectin

solution on Day 0 and a placebo treatment on Day +28. Group 3 was treated with the Imidacloprid/Moxidectin solution on Days 0 and +28. Treatments were blinded. The cats were kept individually in stainless steel cages in environmentally controlled rooms. The three different treatment groups were kept under similar conditions in adjacent rooms. Eight days after the first treatment and thereafter at fortnightly intervals, the ears of the cats were examined for the presence of ear mites by using an otoscope, ear scrapes and dry cotton swabs. Assessments on Day +50 were performed on anaesthetized cats. The study schedule is summarized in Table 1. Efficacy evaluation was based on the presence of mites in the ears of cats. The success rate in each treatment group for Days +22 and +50 was calculated as follows:

$$\text{Success rate (\%)} = \frac{x}{y} \cdot \frac{100}{1}, \text{ where}$$

x = number of cats observed with no live mites

y = total number of cats in group

**Table 1** Synoptic overview of the study layout

Acclimatization	Allocation to groups	Treatment	Pre- and Post-treatment mite assessments
Day: -7	Day: -1	Day: 0; +28	Day: -3; +8; +22; +36; +50



**Figure 1** Debris in the ear of a cat infested with *Otodectes cynotis* mites (left) and the healthy ear of a cat (right) after two treatments with Imidacloprid 10% / Moxidectin 1%, applied 28 days apart at a dose of 0.1 ml/kg body mass

## RESULTS

Live mites, varying in numbers from 1 to 66 were counted in scrapings taken (Day -3) from the ears of all 30 cats included in the study. Live mites were observed in the ears of all the cats in the placebo treated group on all the assessment days. In the case of the group of cats which received a single treatment either one or two cats still harboured live mites in their ears on the different assessment days. No mites were observed in the ears of cats, which received two treatments, on any of the assessment days. The treatment success (%) for Groups 2 and 3 are summarized in Table 2.

**Table 2** Summary of the treatment success rate (%) for Groups 2 and 3 as assessed on Days +22 and +50, respectively.

Day	Group 2: Treated once (Day 0)	Group 3: Treated twice (Day 0 and +28)
+22	90	100
+50	80	100

## CONCLUSION

A single treatment with the Imidacloprid/Moxidectin solution applied at a dosage of 0.1 ml/kg body mass resulted in a treatment success rate of 80% as assessed 50 days after treatment. Two treatments with the Imidacloprid/Moxidectin solution, four weeks apart, at a dosage of 0.1 ml/kg body mass, was efficacious in curing all cats from *Otodectes cynotis* infestations as assessed 22 days after the second treatment (Day +50). ●



**Figure 2** Female mite with a visible egg



# Larvicidal and Persistent Efficacy of an Imidacloprid and Moxidectin Topical Formulation against Endoparasites in Cats and Dogs

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## INTRODUCTION

Dogs and cats are often co-infected with endo- and ectoparasites. Roundworms of the genus *Toxocara* represent the most prevalent endoparasites in dogs and cats followed by hookworms of the genera *Ancylostoma* and *Uncinaria* (Coati et al., 2003). Worldwide many surveys have shown that fleas are the dominating group of ectoparasites in dogs and cats with infection rates of up to 100% under certain circumstances (Krämer and Mencke, 2001). Recently, in European field studies up to 27% of tested cats and 17% of dogs were found to be co-infected with gastro-intestinal nematodes and fleas (Knoppe, personal communications). In cases like these the concomitant treatment using an ectoparasiticide together with an anthelmintic is appropriate. In the present study we have tested the anthelmintic efficacy of a new topical antiparasiticide which combines the insecticide imidacloprid and the macrocyclic lactone moxidectin for the simultaneous treatment and prevention of ecto- and endoparasitic infections in cats and dogs. Here, the efficacy against larval and immature stages of hook- and roundworms in cats was specifically investigated. Furthermore, the potential use of this product for the prevention of patent endoparasitic infections was demonstrated by showing the persistent efficacy against *Uncinaria stenocephala* infections in dogs.

## MATERIALS AND METHODS

The test products were clear solutions with imidacloprid 10% (w/v)/moxidectin 2.5% (w/v) for spot on treatment of dogs and imidacloprid 10% (w/v) /moxidectin 1.0% (w/v) for cats. The solution was applied once cutaneously at recommended minimum dosages of 0.1 ml/kg body weight, i.e. 10 mg imidacloprid and 2.5 mg moxidectin per kg body weight in dogs and 1 mg moxidectin per kg body weight in cats.

Four groups of eight less than five month old cats each were artificially infected with 300 infective stages of *Ancylostoma tubaeforme* and four further groups with *Toxocara cati*, respectively. One of the *A. tubaeforme* infected groups was treated at 7 and together with a placebo treated control group necropsied 12 days post infection (d.p.i.). The two remaining groups were treated at 11 and necropsy at 16 d.p.i.. The *T. cati* groups were

treated at 14 and 24 d.p.i., while necropsy was at 19 and 29 d.p.i., respectively. Parasitic stages were counted for the complete intestinal contents. Following the intestinal washings the small intestines, suspended by wire, were soaked at 37°C in 0.9% saline for two hours to allow migration of mucosal larval stages. In addition to that the mucosa of the small intestines of the *A. tubaeforme* infected cats were stripped of and processed with pepsin-digestion for detection of mucosal stages.

To assess the persistence of efficacy eight of sixteen dogs were treated with the test product at 18 days prior to experimental infection with 300 *U. stenocephala* larvae. At 21 d.p.i. all animals were necropsied and worms were counted.

## RESULTS AND DISCUSSION

For the control groups the following *A. tubaeforme* stages (geometric means) were found per test animal at 12 d.p.i. 9.1 (SD ±15.1) third stage larvae, 36.2 (SD ±23.3) fourth stage larvae and 2.8 (SD ±3.8) immature adults (Fig. 1). At 16 d.p.i. 5.0 (SD ±4.2), 45.9 (SD ±26.4) and 4.6 (SD ±7.0) third, fourth and immature adult stages, respectively, were counted per cat. Adult stages were present neither at 12 d.p.i. nor at day 16 d.p.i. in any cat. Earlier investigations proved that the prepatency period of this strain is approximately 28 days. The mean cumulative worm counts in the control groups were 58.5 and 65.3 at 12 and 16 d.p.i., respectively. No parasite stages were found in any of the moxidectin/imidacloprid treated animals.

Within the *T. cati* infected cats a mean of 5.1 (SD ±3.7) fourth stage larvae and no immature adult stages were found at 19 d.p.i., while 5.8 (SD ±7.3) and 3.5 (SD ±5.3) fourth and immature adults (Fig. 2) were present at 29 d.p.i., respectively. For this strain the prepatency period was found to be approximately 42 days. The average overall worm counts were 7.3 and 15.4 at 24 and 29 d.p.i., respectively. In the animals treated with the test product and necropsied at 19 d.p.i. no parasites were found, whereas in those necropsied at 29 d.p.i. 0.2 (SD ±0.01) and 0.3 (SD ±0.02) fourth and immature adult stages, respectively were found. All test group worm counts differed significantly ( $p < 0.05$ ) from all respective control group worm counts. These data clearly demonstrate high efficacies of the



**Figure 1** (left) Female *A. tubaeforme* L4 with a length of 2.14 mm isolated from the small intestines at 12 d.p.i..

**Figure 2** (top) Female *T. cati* immature adult with a length of 5.12 mm isolated from the small intestines at 29 d.p.i..

imidacloprid/moxidectin solution against early but also against later larval and immature adult stages of the two parasite species tested in this study (Table 1).

In contrast to our results Okoshi and Murata (1957) found far less *A. tubaeforme* L3 in cats necropsied 12 and 16 d.p.i., whereas similarly to this study the L4 stages represented the most prominent group. The recovery rates of 19.5 and 21.7 for 12 and 16 d.p.i., respectively, found in the present study are similar to those described in the earlier publication. The large standard deviations observed in all groups indicate that both strains exhibit extensive differences concerning the progress of development within individual hosts.

The untreated *U. stenocephala* infected dogs showed geometric mean immature adult and adult parasite counts of 4.6 (SD ±3.9) and 8.1 (SD ±4.3), respectively, while in the test product treated animals no worms were found. Thus the product proved to have a persistent efficacy over at least 18 days which completely prevented the development of intestinal stages for this species.

To our knowledge this is the first time that an anthelmintic product was shown to completely remove early larval stages of hookworms and ascarids in cats and furthermore to prove persistence of efficacy. Similar findings concerning the larvicidal efficacy were observed also in additional studies employing the same approach in dogs (data not shown). Furthermore, complete adulticidal efficacies against the respective adult stages of the parasite species used here were found in multiple investigations (data shown elsewhere). Most recently the potential of human infection with *T. canis* by direct contact with dogs was illustrated by Wolfe & Wright (2003). The authors found *T. canis* eggs in the hair of every fourth dog investigated. More

**Table 1** Efficacies of the test product against larval and immature adult stages of *A. tubaeforme* and *T. cati* in cats.

Species	<i>A. tubaeforme</i>			<i>T. cati</i>	
Stage	L3	L4	Immature adults	L4	Immature adults
Efficacy (%)	100	100	100	100 (19 d.p.i.) 97 (27 d.p.i.)	91

than one quarter of eggs were embryonating or already embryonated and thus a potential cause of infection. In response to this, the larvicidal and persistent efficacy of the test product can be exploited especially for the prevention of hook- and roundworms in cats and dogs. ●

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# Evaluation of K9 Advantix™ vs. Frontline Plus® Topical Treatments to Repel Brown Dog Ticks (*Rhipicephalus sanguineus*) on Dogs

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## INTRODUCTION

The ability of acaricides to repel or kill ticks before they attach to a host and feed is important for the prevention of transmission of tick born pathogens. A study design to compare the repellency/acaricidal properties of topically applied products against the castor bean tick (*Ixodes ricinus*), the primary vector of Lyme disease in Europe, following exposure to treated dogs has been previously reported.<sup>1,2</sup> A similar study design was reported to demonstrate repellency and acaricidal properties of a topical 45% w/w permethrin solution (Kiltix®, Bayer Corp. USA) used concurrently with a 9.1% w/w imidacloprid spot on solution (Advantage®, Bayer) against American dog ticks (*Dermacentor variabilis*) the primary vector of *Rickettsia rickettsii*, the causative organism of Rocky Mountain spotted fever in dogs.<sup>3</sup> The present study was conducted to compare the tick repellency/acaricidal properties of a combination of 8.8% w/w imidacloprid and 44% w/w permethrin (K9 Advantix™, Bayer Corp. USA) with the combination of 9.8% w/w fipronil and 8.8% w/w S-methoprene (Frontline Plus®, Merial) against the Brown dog tick (*Rhipicephalus sanguineus*) the vector of *Ehrlichia canis* in dogs.

## MATERIALS AND METHODS

A total of 18 healthy laboratory dogs of both genders with a body weight between 6.9 and 24.8 kg and the ability to harbor tick infestations were included in the study. The dogs had not been treated with any acaricide within 60 – 90 days prior to the study. On test day –12 all dogs were bathed with a mild, non-medicated shampoo. The dogs were experimentally infested with unfed adult brown dog ticks (*Rhipicephalus sanguineus*). This study design evaluated the transfer and attachment of ticks from the environment to dogs. Individual heavy-gauge plastic pet transport carriers were used for tick challenges. Each carrier contained a light colored nylon carpet with a nap of 12 – 15 mm to cover 50 – 60% of the floor area. For each tick challenge, 50 adult *R. sanguineus* (approximate male/female sex ratio of 1:1) were placed on the carpet. The carpet was sprayed lightly



**Figure 1** Mouthpart of a brown dog tick (*Rhipicephalus sanguineus*)

with water prior to placement with the ticks. After a 15 minute acclimation period, dogs were placed in the carriers for a 2 hour period to expose them to the live ticks. The dogs were then removed, examined visually and combed for ticks. The number of live ticks (attached and unattached) and dead ticks on the dogs were counted and removed. Live and dead ticks remaining on the carpet and in the carriers were counted. Carriers were thoroughly washed and carpets were discarded after each use.

On test day -3 each dog was exposed to ticks as described above. Pretreatment live tick counts (attached and unattached on each dog) were recorded. The dogs were then ranked by the total number of live ticks and were blocked into groups of three. Within each block the dogs were randomized to one of the three treatment groups and treated on study day 0 with the recommended dosage:

- Group 1:** 8.8% w/w imidacloprid plus 44.0% w/w permethrin (K9 Advantix™, Bayer Corp. USA).
- Group 2:** 9.8% w/w fipronil plus 8.8% w/w S-methoprene (Frontline® Plus, Merial).
- Group 3:** Control (untreated).

Tick challenge was performed on days 3, 7, 14, 21, 28 and 35 post treatment. On each of these days the dogs were placed in the pet carriers for 2 hours and exposed to 50 adult *R. sanguineus* as previously described. Following this time interval the dogs were removed. Live and dead ticks observed on the dog and ticks remaining in the carrier were counted. Percent efficacy was calculated for the two treatment groups on each evaluation day with the following formula using the geometric mean tick counts:

$$\% \text{ Efficacy} = \frac{\text{Geo Mean No. Live Ticks/Dog(Control)} - \text{Geo Mean No. Live Ticks/Dog(Treated)}}{\text{Geo Mean No. Live Ticks/Dog(Control)}} \times 100$$

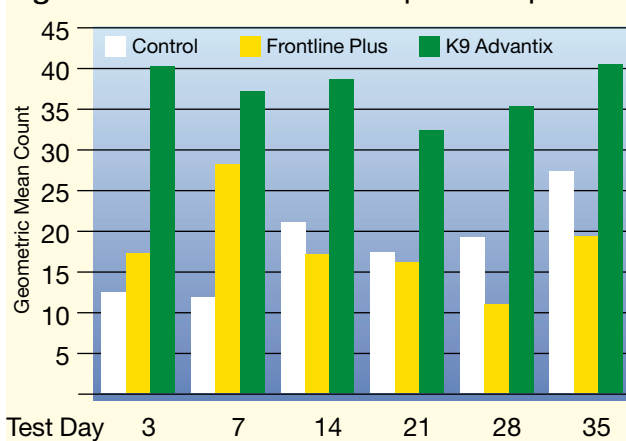
Results of this study were evaluated by descriptive and inferential statistical methods. Means, variability parameters, and range of values were calculated for tick counts. In addition, repeated measures mixed model analysis of variance was used on log (count + 1) transformed tick counts testing for treatment group differences, using contrast statements to control group comparisons.

## RESULTS

The geometric mean number of live ticks per dog (attached and unattached) and percent tick efficacy is displayed in Table 1. Pre-treatment (test day -3) live tick counts ranged from 27 – 46 (Geo mean = 35.0 ticks/ dog) following 2 hours of exposure to ticks in the pet carriers. There were significantly fewer numbers of live ticks on the K9 Advantix treated dogs than on the control dogs for all 6 post-treatment evaluations ( $P < 0.05$ ). The control dogs had a range of 12.0 – 29.5 live ticks/dog during each of the 6 post-treatment tick exposure intervals. A range of 0.4 – 3.6 live ticks/dog were recorded for the K9 Advantix treated dogs through day 35. The Frontline Plus treated dogs had a range of 12.8 – 27.4 live ticks/dog through day 35. There were no significant differences between the number of live ticks observed on the control vs. Frontline Plus treated dogs during the post-treatment tick exposure periods for days 3, 14, 21, 28 and 35. On test days 14, 21, 28 and 35 more live ticks were observed on the Frontline Plus treated dogs than on the control dogs.

The geometric mean number of repelled ticks (dead plus live ticks remaining in the carrier) are displayed in Table 2. There were significantly more ticks repelled on the K9 Advantix treated group than the control group for all post-treatment evaluations except day 35. There were no differences in the repelled ticks between the Frontline Plus treated dogs and control dogs for days 3, 14, 21 and 35. Significantly more ticks were repelled on the control dogs than the Frontline Plus treated dogs on day 28. The repellency values represent (2.3, 1.3, 2.3, 2.0, 3.2, 2.1) fold greater repellency of K9 Advantix vs Frontline Plus for test days 3, 7, 14, 21, 28 and 35, respectively.

**Fig. 2** Geometric mean number of repelled ticks per carrier



**Table 1** Geometric mean number of live *R. sanguineus* per dog and percent efficacy

Test Day	Group 1 K9 Advantix		Group 2 Frontline Plus		Group 3 Control
	$\bar{x}$ Ticks/Dog	% Efficacy	$\bar{x}$ Ticks/Dog	% Efficacy	$\bar{x}$ Ticks/Dog
-3	35.2	–	34.5	–	35.2
3	0.4*	98.5	20.5	25.9	27.6
7	1.3*	95.4	12.8*	56.8	29.5
14	1.8*	90.6	24.0	-28.1	18.8
21	3.6*	84.0	24.1	-7.8	22.4
28	2.7*	89.1	27.4	-11.4	24.6
35	1.7*	85.9	14.6	-21.9	12.0

\*Statistically different versus control group ( $P < 0.05$ )

**Table 2** Geometric mean number of repelled ticks<sup>a</sup> per dog carrier

Test Day	K9 Advantix	Frontline Plus	Control
	Geo Mean No. Ticks	Geo Mean No. Ticks	Geo Mean No. Ticks
3	40.1*	17.1	12.3
7	37.0*	28.1*	11.7
14	38.5*	17.0	21.0
21	32.3*	16.0	17.3
28	35.2*	10.9*	19.1
35	40.4	19.2	27.2

<sup>a</sup>Dead plus live ticks remaining in the carrier.

\*Statistically different versus control group ( $P < 0.05$ )





**Figure 3** Brown dog tick (*Rhipicephalus sanguineus*)

## DISCUSSION

The use of permethrin containing formulations to repel ticks, including three species of *Ixodes*, *Dermacentor variabilis*, and *Amblyomma americanum*, has been previously described<sup>1-6</sup>. The activity of imidacloprid alone against ticks is inconsistent<sup>7</sup> although recent data indicates imidacloprid is able to enhance the activity of permethrin when the two active ingredients are combined<sup>8</sup>. There is no published information on the repellent activity of fipronil against ticks. Fipronil does provide a high level of efficacy against ticks, however, data demonstrate that optimal tick control is achieved 24 – 48 hours after ticks have the opportunity to attach and feed<sup>9</sup>.

There are relatively few reports to indicate the duration of blood feeding by ticks to successfully transmit pathogens. Ticks may only require 5 – 20 hours of feeding activity to transmit *R. rickettsii* to dogs<sup>10</sup>. Human and laboratory animal studies indicate *Borrelia burgdorferii* may be transmitted 24 hours after *Ixodes* spp. attach and feed.<sup>11,12</sup> The results of the current study are consistent with the results of previous studies and demonstrate that permethrin containing products offer protection against transmission of tick born pathogens by preventing ticks from attaching and feeding. ●

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# The Effects of an Imidacloprid and Permethrin Combination against Developmental Stages of *Ixodes ricinus* Ticks



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## INTRODUCTION

Ticks and especially the worldwide distributed members of the species of the genus *Ixodes* threaten the health of man and his companion animals, since they are able to transmit several agents of diseases (e.g. *Borreliosis*, *Ehrlichiosis*, *Babesiosis*) (Lane and Crosskey 1995, Mehlhorn 2001a, 2001b; Piesman et al. 1987). Thus exposition prophylaxis is needed in order to avoid infestation with ticks and the subsequent transmission of tick-borne diseases.

The combination of 10% imidacloprid and 50% permethrin (K9 Advantix™, trade mark Bayer AG, Leverkusen, Germany) was recently registered by the EPA for the United States market. The product is indicated for the prevention and treatment of ticks, fleas and mosquitoes and is for monthly use in dogs. The repellent effect of synthetic pyrethroids, known for acaricides, was recently published for permethrin topically applied to dogs (Endris et al. 2000, 2002). In the present paper the effects of this new product were tested *in-vitro* and *in-vivo* as reported to some extent in a recent publication (Mehlhorn et al. 2003).

**Fig. 1** Unfed female of *Ixodes ricinus* questing for a host at the tip of a plant



## MATERIAL AND METHODS

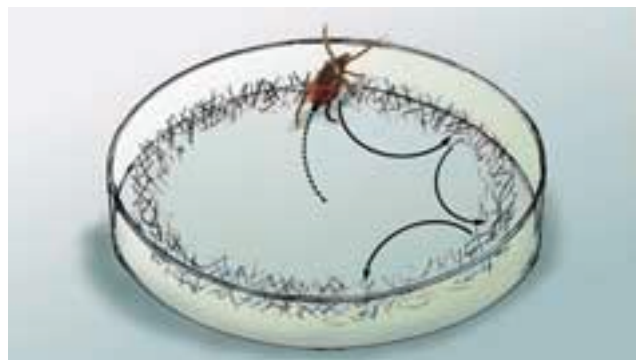
**Ticks** Specimens of *Ixodes ricinus* – the so-called castor bean tick – were collected from nature, where they lurk at the tip of plants (Fig. 1) and were propagated in the institute. Unfed (hungry) larvae, nymphs and adults were used for the experiments.

**Dogs** Four dogs were treated topically with K9 Advantix at the recommended dosage of 0.1 ml/kg body weight. This corresponds with a dosage of 10 mg imidacloprid/kg and 50 mg permethrin/kg. Four dogs remained untreated as controls. At 7, 14, 21 and 28 days after this treatment, both flanks of one of these dogs were shaved, producing a circular, hairless region of about 20 cm in diameter. The hair were collected separately and used for *in-vitro* studies (Fig. 2).

**Fig. 2** Ticks were placed into a petri dish with hair of treated and untreated dogs arranged in a circular ring



**Fig. 3** In the case of K9-Advantix-treated hair the ticks move back immediately, when approaching the hair and try to approach again (arrows)





## RESULTS

### In-vitro studies

#### Test for repellent activity

The hair taken from the treated dogs at 7, 14, 21, or 28 days post-treatment and additionally from the untreated dogs was arranged as a circular barrier on a filter paper in individual glass Petri dishes (Fig. 2). Into the centre of this circular barrier of hair, 50 larvae, 8 – 10 nymphs or adults of *Ixodes ricinus* were placed and their behaviour was followed for at least 1 h. In all cases, it was noted that the ticks immediately started to run in the direction of the hair. However, at a distance of 1 – 10 mm before reaching the hair, they stopped, raised their pair of front legs, where the Haller's organ (a chemosensitive organ) is located, moved these two legs and went backwards (Fig. 3). Then they tried to approach the hair barrier for 1-2 min at other places. Later, 2-3 ticks entered the hair, while the rest remained in the hairless centre of the Petri dish. This „hot-foot“ reaction indicates the existence of an initial repellent effect. This effect is very valuable for the protection against an infestation with ticks, since a tick in the host-seeking position has only seconds to decide whether it will attach or not to a host passing by. Control ticks, however, immediately entered the untreated hair barrier and thus found shelter there.

#### Acaricidal activity of the hair of treated dogs

Hair samples from dogs treated 7, 14, 21, or 28 days previously with K9-Advantix were mixed with larvae, nymphs and adults of *I. ricinus* ticks in Petri dishes. While larvae and nymphs died within 48 h (very often within 24 h), the adult *Ixodes* ticks survived for a longer period, although completely paralysed. *Ixodes* adults (Fig. 4) became paralysed within 3 – 24 h, showing only slow movements of the legs without true body-motion and never recovered.

### In-vivo studies

Individual adult *Ixodes ricinus* ticks were put by means of tweezers onto the hair of treated and untreated dogs. In the case of treated dogs, the ticks dropped from the hair immediately after being put in position. On untreated control dogs, the ticks moved over the hair to reach the skin for attachment within a few seconds.

On days 7, 14, 21 and 28 after treatment of the dogs, Petri dishes containing ten freshly caught adult female *I. ricinus* ticks were fixed on the shaved lateral thorax of the dogs. The behaviour of the ticks was observed for 15 min and the Petri dishes were then covered by blackout/ bandaging material. Ticks were allowed to attach on the shaved skin area for 1 h in the dark. After this period, the Petri dishes were removed and the position of the ticks was noted. All ticks were collected in another Petri dish, transferred into an incubator and observed after 3, 24 and 48 h by means of a stereo microscope.

When considering the situation after placing the ticks onto the shaved skin, it was seen that the ticks tried to avoid contact with the skin and assembled along the border or the inner lid of the



**Fig. 4** Light micrographs of *Ixodes* ticks, which had contact to untreated hair (a) or treated hair (b). In the first case the ticks crawled around, while in the second case ticks became paralysed and later died

plastic Petri dish. However, after 3 – 5 min, some ticks started to crawl around on the shaved skin. After 1 h, when removing the blackout/ bandaging material, it was noted that in both groups (treated or non-treated dogs) only a few ticks (mainly two or three out of ten ticks) had attached on the skin, while the rest was still unattached. The results were independent of the length of the period after treatment with the test product (i.e. the number of attached ticks was similar on the dogs at 7, 14, 21, or 28 days after the compound was applied to the hair). However, all *Ixodes* ticks were alive and motile after this first period.

After 3 h, all *Ixodes* ticks in the Petri dishes collected from the treated dogs were found lying on their backs and showing only slight, paralytic movements of all eight legs. This finding was identical on the ticks deriving from dogs being treated 7, 14, 21, or 28 days previously. These ticks never recovered from this paralysis and died mostly within 24– 48 h after first exposure to the skin, even when deriving from dog treated 28 days previously. Thus, it can be concluded that a contact of only 1 h to the shaved skin of an imidacloprid/permethrin-treated dog is sufficient to kill *Ixodes* ticks.

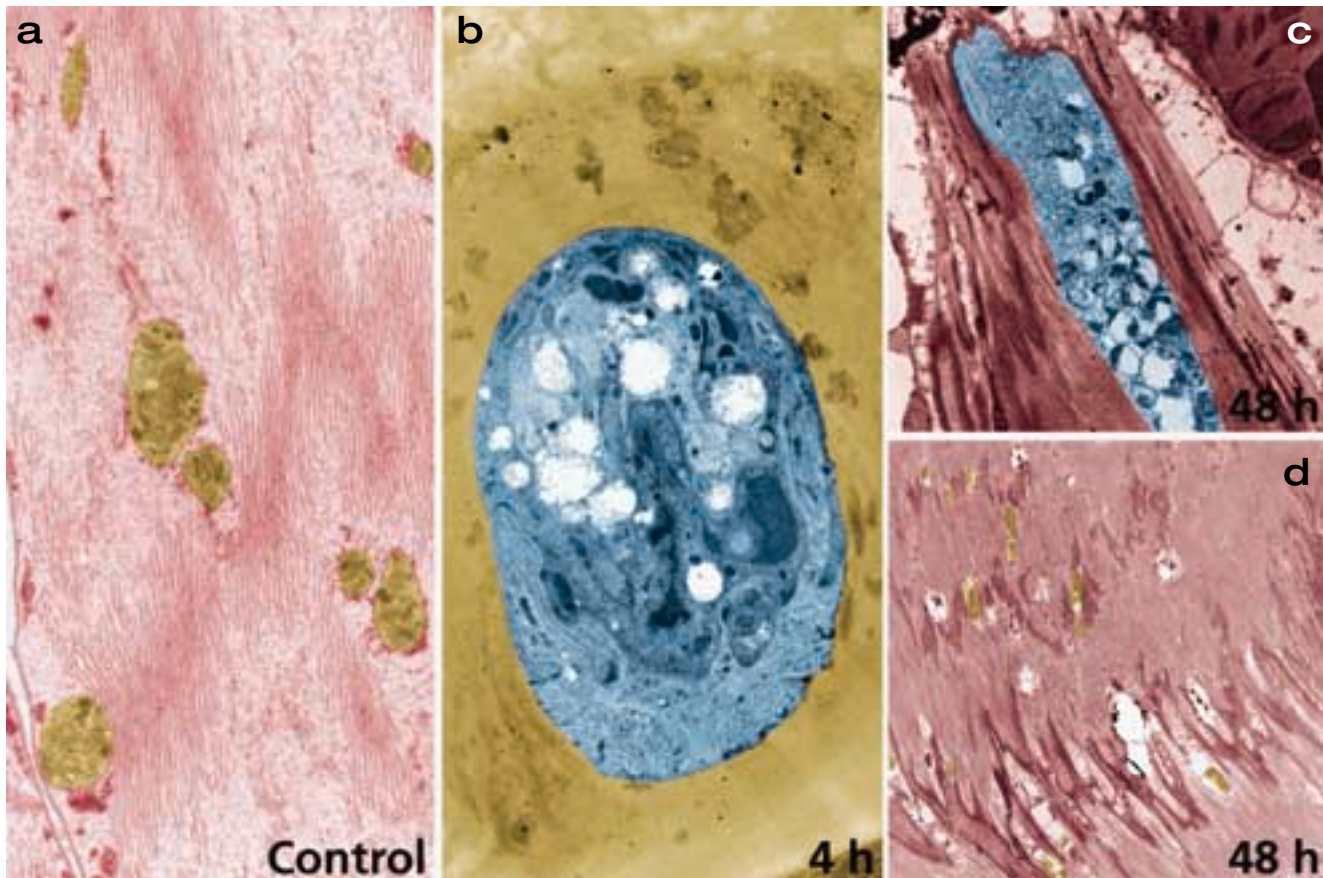
#### Electron microscopic studies

When examining (by means of electron microscopy) the tissues of *Ixodes* ticks having had contact with K9-Advantix® treated hair or not (controls), it was seen that damages were found at the level of muscle and nerve cells. The grade of damages increased with the time of exposure leading to a final complete morphological and functional destruction (Fig. 5).

## CONCLUSIONS

Our in-vivo and in-vitro experiments offered some significant results:

1. The new combination of 10% imidacloprid and 50% permethrin, applied topically to dogs, resulted in a significant repellent effect, lasting for at least 4 weeks against *Ixodes ricinus* ticks.



**Fig. 5** Transmission electron micrographs of tissues of ticks that had been exposed to K9-Advantix or remained untreated.  
**a)** Control. The muscle fibres and the mitochondria (yellow) are unchanged.  
**b)** Cross section through a nerve (=peripheral sensillum) after 4 h of contact with K9-Advantix. Note the initiation of a vacuolization.  
**c)** Longitudinal section through a nerve of a tick after 48 h of exposure to K9- Advantix. Note the intense degeneration.  
**d)** Section through a muscle fibre after an exposition of 48 h. Note the contraction of the fibre and the destruction of mitochondria.

2. For dogs resting at tick-contaminated places (thus offering the ticks time for delayed entry into the coat), the acaricidal activity of the new product considerably limits the attachment of ticks. This efficacy is independent from life cycle stages of the *I. ricinus* tick, the known vector of pathogens that cause borreliosis, ehrlichiosis and babesiosis.

3. Transmission of e.g. *Borellia burgdorferi* by *Ixodes* spp. ticks is dramatically increased by the duration of tick-feeding on the host (Piesman et al. 1987). The repellency and acaricidal effect after 1 h of exposure reported here is expected to be sufficient to prevent transmission of tick-borne diseases.

4. The electron microscopical analysis of nerve and muscles of ticks having had contact to treated hair shows significant, irreversible damages.

5. Together with the fast onset of efficacy against fleas published for imidacloprid (Mehlhorn et al. 2001), the new combination now offers a high level of protection for at least 28 days against fleas and ticks, thus limiting their activities as vectors of diseases. ●

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# Efficacy of the Compound Preparation Imidacloprid 10% (w/v) / Permethrin 50% (w/v) Spot-on against Ticks (*I. ricinus*, *R. sanguineus*) and Fleas (*C. felis*) on Dogs

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## STUDY OBJECTIVES AND RATIONALE

These studies were designed to confirm the acaricidal efficacy of the compound preparation Imidacloprid 10% (w/v) / Permethrin 50% (w/v) spot-on.

Dogs, artificially infested with Brown Dog Ticks (*Rhipicephalus sanguineus*) and Castor Bean Ticks (*Ixodes ricinus*), and, in another study set infested with Cat Fleas (*Ctenocephalides felis*), were used as test system.

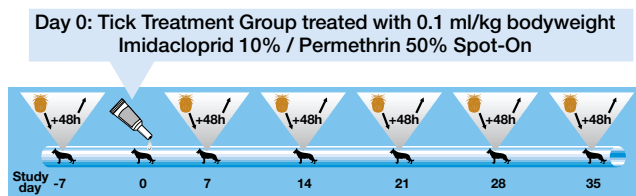
The acaricidal efficacy against the two tick species and the flea species following a single topical treatment was assessed by

repeated tick and flea infestations and subsequent tick and flea counts over a period of five weeks.

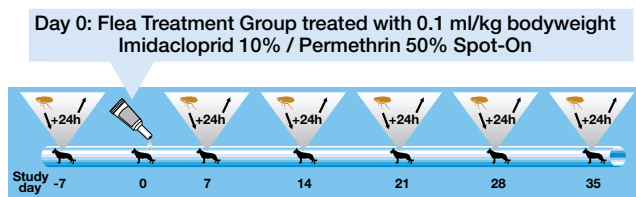
These studies were conducted in accordance with the Principles of the VICH Guideline on Good Clinical Practices (GCPV) and the recommendations of the Guidelines for the Testing and Evaluation of the Efficacy of Antiparasitic Substances for the Treatment and Prevention of Tick and Flea Infestation in Dog and Cats (CVMP).

The test facility was inspected and certified as working in compliance with the Principles of GCPV. All procedures concerning animal husbandry and maintenance, hygienic measurements, randomisation, scalings, handling of test substances, retain samples, archiving and quality assurance are written down in current SOPs.

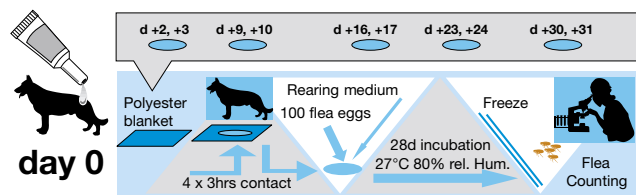
### 1 STUDY DESIGN Tick Infestation and Counting



### 2 STUDY DESIGN Flea Infestation and Counting



### 3 STUDY DESIGN Blanket Incubation Test



## STUDY DESIGN

This blinded, negative controlled clinical laboratory GCP-study was designed with two parallel groups for each study set with tick infestation and flea infestation. After passing the study inclusion examination for each set 20 dogs were randomly allocated to two study groups of 10 animals per group.

Group 1 of each set was treated with the investigational product in a dosage of 0.1 ml/kg, the other group remained untreated. Dogs of both sets were reinfested weekly with 50 adult *Rhipicephalus* and 50 adult *Ixodes* ticks (sex ratio: 1:1) or 100 adult *Ctenocephalides felis* fleas, respectively, for a period of five weeks. Efficacy against two tick species (*I. ricinus*/*R. sanguineus*) was tested 48 hours after regularly reinfestation on a weekly basis. (Fig. 1)

Efficacy against adult fleas (*C. felis*) was tested 24 hours after treatment or regularly reinfestation on a weekly basis. (Fig. 2)

Efficacy against juvenile fleas (larvae of *C. felis*) was tested with a blanket incubation test on a weekly basis. (Fig. 3)

Tolerance was tested as second criterion also on a weekly basis.

## MATERIAL AND METHODS

### Ticks

1. Brown Dog Ticks (*Rhipicephalus sanguineus*) originated partly of the laboratory strain in Monheim, reared on rabbits and

partly of the laboratory strain of EL Labs Soquville in California/USA (ratio 50:50 Monheim: USA strain). The ticks for infestation were unengorged adult males and females (ratio 1:1) that have moulted at least 14 days before to the adult stage.

2. Castor Bean Ticks (*Ixodes ricinus*) originated from the Charité, Berlin. The ticks for infestation were unengorged males and females (ratio 1:1) in which the transmission of the spermatophore had already occurred.

## Fleas

Cat fleas (*Ctenocephalides felis felis*) of the laboratory strain in Hanover, reared on cats, were used as test parasites. The adult unfed fleas were held in polyvinyl vials at  $\approx 27^{\circ}\text{C}$  /  $\approx 80\%$  relative humidity until they were used for infestation (maximum for 12 days).

Viable flea eggs from the routine breeding of this flea strain were used as test parasites for the blanket larvicidal test. The flea eggs were at an age of max. 48 hours.

## Tick Infestation procedure

After sedation dogs of study set 1 were placed in individual transport boxes. The ticks were released onto the back of the dogs and were allowed to disperse and move into the hair without disturbance. Dogs were released from the transport boxes after approx. 30 minutes.

## Flea Infestation procedure

All dogs of study set 2 were artificially infested with about 100 unfed cat fleas by pouring the fleas together with cocoon material out of the vials onto the dog's coat.

## Tick Counting Procedures

The following examination procedure was followed: Protective clothing and disposable hand gloves were worn during the clinical examinations; gloves were changed between each dog. The dog was placed on a single-use paper pad covered table and was identified by ear tattooing.

All ticks were counted. Total body surface of the dog was examined by thumb counting, parting the hair with the fingers and removing the ticks with a forceps (excepting day 0 counts where the ticks were left in situ). The following regions were examined: head, ears, neck, lateral areas, dorsal strip from shoulder blades to base of tail, tail and anal area, fore legs and shoulders, hind legs and abdominal area from chest to inside hind legs. Removed ticks were placed into individually marked vials and differentiated and counted in the laboratory. There was no time dictated for the counting procedure.

## Flea Counting:

The following examination procedure was followed: Total body surface was combed with a flea comb in the following



**Figure 1** Cat fleas (above); Castor bean ticks in all stages (below)

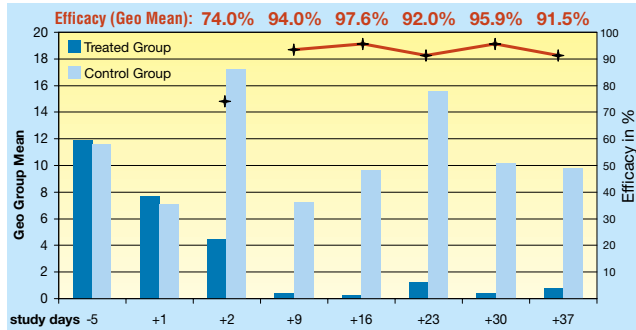


sequence: Head, ears, lateral areas, dorsal strip from shoulder blades to base of tail, tail and anal area, fore legs and shoulders, hind legs abdominal area from chest to inside hind legs, neck (application site). Dogs were combed until fleas were no longer found but for a minimum of 5 minutes. Live fleas were collected, removed and counted. Total counts of fleas were recorded.

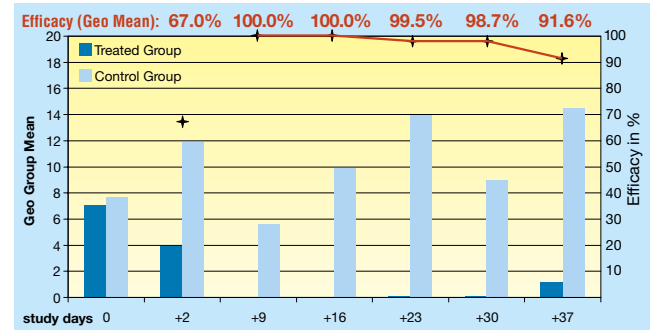
## Blanket Incubation Test

For assessment of the larvicidal properties of the test formulation in the surroundings of the dogs during the weeks after treatment, the dogs were placed on blankets for twelve hours a week divided into four contact intervals of 3 hours each. Blankets were exchanged weekly. One circular sample was cut from the middle part of each blanket after each study week, placed into individually marked plastic dishes and frozen at about  $-18^{\circ}\text{C}$  for 24 hours to kill possible living fleas, larvae or eggs on the samples. For the incubation test approximately 50 flea eggs (originated from the same flea strain which was used for the adulticidal tests) were placed together with flea rearing medium on each fleece sample and were incubated at  $27^{\circ}\text{C}$  and 80% rel. hum. for four weeks. The number of the developing fleas was counted on day 28 after start of incubation. >>

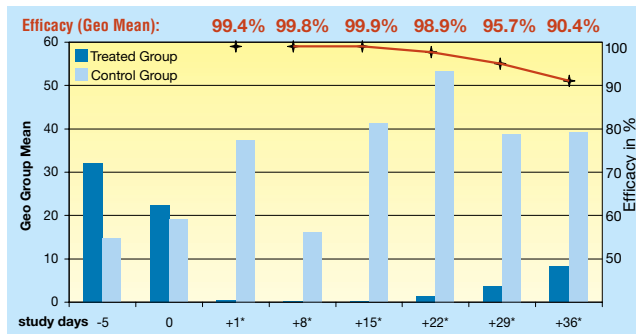
**Tab 1 Efficacy against Ticks (*Rhipicephalus sanguineus*)**



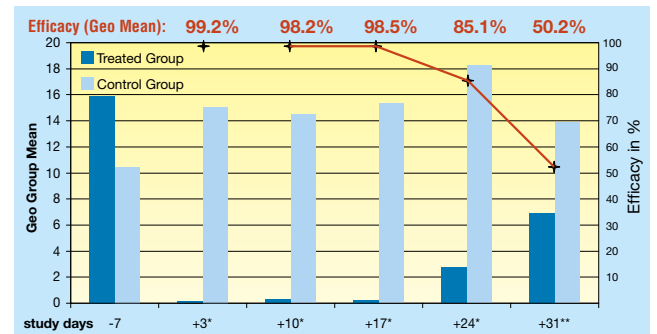
**Tab 2 Efficacy against Ticks (*Ixodes ricinus*)**



**Tab 3 Adulticidal Efficacy against Fleas (*Ctenophalides felis*) – Flea Counts**



**Tab 4 Larvicidal Efficacy against Fleas (*Ctenophalides felis*) – Blanket Incubation**



## RESULTS

Efficacy was calculated comparing the tick counts in the treated group to the tick counts in the untreated group based on the geometric means as recommended in the guidelines.

The product's curative efficacy against *Rhipicephalus sanguineus* was 74.0% (day 2); the preventive efficacy was 94.0% (day 9), 97.6% (day 16), 92.0% (day 23), 95.9% (day 30) and 91.5% (day 37) (Tab. 1).

The product's curative efficacy against *Ixodes ricinus* was 67.0% (day 2); the preventive efficacy was 100.0% (day 9), 100.0% (day 16), 99.5% (day 23), 98.7% (day 30) and 91.6% (day 37) (Tab. 2). The curative efficacy of the treatment against fleas was 99.4% (d+1); the preventive efficacy was 99.8% (day 8), 99.9% (day 15), 98.8% (day 22), 95.7% (day 29) and 90.4% (day 36) (Tab. 3).

The larvicidal efficacy on the blankets after 12 hours dog contact was 99.2% (d+3); 98.2% (day 10), 98.5% (day 18), 85.1% (day 24) and 50.2% (day 31) (Tab. 4).

The general and dermal tolerance of the product was very well and no adverse reactions were observed in any of the treated dogs during the study.

## CONCLUSIONS

### 1) Efficacy against ticks

The investigational veterinary product had a curative efficacy against ticks of 74.9% in case of *Rhipicephalus sanguineus* and 67.0% in case of *Ixodes ricinus*.

The preventive efficacy was clearly above 90% for a period of

five weeks for both tick species. Preventive efficacy values were between 97.6% and 91.5% in case of *Rhipicephalus sanguineus* and 100% and 91.6% in case of *Ixodes ricinus*.

Therefore the product proved to be effective against these tick species for a period of five weeks.

### 2) Efficacy against fleas

#### Adulticidal Flea Efficacy

The investigational veterinary product had an adulticidal curative efficacy against fleas on animals of 99.4% within 24 hours after treatment.

The preventive efficacy was above 95% for a period of four weeks. Preventive efficacy values were between 99.9% and 95.8%.

Therefore the product proved to be effective against fleas for a period of four weeks.

#### Larvicidal Flea Efficacy

The investigational veterinary product had a remarkable larvicidal efficacy against fleas on blankets that had been in contact for 12 hours to a treated animal.

The efficacy values that were achieved were above 85% for a period of four weeks and ranged between 99.2% and 85.1%.

Therefore the product proved to have a high protective effect against larval development in the animal's direct surroundings throughout.

### 3) Tolerance

Imidacloprid 10% / Permethrin 50% spot-on was well tolerated by all ten dogs of the investigational veterinary product group concerning the general and dermal tolerance. ●

# European Multicenter Field Trial on the Efficacy and Safety of a Topical Formulation of Imidacloprid and Permethrin (Advantix™) in Dogs naturally infested with Ticks and/or Fleas



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## INTRODUCTION

The efficacy and safety of the combination of Imidacloprid and Permethrin spot-on was assessed in dogs naturally infested with ticks and/or fleas in comparison to the marketed control product Frontline® spot-on containing Fipronil as a positive control. The study was conducted as a multicenter, multiregional, controlled, randomised and blinded non-inferiority clinical field study according to VICH GL 9 (Good Clinical Practice) and Directive 2001/82/EC.

## MATERIAL AND METHODS

Twelve veterinary clinics in three different areas (North-East, East and South) of Germany, nine clinics in three areas (Central, West Coast and South) of France and two clinics in two different areas (Central and South) of Italy enrolled patients to the study (Fig. 1). A total of 363 dogs showing tick and/or flea infestations were randomly allocated to one of the two treatments in a ratio of 2:1 for the investigational veterinary product and the control product. 229 dogs were treated with 10% (w/v) Imidacloprid / 50% (w/v) Permethrin (group G1) and 134 dogs were treated with 10% (w/v) Fipronil (Frontline®, group G2). Treatments were administered once on day 0 according to body weight using prefilled applicator tubes. The randomisation was done separately for animals hosting ticks on test day 0 and households hosting fleas. Personnel were blinded regarding treatments.

In case that additional dogs and/or cats were sharing the same household with flea infested study dogs, these supplementary animals were also treated with the same product as the primary patient of the household (dogs with either Imidacloprid/Permethrin or Frontline® for dogs;

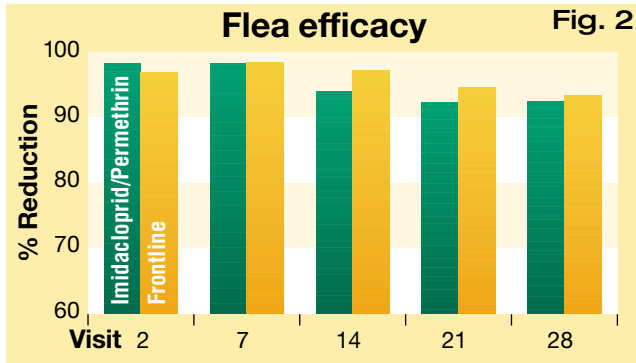
cats with marketed products Advantage® (10% (w/v) Imidacloprid) for cats or Frontline® for cats).

Clinical examinations and parasite counts were performed by the examining veterinarian. The animal owners returned the dogs for re-check examination on day 2, day 7 ( $\pm 2$ ), day 14 ( $\pm 2$ ), day 21 ( $\pm 2$ ) and on day 28 ( $\pm 2$ ) for study completion. Parasites were counted on each visit and collected for species identification at the central laboratory.

Dogs with concurrent tick and flea infestation on day 0 were randomised as flea households when  $\geq 5$  fleas were found on these dogs. All dogs showing  $\geq 1$  viable tick on day 0 were included in the 'tick efficacy population' (n=170). From each flea household, the dog with the highest flea count on day 0 was defined the main patient and included into the 'flea efficacy population' (n=108). Dogs with protocol violations were excluded from both populations. >>

**Figure 1** Locations of the investigational sites within Europe





For all tick and flea counts, geometric means were calculated and used to determine the percentage reduction of tick and flea counts within each treatment group. Day 2, day 7, day 21 and day 28 were compared to baseline, day 0. The hypothesis stated that the efficacy of Imidacloprid/Permethrin was on an average more than 90% over the treatment period. The Mann-Whitney test was used to show non-inferiority.

## RESULTS AND DISCUSSION

For both, tick and flea efficacy population, greater than 90% efficacy was achieved for Imidacloprid/Permethrin. Non-inferiority was shown for the investigational product for both efficacy populations (lower 97.5% confidence bound > 0.29).

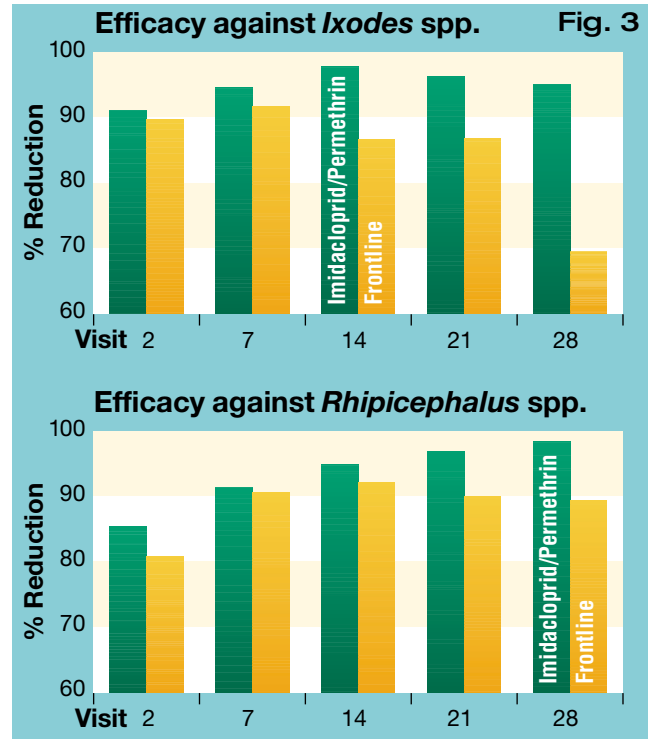
Individual efficacy values for flea infestation are shown in Fig. 2. Imidacloprid/Permethrin values were significantly non inferior to the positive control.

Individual efficacy values for tick infestations are shown in Fig. 3.

Ixodes efficacy was highly significantly ( $p = 0.0062$ ) different from positive control group at day 28 and the Rhipicephalus efficacy was near to significantly ( $p = 0.0508$ ) different from the positive control at day 28.

All 363 study dogs (229 dogs treated with Imidacloprid/Permethrin, 134 dogs treated with Fipronil (Frontline®) represented the 'safety population'. No suspected adverse drug reaction was reported for Frontline® (0%) whereas one suspected adverse drug reaction was reported for Imidacloprid/Permethrin (0.4%) (local reaction on one out of four application sites). Sixty-four cats were living in households with Imidacloprid/Permethrin treated study dogs. None of these cats showed any adverse event as reported by the owners.

Sixty-five of the treated dogs were observed to have concurrent tick and flea infestation on day 0. The prevalence of mixed infestations was calculated based on all study dogs (15.4%) and based on the households (14.2%). There was a permanent environmental tick and flea infestation pressure during the study period based on the presentation of tick and flea infested dogs in 15 of the 23 veterinary centres.



## CONCLUSION

The combination of Imidacloprid and Permethrin spot-on was efficacious and safe in the treatment of flea and tick infested dogs for a period of 28 days. The product was also safe in cats sharing households with Imidacloprid/Permethrin-treated dogs. Non-inferiority was shown for the investigational product compared to Frontline®.

Concurrent tick and flea infestation occurred regularly in the participating veterinary clinics from April to October 2002. This combination of two substances, one effective against fleas, the other effective against ticks, is justified based on this data.

## Progress of the International Work of the “Imidacloprid Flea Susceptibility Monitoring Team”



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 RUST M.K.<sup>5</sup> & VAUGHN M.B.<sup>8</sup>

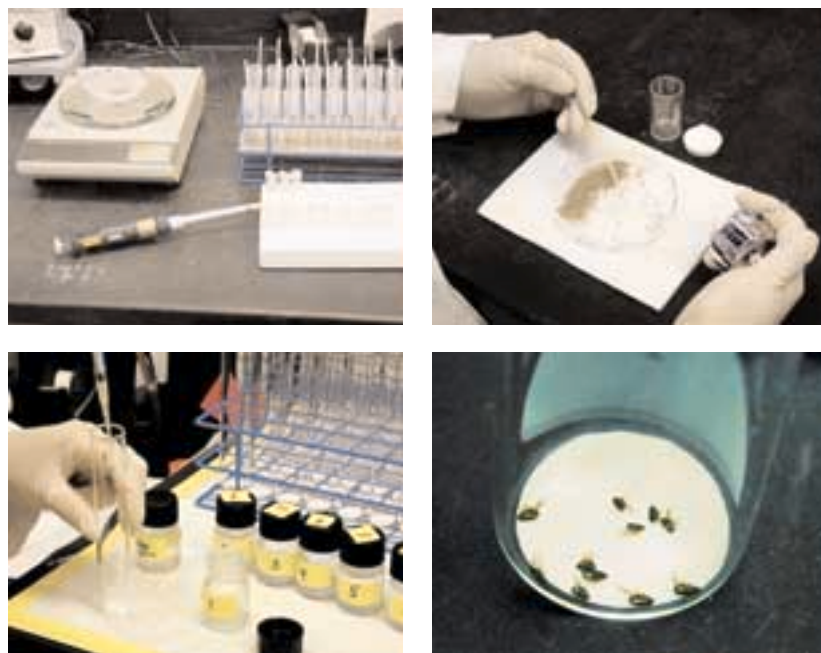
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The cat flea, *Ctenocephalides felis felis* Bouché (Siphonaptera: Pulicidae) is the most important ectoparasite of domestic cats and dogs worldwide, and is responsible for flea allergy dermatitis and for transmission of infectious agents<sup>1,2</sup>. The introduction of highly effective insecticides for on-animal treatment has eliminated established flea infestations and safeguarded pets for weeks from new infestations, even in the most difficult climatic conditions. One of those effective insecticides is the neonicotinoid imidacloprid (Advantage®) which was introduced to the Animal Health market in 1996 as the first neonicotinoid (chloronicotinyl)<sup>3</sup> and has since become one of the most successful and largest selling veterinary products for flea control.

As a consequence of extensive exposure to insecticides, *C. felis* has developed resistance to a wide range of compounds, such as pyrethroids, organophosphates, carbamates and fipronil<sup>4,5</sup>. Resistance is defined by the WHO as ‘development of an ability in a strain of some organisms to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species’<sup>6</sup>. Besides the above mentioned reports on resistance, different resistance ratios have been published<sup>7</sup>. The prevalence of resistant genes within a cat flea population, and their clinical relevance, has been the subject of much speculation and discussion in numerous publications with often controversial conclusions. We must be ever conscious that the potential exists for selection for reduced susceptibility of any flea product against cat fleas<sup>8</sup>.

In 1999, an international team of veterinarians, parasitologists, and entomologists, supported by Bayer Animal Health, was formed to acquire fundamental scientific knowledge by surveying imidacloprid susceptibility among cat fleas collected from the field. A larval bioassay was first developed to determine the baseline susceptibility of established laboratory flea strains to imidacloprid<sup>9,10</sup>. This basic research was necessary since laboratory methods used previously to report resistance to flea isolates lacked comparability. This baseline work was followed by the evaluation of dose-response, over a range of imidacloprid concentrations (0.005 ppm – 3 mg litre<sup>-1</sup>), using 17 cat flea field isolates selected and collected from a large geographic area. The LD<sub>50</sub>-values of these field isolates (0.06 – 1.51 mg litre<sup>-1</sup>) differed little from those of the laboratory strains (0.07 – 0.77 mg litre<sup>-1</sup>).

Since the objective of the project is to monitor large numbers of cat flea field isolates, the bioassay had to be slightly modified. A single discriminating dose of 3 mg imidacloprid



**Fig. 1** Development of a larval bioassay. Serial dilutions of imidacloprid in the flea media.

**Fig. 2** Development of a larval bioassay. Counts of flea pupae in the media.

**Fig. 3** Serial dilutions of imidacloprid for an adult *in-vitro* contact test.

**Fig. 4** Dead fleas exposure to the active imidacloprid on filter paper



**Fig. 5** Head of a cat flea (*Ctenocephalides felis felis*)

litre<sup>-1</sup> in the flea media was chosen as the standard assay concentration. To determine the susceptibility of a field isolate, a minimum of 40 viable flea eggs are necessary. Imidacloprid dissolved in acetone is accurately mixed with larval flea rearing media and the acetone allowed to evaporate totally before the media is transferred into glass petri dishes. Twenty viable cat flea eggs are then exposed to the treated media, twenty to an acetone control. Petri dishes are covered and incubated at 26 – 28°C, 75 – 80% RH for 28 days. Petri dishes are examined after 5 days and again at 11 – 14 days for hatching of larvae and/or pupae development. After 28 days, live adult fleas are counted in both control and treated plates and recorded. If survivorship occurs in the treated plates, the fleas reared in the control media are placed on laboratory cats for breeding. Rearing on laboratory cats is necessary to increase numbers for follow up assays and comparisons to existing laboratory strains. If survivorship in the 3 ppm assay is confirmed, the LD<sub>50</sub> values of this isolate and the laboratory strains will be determined by a dose-response study in the range of 0.005 to 3 mg litre<sup>-1</sup> imidacloprid. By comparison of these LD<sub>50</sub> values it can be estimated whether a shift in susceptibility to imidacloprid has occurred. In either case the flea isolate will be researched extensively.

Currently an *in-vivo* test which will evaluate the on-host efficacy of Advantage® is being established. Genetically established resistance mechanisms in insects consist of enhanced degradation of insecticides through increased rate of metabolic detoxification are well described. In cooperation with Bayer Crop Science numerous experiments have been made to characterize metabolic resistance in fleas. Further on molecular change in the target sites of insecticides are intensively examined at Biotechnology and Biological Sciences Research Council (BBSRC), Rothamsted, UK to establish a molecular screening assay. The advantage of this assay is the need of only a single flea to determine a possible mutation in the target site whereas enzymatic assays can only be conducted with a large number of individuals. A widespread occurrence of mutations conferring resistance to pyrethroids and cyclodiene, and a significant increase of production of enzymes responsible for resistance to

organophosphates and carbamates are well known for fleas. However, no resistance could be detected against the neonicotinoid imidacloprid in cat fleas.

In 2001 and 2002, more than 190 separate egg collections from individual flea isolates from USA, UK and Germany were tested in the laboratory with a discriminating dose of 3 mg litre<sup>-1</sup>. None of the isolates revealed reduced susceptibility to imidacloprid<sup>8,11</sup>.

By the establishment of a combination of the approved bioassay and the currently evaluated metabolic and molecular assays the cause of any apparently susceptibility reduction may be characterized in due time. ●

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# Flea Allergy Dermatitis in Cats: Establishment of a Functional *In Vitro* Test

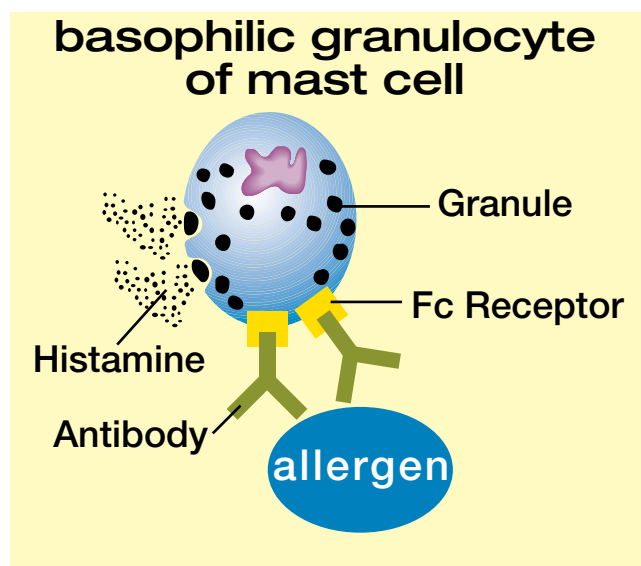


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The cat flea, *Ctenocephalides felis felis* (Order: Siphonaptera) is an almost ubiquitous nuisance to cats and dogs. Fleas feed on the blood of their host by puncturing small blood vessels. Blood is sucked up into the food channel and periodically saliva is emitted through a small salivary canal. In some individuals exposure to fleas leads to the condition of flea allergy dermatitis (FAD). It is assumed that type I hypersensitive reactions to antigenic components contained in the saliva of fleas play a major role in FAD (Arlian, 2002, Ribeiro, 1987). The diagnosis of feline FAD is frequently made by the presence of the classical triad of pruritus, fleas and “typical distribution” of clinical signs. Unfortunately clinical signs can mimic almost any pruritic dermatosis and evidence of fleas is not always present. Intracutaneous testing with an aqueous flea extract may be used to support a presumptive diagnosis of FAD. However, generally it can be seen that the cat appears to react to intracutaneous tests with poorly defined wheals and very little erythema compared to dogs (Foster and O’Dair,

1993). Yet *in vitro* methods are comparatively attractive, as there is no allergen boosting of patients. Moreover they can be tested despite of severe skin disease, and sedation is usually not required. Furthermore, serological testing by determination of allergen specific free serum antibodies including IgE has very little clinical relevance. Therefore, we are attempting to develop a more reliable and for the patient less invasive allergy test in the cat: The functional *in vitro* test (FIT) (Kaul, 1998) is monitoring exclusively those antibodies sensitizing basophiles and mastcells known as the prime initiators of type I allergies. By means of their Fc-receptors they accumulate antibodies of selected isotypes on their surface. Depending on their specificity these antibodies may bind the “fitting” antigens as bridging “allergens” causing the release of various mediators and, thus, the induction of type I allergy reactions. Histamine is one of these mediators and the only one being stored in considerable amounts in basophils and mastcells exclusively (Fig. 1).



**Fig. 1** Principle of triggering histamine release from sensitized basophilic granulocytes or mast cells by allergens

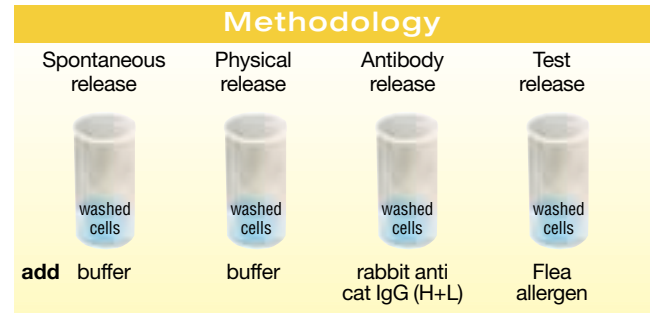
## MATERIAL AND METHODS

Samples were taken from clinically healthy laboratory cats (*Felis domestica*) with no history of skin disease and not infested with fleas for at least 12 months and from individuals that have been continuously exposed to *Ct. felis* for the last 12 months. The FIT was performed with 2,2 ml blood collected in EDTA tubes. Cats were bled by puncturing the *Vena cephalica antebrachii* of the right or left foreleg without anesthesia. Histamine release of feline peripheral blood basophils was measured upon application of the following treatments: Spontaneous release of histamine was obtained by incubating 200  $\mu$ l washed, plasma free blood cells 1:2,5 diluted in releasing buffer (Pipes B) at 37°C for 60 minutes. Physical histamine release was obtained by boiling 200  $\mu$ l washed, plasma free blood cells 1:5 diluted in Pipes B for 10 minutes in a water bath. Antibody mediated release was obtained by cross linking membrane bound antibodies with a polyclonal rabbit antiserum against cat immunoglobulin G and light chains (RaC IgG [H+L]). 200  $\mu$ l washed, plasma free blood cells 1:2,5 diluted in Pipes B containing antiserum at final concentrations of 100 and 30  $\mu$ g/ml were incubated at 37°C for 60 minutes. The actual

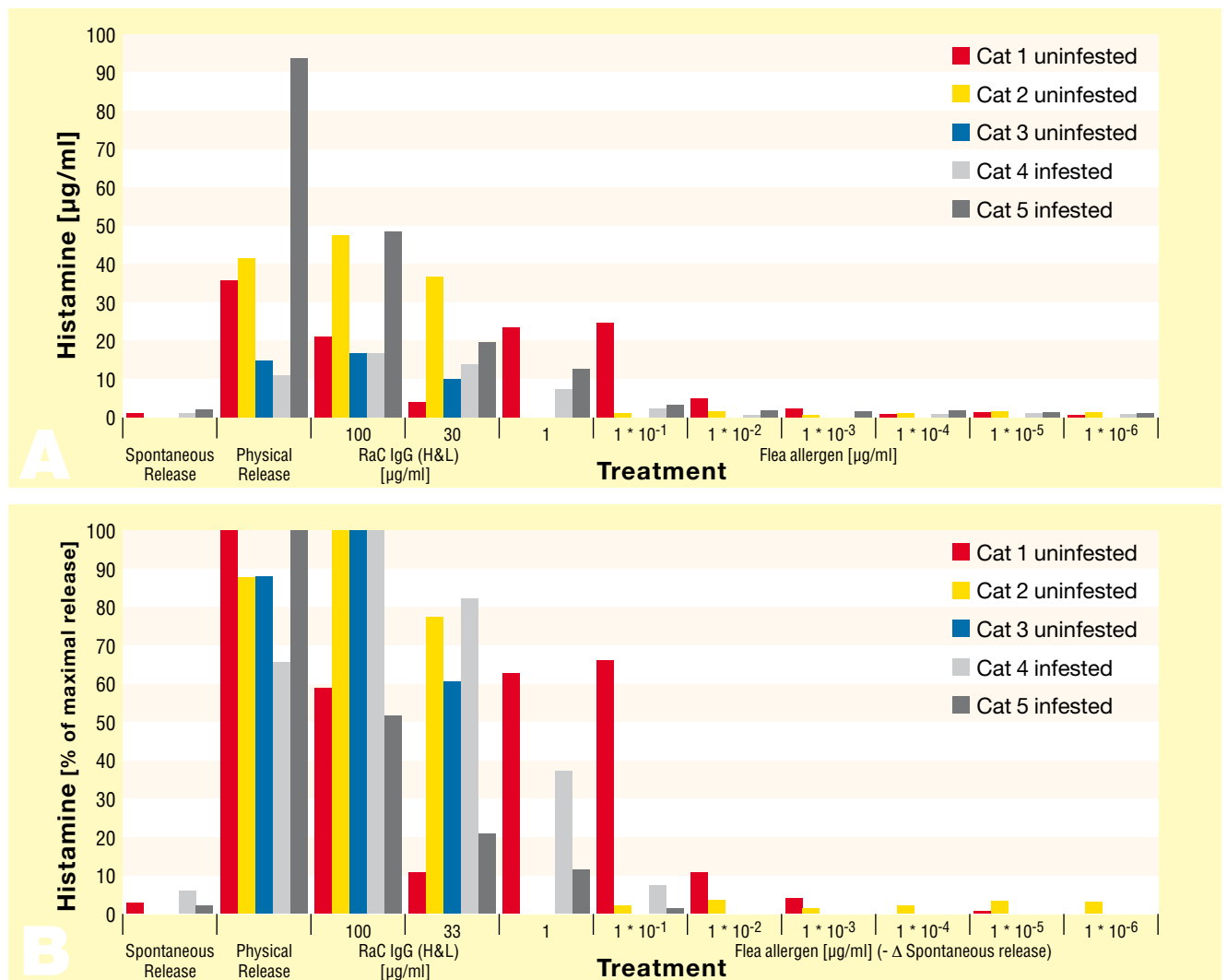


## Flea Allergy Dermatitis in Cats: Establishment of a Functional *In Vitro* Test

testing for allergen specific reactivity was monitored by bridging of a sufficient amount of allergen binding antibodies which were bound *in vivo* on the cell surface of basophils. For this approach a commercially available aqueous whole flea extract of *Ct. felis/canis* (Greer Laboratories Inc., Lenoir, USA) was used. 200µl washed, plasma free blood cells 1:2,5 diluted in Pipes B containing flea extract at final concentrations between 1 and  $1 \times 10^{-6}$  µg/ml were incubated at 37°C for 60 minutes. Before application the aqueous flea extract was separated from preservatives and reagents by gel filtration technique using a Sephadex G PD 10 column. After centrifugation of samples the histamine containing cell free supernatant was collected. The amount of released histamine was quantified by a radioimmunoassay (RIA) (Fig. 2).



**Fig. 2** Principle set up of the functional *in vitro* test (FIT) comprising spontaneous, physical, antibody mediated and antigen induced release for each individual blood sample



**Fig. 3** Allergen specific histamine release of washed peripheral blood cells from five cats (3 recently uninfested and 2 flea-infested individuals) following incubation with *Ctenocephalides* spp. extract at concentrations from 1 to  $1 \times 10^{-6}$  µg/ml. Spontaneous, physical and antibody release with RaC IgG [H+L] (100 and 30 µg/ml) serve as negative and positive controls, respectively. Histamine in the cell free supernatants is quantified by means of a radioimmunoassay in ng/ml blood (A) and as percentage of the maximal histamine release (B) per cat.

## RESULTS AND DISCUSSION

As an example of variation in histamine release between cats five individuals with different history of flea-infestation were selected. Cat 1, 2 and 3 were clinically healthy with no history of skin disease and free of *Ct. felis* for at least 12 months. However, it was known that cat 1 had served to maintain the colony of *Ct. felis* in the Department of Parasitology, Hannover School of Veterinary Medicine earlier. Cat 4 and 5 have been continuously exposed to cat fleas for the last 12 months. At the time of sampling moderate dorsal lumbosacral hair loss was noticed in these cats.

All cats reacted dose-dependently with RaC IgG [H+L] antiserum. In cat 2, 3 and 4 the maximal release was achieved by antibody release. In cat 1 and 5 the maximal release was obtained by boiling. Physical releases showed strong inter-individual variations (from 11 to 94 ng/ml blood). The allergen preparation of *Ctenocephalides* spp. tested proved to be free of unspecific triggering of feline basophils in concentration from 1 to  $1 \times 10^{-6}$  µg/ml. Applied in the same range it caused dose dependent and individually very different reactions. For example a dose-dependent histamine release could be observed in cat 1, 4 and 5 whereas cat 2 and 3 did not react to such preparations with a detectable histamine release.

In order to assess the different forms of histamine release it has to be considered that physiological variations of histamine contents among blood samples require a relative evaluation of allergen released histamine in relation to the maximal and spontaneous release from each blood specimen. The maximal histamine release achieved by physical or antibody controls provides the 100% value. In relation to the relevant maximal release a threshold has still to be determined for the cat above which an allergen induced histamine release can be considered positive. In the horse an allergen induced histamine release above 10% of the relevant maximal release is weighed as positive (Kaul, 1998). Extrapolating that to the animals no. 1, 4 and 5 would be considered to be functionally sensitized against flea allergens while cat 2 and 3 are not sensitized. Initial results indicate that the FIT might provide a sensitive monitoring system for flea allergy dermatitis in the cat. Further comparative studies with samples of cats with and without signs of FAD will have to be carried out to determine cat related cut offs and to evaluate the clinical relevance of the results. Applicability, sensitivity and reliability have to be ascertained. ●

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# The Feline Leukemia Virus (FeLV) and the Cat Flea (*Ctenocephalides felis*)

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## INTRODUCTION

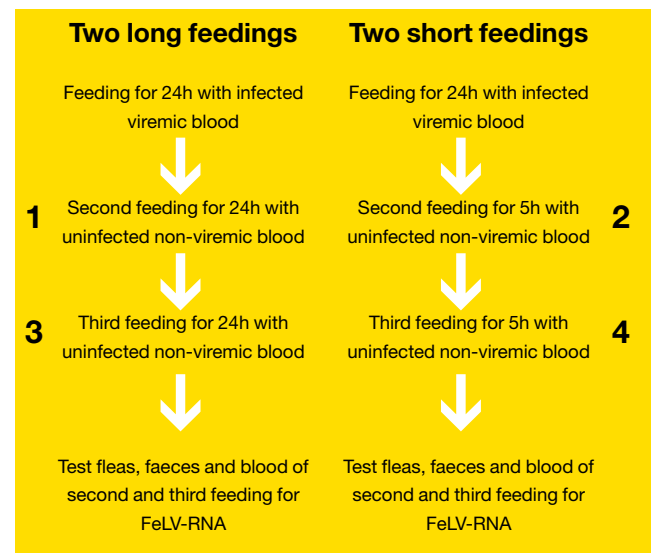
Cat leukemia is a widespread and feared infectious disease of cats, that is caused by the feline leukemia virus (FeLV) (Jarret 1975). FeLV was first discovered in 1964 (Jarret et al. 1964) and is a naturally occurring retrovirus that in some countries is still considered to be responsible for most of disease related deaths among pet cats. Clinical studys could not show a connection between human leukemia and FeLV (Sordillo and Markovich 1982), but human bone marrow cells could be infected with the virus in vitro (Morgan et al. 1993). An infection with FeLV can lead to persistent or transient infection (Hoover et al. 1975; Lutz et al. 1980; Hoover and Mullins 1991). Persistently infected cats die mostly within 4 years after infection (McClelland et al. 1980). Transiently infected cats become immune to the virus after the first viraemia and are considered immune to reinfection (Charreyre and Pedersen 1991), where another group of cats become immune after the first exposure to FeLV and show no clinical signs of infection (Rojko and Kociba 1991). FeLV is spread horizontally or vertically through saliva, blood, tears, breast milk or other body fluids and requires intimate contact between cats (Hardy 1981). Also transplacental transmission has been reported (Hoover and Mullins 1991). An alternative way of transmission appears to be possible by blood sucking parasites, that could transmit the virus from cat to cat without the need of intimate contact. Fleas are well known as vectors for bacteria, rickettsiae or even tapeworms (Hinaidy 1991; Mehlhorn 2001). However, little is known about the transmission of viruses via the flea. In this study, the vector potential of the cat flea *C. felis* for feline leukemia virus was investigated.

## MATERIAL AND METHODS

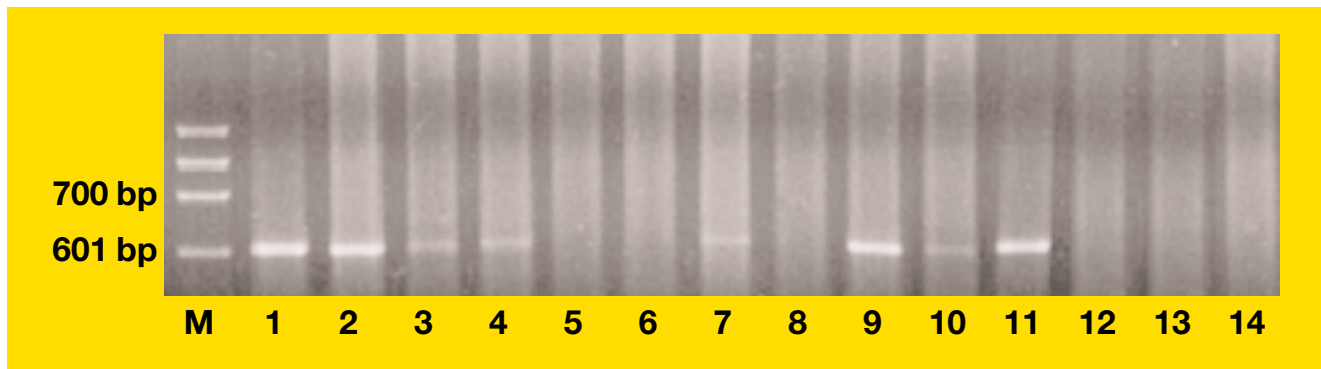
Fleas were artificial reared and fed via membranes in the so-called “artificial dog” from FleaData Inc. (Freeville, USA). The air temperature inside the “artificial dog” was adjusted to 39°C, leading to a blood temperature of 37°C.

Viral RNA was isolated from blood samples using the QiAamp® Viral RNA Mini Kit according to the protocol of the manufacturer (Qiagen, Hilden, Germany). Preparation of viral RNA from fleas was performed by freezing the fleas in liquid nitrogen and pulverise them in a steel mortar. The pulver was then mixed with buffer and viral RNA preparation was performed according to the protocol by the manufacturer.

Preparation of viral RNA from feces was performed by simply mixing the feces with buffer and continue preparation of RNA according to the protocol of the manufacturer. To prevent DNA contamination, all preparations were subsequently treated with DNase I with the DNasefree Kit according to the protocol by the manufacturer (Ambion, Austin, USA). Reverse transcription of the viral RNA was performed using the 1st strand cDNA Synthesis Kit for RT-PCR according to the protocol by the manufacturer (Roché, Indianapolis, USA). Amplification and detection of the FeLV cDNA was performed with a high sensitive nested PCR as previously described (Miyazawa and Jarret 1997). The DNA amplification was performed in 100 µl reaction tubes in a total volume of 50 µl using the Taq PCR Core Kit according to the protocol by the manufacturer (Qiagen, Hilden, Germany).



**Fig. 1** Overview of the experimental setup. An initial population of fleas was fed for 24h with FeLV infected blood. Fleas and feces were tested for viral RNA. One population was subsequently fed for 24h (step 1), the other for 5h (step 2) with uninfected non-viremic blood. Fleas and feces were tested for viral RNA (test for uptake of virus), as well as the two resulting blood samples (test for transmission of virus). In a third feeding, fleas were transferred again to fresh and uninfected blood and were fed for 24h (step 3) and 5 h (step 4), respectively. Fleas, feces and the resulting blood samples were tested for FeLV-RNA like in feeding two.



**Fig. 2** PCR amplification of cDNA with inner primer pair targeting a 601bp fragment between U3 and gag region of FeLV-RNA. Prior to RT-PCR, all RNA-preparations were treated with DNase I to ensure, that no cellular proviral DNA was detected instead of viral RNA. **1:** Feces of fleas after feeding for 24h with FeLV infected blood. **2:** Fleas after feeding for 24h with FeLV infected blood. **3:** Uninfected blood after feeding from step 1. **4:** Fleas after feeding from step 1. **5:** Uninfected blood after feeding from step 3. **6:** Fleas after feeding from step 3. **7:** Feces of fleas after feeding from step 1. **8:** Feces of fleas after feeding from step 3. **9:** Uninfected blood after feeding from step 2. **10:** Fleas after feeding from step 2. **11:** Feces of fleas after feeding from step 2. **12:** Uninfected blood after feeding from step 4. **13:** Fleas after feeding from step 4. **14:** Feces of fleas after feeding from step 4. All experiments were also performed with non-viremic blood as a negative control (not shown). No FeLV-RNA was detected. **M:** PeqGold 100bp ladder (Peqlab).

## RESULTS

In a first feeding, an initial population of 110 fleas were infected with FeLV through feeding for 24h with 2 ml blood from an viremic cat (Fig. 1). To verify successful uptake of viruses, 10 fleas and whole feces of this initial population were tested for FeLV RNA. The viral RNA could be detected in both samples, fleas and feces (Fig. 2, lane 1 and 2). The FeLV incorporated by the fleas via blood feeding and was excreted with the feces. The remaining 100 infected fleas were subsequently divided in two populations of 50 where population 1 was fed for 24 hours (Fig. 1, step 1), and population 2 for 5 hours (Fig. 1, step 2) with 300 µl uninfected blood from a healthy cat. After the defined time, 10 fleas and feces of each population were tested for FeLV. Viral RNA could be detected in fleas of both populations, as well as in their feces (Fig. 2, lane 4,7,10, and 11). FeLV RNA was detectable for 5 and 24 hours respectively after uptake by the flea and feeding with uninfected blood. To investigate, whether transmission of FeLV occurred, the remaining blood from transfection 1 and 2 was examined. Viral RNA could be detected in both samples of the previously uninfected blood from a clinically healthy cat (Fig. 2, lane 3 and 9). This shows, that the cat flea *C. felis* can function as a vector for feline leukemia virus RNA in vitro. Viral RNA was detectable in the fleas and its feces for up to 24 hours. In addition, the fleas successfully transmitted FeLV RNA directly from an infected blood sample to an uninfected one. In a third feeding study, the same fleas were fed again with fresh and uninfected blood (Fig. 1, step 3 and 4). After 5 and 24 hours, respectively, fleas, feces and the blood samples were examined for FeLV. After this third feeding, viral RNA could not be detected, neither in the fleas, their feces nor in the blood samples (Fig. 2, lane 5,6,12 and 8,13,14). FeLV RNA was detectable in the fleas up to 24

hours after feeding. During this time, fleas also excreted viral RNA with their feces. In addition, the fleas were able to transmit FeLV RNA directly from one blood sample to another and functioned therefore as a vector for the virus.

## DISCUSSION

Feline leukemia virus is known to be spread from cat to cat via saliva (by licking), blood (scratching or fighting) and all other body fluids (Hardy et al. 1975). A close and intimate contact between healthy and infected cats is sufficient to disperse the virus within a population or household (Essex et al. 1977). The results of our work show, that there may be another, from close cat to cat contact independent transmission of feline leukemia virus by the cat flea *C. felis*. The flea is an important vector for various pathogens, such as bacteria and rickettsiae. Due to the wide host range of some fleas, pathogens can even be spread across species, e.g. from rat to man (plague). Recently, even an infection of human with *Rickettsia felis* through the flea has been discussed (Richter et al. 2002). However, not much is known about the flea as a vector for viruses. It has been reported that some viruses can outlast intact in the flea for at least 24 hours (Smetana 1965). The possible transmission through the flea of the feline leukemia virus, or the HIV related feline immunodeficiency virus, is unknown. One result of our study is, that if fleas are fed for 24 hours on FeLV positive blood, the flea excretes viral RNA with its feces. Under the view of the various infection possibilities of FeLV, this aspect may gain more importance. There may be risk that a healthy cat scratches flea feces into its skin, as a result of itching flea bites or while fighting with against other cats.



Another result of our studies might be the direct transmission of FeLV through the flea and the flea bite. The feline leukemia virus may be ingested by the flea and excreted with the feces, but also directly transmitted during the blood sucking activity. Whether this route of FeLV infection is of importance in-vivo and has clinical significance remains unanswered and further investigations on this aspect are necessary. While in principle the risk of pathogen transmission via flea bites to both pet animals and human is known, it can be concluded that prevention of flea infestation in pet animals is recommended. Regular application of effective topical spot on's using insecticides like imidacloprid with its proven therapeutic and preventative properties eliminates flea infestation and thus transmission of diseases. ●



**Fig. 3** Frontal view of a cat flea (*Ctenocephalides felis felis*)

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# Evaluation of the Efficacy of an Imidacloprid 10% / Moxidectin 2.5% Spot-on against *Sarcoptes scabiei* var *canis* on Dogs



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## OBJECTIVES

The objectives of the study were firstly to determine whether two treatments with a novel formulation of Imidacloprid 10% plus Moxidectin 2.5%, administered four weeks apart, would be effective against *Sarcoptes scabiei* var *canis* on dogs. Secondly to compare the results with that of a positive control group of dogs treated with Selamectin.

## STUDY DESIGN AND METHODS

This study was performed in compliance with VICH GL9 “Good Clinical Practice, June 2000” at ClinVet International (Pty) Ltd, situated in Bloemfontein, Republic of South Africa. Thirty dogs naturally infested with *Sarcoptes scabiei* var *canis* were allocated to two groups of 15 dogs each, according to randomisation through minimization with Day -1 body weight as primary criterion. One group was treated with the Imidacloprid/ Moxidectin Spot-on at 0.1 ml/kg body weight and the second group with Selamectin (Stronghold®) at 0.05 ml/kg (6 mg/kg) body weight. Treatments were blinded and were administered as topical applications on Day 0 and + 28. All the dogs were housed individually in pens, under strict quarantine conditions and no contact between the dogs was possible.

The presence or absence of mites was assessed by taking skin scrapings from five body regions suspected of being infested on each dog, and mite counts were done on these. Clinical symptoms and the extent of sarcoptic lesions and pruritus were assessed and recorded. Comprehensive photographic documentation was done (Fig. 1). The schedule followed is summarized in Table 1.

The primary assessment variable used in this study was the presence or absence of live mites or eggs on the dogs on each assessment day following treatment. The success rate for each group was calculated as follows:

$$\text{Success rate (\%)} = \frac{x}{y} \cdot \frac{100}{1}, \text{ where}$$

x = number of dogs observed with no live mites / eggs

y = total number of dogs in the group

Due to the nature and uncertainty factor of the mite count assessment, false negatives could have been recorded, resulting in an overestimation of the success rate. Consequently, clinical symptoms as well as the frequency of pruritus were used to confirm the success rate described above. The above-mentioned parameters were used to categorize a dog as an “overall success” or “failure”. An overall success in terms of treatment was defined as a dog who complied with all of the >>

**Fig. 1** A dog naturally infested with *Sarcoptes scabiei* var *canis* before (Day -3) (left) and after (Day +50) (right) treatment (Imidacloprid 10% / Moxidectin 2.5% applied four weeks apart at a dose of 0.1 ml/kg body weight)



**Table 1** Synoptic overview of the study layout

\* Only five dogs retained for extended periods of observation

Acclimatization	Allocation to Groups	Treatment	Pre- and Post-treatment assessments		
			Pruritus Index	Mite counts and clinical assessments	Photographic Documentation
Day -7	Day -1	Day 0; Day + 28	Day -1; +7; +21; +35; +42; +57*; +63*	Day -3; +8; +22; +36; +50; +64*	Day -3; +22; +50; +64*

**Table 2** Summary of the treatment and overall treatment success rates (%) for the study groups

\* Pooled data for Days +50 and +64

	Group 1: Treated with Selamectin			Group 2: Treated with Imidacloprid/Moxidectin		
	Day +22	Day +50	Day +64*	Day +22	Day +50	Day +64*
Treatment Success rate (%)	100	100	100	100	100	100
Overall Treatment Success rate (%)	6.7	66.7	66.7	7.1	92.9	100

following conditions: No live mites or eggs; a complete resolution of papules and crusts as assessed on Day +22 and +50 or +64; a marked (> 80%) reduction in body areas showing alopecia; and the frequency of pruritus not consistently high.

in the Selamectin treated group displayed prominent alopecia and two of these displayed localized areas with crusts on Day +64. Three of these dogs were infested with *Demodex* spp. mites, which may have been the cause of the alopecia.

## RESULTS

The treatment success rate and overall treatment success rate for the two study groups are summarized in Table 2. On Day +8 *S. scabiei* was found in skin scrapings of two dogs treated with the Imidacloprid / Moxidectin solution and of three dogs treated with Selamectin. From Day +22 and onwards, no *S. scabiei* mites were detected in the skin scrapings of any of the dogs, giving 100% treatment success rates for both groups. Based on the criterion defined above, 7.1%, 92.9% and 100% of the dogs treated with the Imidacloprid / Moxidectin solution were categorized as an overall success in terms of treatment on Days +22, +50 and +64, respectively. Five dogs

## CONCLUSION

The Imidacloprid / Moxidectin solution applied twice (four weeks apart) at a dosage of 0.1 ml/kg body weight, was highly efficacious in curing *Sarcoptes scabiei* var *canis* infestations on all the treated dogs, and resulted in an almost complete resolution of clinical symptoms within 50 to 64 days after the first treatment. ●

# Feline Advantage Heart™ (Imidacloprid and Moxidectin) Topical Solution as Monthly Treatment for Prevention of Heartworm Infection (*Dirofilaria immitis*) and Control of Fleas (*Ctenocephalides felis*) on Cats



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## INTRODUCTION

Feline Advantage Heart™ is a combined formulation of 10% w/v imidacloprid plus 1% w/v moxidectin spot-on for dermal application to kittens and cats. The combination product has been developed by Bayer, using the insecticide, imidacloprid, and the macrolide anthelmintic, moxidectin. It is intended for monthly application to provide treatment and control of flea infestations, intestinal nematodes, and for prevention of feline heartworm disease. Imidacloprid is already marketed worldwide as Advantage® (10 w/v imidacloprid = 9.1% w/w), a spot-on treatment for the control of fleas on cats and dogs. Imidacloprid applied monthly as a low volume topical application to cats or dogs provides highly effective flea control.<sup>3,4</sup> Fleas are immobilized and killed after contact with imidacloprid on the skin or hair of cats. For small animal veterinary medicine, a moxidectin tablet formulation has been marketed for some time as monthly prophylactic treatment for dogs, (Proheart®, Ft. Dodge) and more recently as an injectable slow release formulation<sup>1</sup>. Moxidectin is also available as an endectocide for ruminants and an anthelmintic for horses<sup>2</sup>. The new formulation of 10% imidacloprid plus 1% moxidectin spot-on is produced by replacing a proportion of the pharmaceutically inactive component of the Advantage® formulation with moxidectin. With this additional component Bayer has extended the Advantage® spectrum of activity to include activity against intestinal nematodes and heartworm disease prevention.

The studies presented here were conducted to evaluate flea control and heartworm prevention in cats with topically applied imidacloprid and moxidectin formulations.

## MATERIAL AND METHODS

### Flea Study No 1

Thirty two short-haired cats were randomized into 4 treatment groups balanced by sex and pretreatment flea infestations: (1) 10% imidacloprid + 1.0% moxidectin (2) 10% imidacloprid

only (3) 1.0% moxidectin only (4) control (placebo without active ingredient). Each cat was infested with 100 recently emerged adult fleas (*Ctenocephalides felis*) on day -1. Each cat was treated on test day 0 with the appropriate test article provided in pre-filled applicator tubes designed to deliver a minimum of 0.1 mL of solution/kg body weight. Treatments were applied by dermal application of the product on the skin at the base of the skull. Live fleas were combed from the cats, counted and removed on test day 1. Each cat was reinfested with 100 live fleas on test days 6, 13, 20, 27 and 34. Live fleas were combed from the cats, counted and removed on test days 7, 14, 21, 28 and 35. Percent flea control efficacy was calculated by comparing the geometric mean number of live fleas on test groups 1 – 3 with the geometric mean number of live flea on the control cats with the following formula:

% Efficacy =

$$\frac{\text{Geo Mean No. Fleas (Controls)} - \text{Geo Mean No. Fleas (Treated)}}{\text{Geo Mean No. Fleas (Controls)}} \times 100$$

### Flea Study No 2

Sixteen cats were randomized into 2 treatment groups balanced by sex and pretreatment flea infestations: (1) 10% imidacloprid + 1.0% moxidectin or (2) control (placebo without active ingredient). Each cat was infested with 100 recently emerged adult fleas on day -1. Each cat was treated on test day 0 with the appropriate test article provided in pre-filled applicator tubes designed to deliver a minimum of 0.1 mL of solution/kg body weight. Treatments were applied by dermal application of the product on the skin at the base of the skull. Live fleas were combed from the cats, counted and removed on test day 1. Each cat was reinfested with 100 live fleas on test days 6, 13, 20, 27 and 34. Live fleas were combed from the cats, counted and removed on test days 7, 14, 21, 28 and 35. Percent flea control efficacy was calculated by comparing the geometric mean number of live fleas on test group 1 with the geometric mean number of live fleas on the control cats (group 2) using the same formula indicated above.



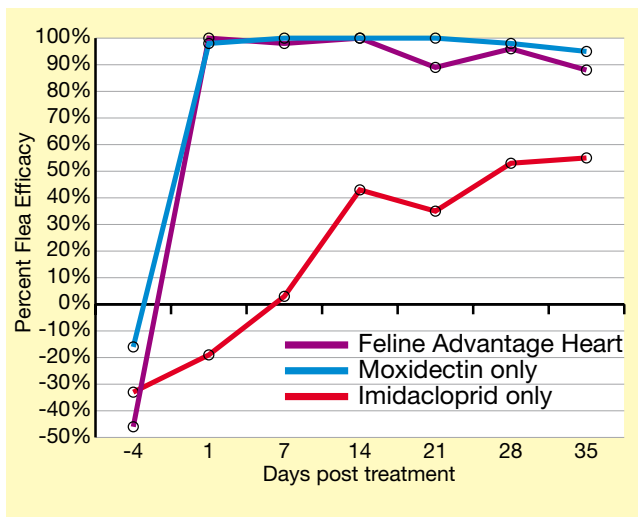


Fig. 1 Flea Study No. 1: Comparative Flea Control

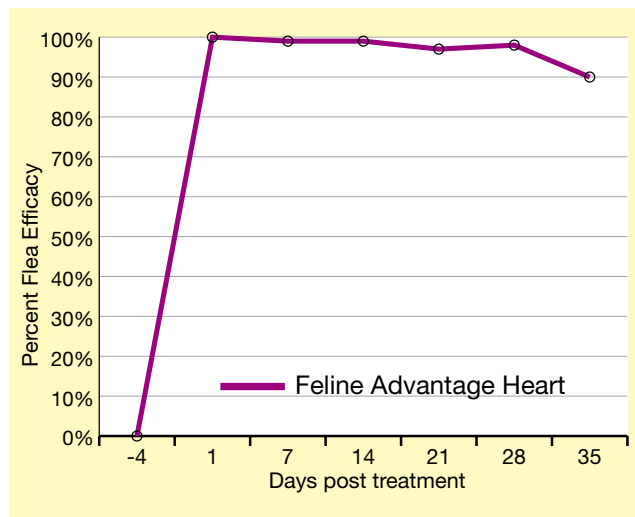


Fig. 2 Flea Study No. 2: Percent Flea Control

### Heartworm Study No 1

A controlled laboratory study was conducted to evaluate the efficacy and safety of topically applied imidacloprid plus moxidectin against experimentally-induced infections with *Dirofilaria immitis* larvae in cats. On test day -30 forty cats (20 males/20 females, 5 to 8 months of age) were each infected with 100 third stage *D. immitis* larvae. On test day -1 the cats were blocked by gender and body weight, and were randomly assigned to the following 4 treatment groups: (1) 10% imidacloprid +1.0% moxidectin, (2) 1.0% moxidectin only, (3) 10% imidacloprid only and (4) control (placebo without active ingredient). On test day 0 (30 days following experimental infections with *D. immitis*), cats were topically treated, at a minimum dose equal to 0.1 ml/kg body weight, with the appropriate formulation. Study animals were maintained until test day 140 (170 days post-infection). At that time, they were euthanized, necropsied, and examined for recovery of adult heartworms within the abdominal and thoracic cavities, heart, lungs and connecting vascular system.

### Heartworm Study No 2

A second controlled laboratory study was conducted to further evaluate the safety and efficacy of topically applied 10% imidacloprid + 1% moxidectin against experimentally-induced *Dirofilaria immitis* infections in cats. On test day -30, twenty cross breed cats (11 males/9 females, 5.5 to 6.5 months of age) were each infected with 100 third-stage *D. immitis* larvae (L3). On test day -1, cats were blocked by gender and body weight and were then randomized to 2 treatment groups: (1) 10% imidacloprid +1.0% moxidectin or (2) control (placebo without active ingredient). On test day 0 (30 days post-infection with *D. immitis* larvae), cats were topically treated, at a minimum dose equal to 0.1 ml/kg body weight, with either the test product or a placebo. Study animals were maintained until test day 140 (170 days post-infection). At that time, they were euthanized, necropsied, and examined for the recovery of adult heartworms within the abdominal and thoracic cavities, heart, lungs and connecting vascular system.

## RESULTS

### Flea Study No 1

Percent flea control efficacy is displayed in Figure 1, group 1 (imidacloprid + moxidectin) and group 2 (imidacloprid only) treatments provided 100 and 98.4% flea efficacy on test day 1. No flea control was observed with group 3 treatment (moxidectin only) on test day 1. Group1 treatments provided 98.7, 100, 89.4, 98.0, and 88.4% flea efficacy on test days 7, 14, 21, 28 and 35, respectively. Group 2 treatments provided 100, 100, 100, 98.6 and 96.5% flea efficacy for the same the five corresponding post-treatment periods. Percent flea control for the moxidectin only treatment (group 3) ranged from 2.9 – 55.4% for test days 7 – 35. The control cats (group 4) retained a geometric mean range of 11.9 to 31.7 live fleas/cat through test day 35.

### Flea Study No 2

Percent flea control efficacy is displayed in Figure 2, group 1 (imidacloprid + moxidectin) provided 100% flea efficacy on test day 1 and then provided 99, 99, 97, 98.0, and 90% efficacy on test days 7, 14, 21, 28, and 35, respectively. The control cats (group 2) retained a geometric mean range of 27.7 – 83.5 live fleas/cat through test day 35.

### Heartworm Study No 1

The recovery of adult heartworms and percent heartworm efficacy for heartworm study number 1 is displayed in Figure 3. No adult heartworms were recovered from the cats in group 1 treated with imidacloprid + moxidectin or from the cats in group 2 treated with moxidectin only. Heartworm efficacy for groups 1 and 2 was 100%. A total of 74 adult heartworms were recovered from 7 of 10 cats in group 3 and 61 adult heartworms were recovered from 6 of 10 cats in group 4.

These results demonstrate that the 1.0% moxidectin plus 10% imidacloprid and 1.0% moxidectin formulations applied topically at a minimum dose of 0.1 ml/kg bodyweight were

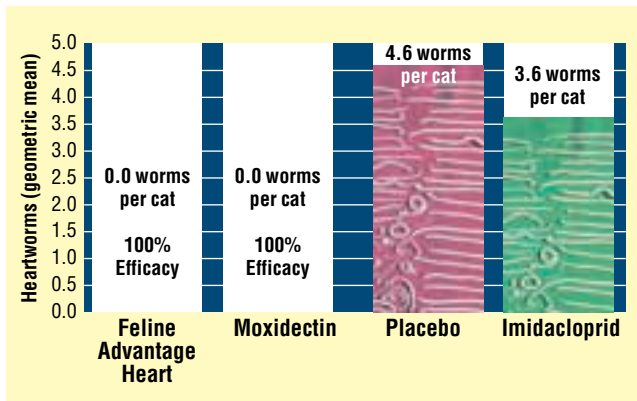


Fig. 3 Heartworm Study No. 1: Adult Heartworm Counts

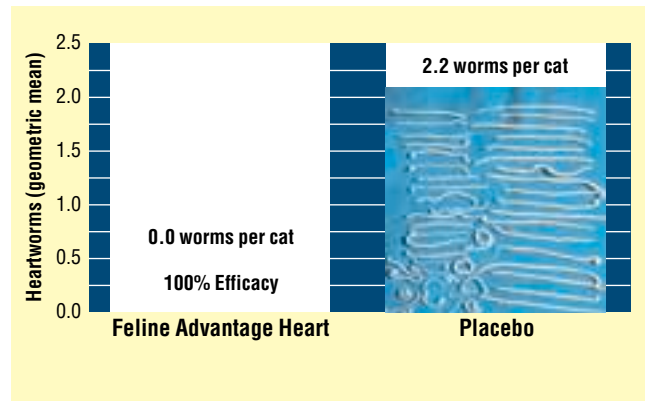


Fig. 4 Heartworm Study No. 2: Adult Heartworm Counts

100% efficacious against the development of adult heartworm infections in cats. The results also demonstrated that 10% imidacloprid, applied topically to cats, had no activity against larval stages of *D. immitis*. Imidacloprid did not interfere with the efficacy of moxidectin against *D. immitis* when combined with moxidectin into one formulation. No adverse or unusual reactions were observed following treatment with any of the test articles.

### Heartworm Study No 2

The recovery of adult heartworms and percent heartworm efficacy for heartworm study number 2 is displayed in Figure 4. No adult heartworms were recovered from the cats in group 1 treated with imidacloprid + moxidectin only. Heartworm efficacy for group was 100%. A total of 31 adult heartworms were recovered from 8 of 10 cats in group 2. These results further demonstrate that the 1.0% moxidectin plus 10% imidacloprid applied topically at a minimum dose of 0.1 ml/kg bodyweight is 100% efficacious against the development of adult heartworm infections in cats.

## DISCUSSION

The results of these studies demonstrate that moxidectin solution applied alone topically to cats provided little or no flea control. Imidacloprid alone provided no activity against the development of heartworm larvae to the adult stage in cats but did not interfere with the activity of moxidectin to prevent the development of heartworm larvae in cats when the two active ingredients were combined. These results are in agreement to similar results observed during efficacy studies conducted with 10% imidacloprid + 0.08% ivermectin for dogs.<sup>5</sup> The results of these studies demonstrated that ivermectin alone had little or no activity against fleas on dogs, imidacloprid alone did not have activity against larval stages of canine heartworm, while the combined formulation of imidacloprid and ivermectin was efficacious for prevention of heartworm disease and the treatment and control of fleas when applied monthly.

The results of these studies demonstrate that 1% moxidectin may be combined with 10% imidacloprid to provide a dosage volume of 0.1 ml/kg (10 mg/kg imidacloprid + 1.0 mg/kg body weight moxidectin). The combined formulation applied as a monthly topical treatment will prevent the development of feline heartworm disease and will provide treatment and control of flea infestations. ●

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# Evaluation of the Efficacy of a Combination of Imidacloprid and Moxidectin against Immature *Toxocara cati* in Cats

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## INTRODUCTION

From a veterinary medical perspective, optimum control of preventable diseases has always been a higher priority than disease therapy. Today, especially in small animal medicine, this objective can only be achieved by providing a product to the pet owner in an easy-to-administer formulation. The combination of 1% Moxidectin and 10% Imidacloprid in a spot-on formulation for dermal application fulfills these criteria. The combination product has been developed by Bayer, using the neonicotinyl insecticide, Imidacloprid, and the macrocyclic lactone, Moxidectin. It is intended to provide a treatment for, and prophylaxis against, fleas and a range of nematode infections of cats. It is desirable to control and treat

not only established adult gastrointestinal parasites, but also the developmental stages including L4 and immature adults of *T. cati*. The importance of controlling feline parasitism is not only to relieve clinical symptoms in infected cats, but also to minimize the zoonotic potential of larval nematode infections in man (Sprent, 1956).

Moxidectin is related to the milbemycins and is believed to exert its effect in the same way as the milbemycins and avermectins. All appear to cause paralysis of susceptible parasite species by altering chloride conductance into cells. It was originally believed that this was brought about by activity of the molecules at the GABA receptors (McKellar and Benchaoui, 1996). However, after further investigation, it is understood that ivermectin and milbemycin D bind to glutamate-gated chloride channels in nematodes, where they can potentiate glutamate or cause an increase of conductance in the absence of glutamate (Martin, 1996).

**Figure 1** *Toxocara cati* in the duodenum of a cat



## STUDY DESIGN

A controlled laboratory study was conducted to evaluate the efficacy of a novel formulation of Imidacloprid + Moxidectin against fourth stage larvae and immature adult *Toxocara cati* infection in cats. Thirty-two animals experimentally infected with *T. cati* were treated topically with a formulation to provide at least 10 mg/kg of Imidacloprid and 1 mg/kg of Moxidectin one time in a controlled anthelmintic evaluation. The four treatment groups were as follows: 1. Imidacloprid + Moxidectin (treated on Day 14, necropsied on Day 19), 2. Placebo (treated on Day 14, necropsied on Day 19), 3. Placebo (treated on Day 24, necropsied on Day 29), and 4. Imidacloprid + Moxidectin (treated on Day 24, necropsied on Day 29). During the five day post-treatment period, cats were observed daily for clinical changes to eyes, feces, respiration, behavioral attitude, locomotion/musculature, and skin conditions. At the end of the five-day observation period, the animals were necropsied and the remaining worms were recovered and counted. At necropsy, alimentary contents and saline incubates of the small intestine were collected, preserved, and examined microscopically. Nematodes were recovered, counted, and identified to genus, species, and stage of development.

## RESULTS

Topical administration of Imidacloprid + Moxidectin proved safe in cats as evidenced by no adverse clinical signs post-treatment. The geometric mean number of *Toxocara cati* larvae for the Day 19 necropsy was 36.9 for Placebo and 1.0 for the combination of Imidacloprid + Moxidectin. The geometric mean number of *Toxocara cati* adults for the Day 29 necropsy was 59.2 for the placebo and 1.0 for the combination of Imidacloprid + Moxidectin. Efficacy for the treatment group that was necropsied on Day 19 (larvae) was 97.2%, while the efficacy for the treatment group that was necropsied on Day 29 was 98.3%.

## DISCUSSION

Infections with gastrointestinal nematodes, esp. *Toxocara cati* are significant and have been recorded not only in stray cats (Coman et al. 1981, Yamaguchi et al, 1996) but also in cats kept as pet animals (Hellmann et al. 2002). Fleas comprise the major ectoparasitic infestation of both cats and dogs worldwide (Rust and Dryden 1997). The combination of these two active parasiticides provides a product to control and prevent infection with both fleas and *Toxocara cati* in cats. ●



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# The Anthelmintic Efficacy and the Safety of a Combination of Imidacloprid and Moxidectin Spot-on in Cats and Dogs under Field Conditions in Europe

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## INTRODUCTION

Roundworms of the genus *Toxocara* and hookworms of the genera *Ancylostoma* and *Uncinaria* are the most common intestinal worms in dogs and cats. Co-infections of intestinal worms and fleas occur in a high proportion of dogs and cats in Europe. For a simultaneous treatment against endo- and ectoparasites a spot on product was developed containing the neonicotinoide (chloronicotiny) Imidacloprid and the macrocyclic lactone Moxidectin. The efficacy and safety have been proven in various experimental studies in dogs and cats. Objective of this investigation was to assess the anthelmintic efficacy and safety of the product under European field conditions considering multiple complicating variables such as home environment, age, breed and hair length.

### DOGS

#### I. MATERIAL AND METHODS

Dogs were allocated in twelve veterinary practices located in various regions of Germany and France. A total of 1,355 faecal samples from dogs were examined for the presence of nematode eggs. 131 worm-positive dogs were enrolled and randomly allocated in a 2:1 ratio to one of the two treatments:

- (a) A combination of 10 % (w/v) Imidacloprid and 2.5% (w/v) Moxidectin, administered topically at the skin surface between the shoulder blades. Pipettes containing a dose volume of 0.4 ml were used for dogs of less than 4 kg bodyweight, 1.0 ml for dogs of 4 to 10 kg bodyweight, 2.5 ml for dogs between 10 and 25 kg bodyweight and 4.0 ml for dogs above 25 kg bodyweight. Dogs weighing more than 40 kg were treated with a combination of a 4 ml pipette and a smaller pipette size.
- (b) A positive reference product (Drontal® Plus, Bayer) containing 50.0 mg praziquantel, 144 mg pyrantel-embonat and 150 mg febantel, administered orally at a dosage of 1/4 of a tablet for 2.5 kg bodyweight.

On days 7 – 1 before treatment and 8 to 13 days after treatment faecal samples were taken for faecal egg count examination. Efficacy was assessed by analysing the reduction in faecal egg counts of the first faecal sample (prior to treatment) in comparison to the second faecal sample taken post treatment.

### CATS

#### I. MATERIAL AND METHODS

Cats were allocated in twenty one veterinary practices located in various regions of Germany and France. A total of 1,292 faecal samples from cats were examined for the presence of nematode eggs. 141 worm-positive cats were enrolled and randomly allocated in a 2:1 ratio to one of the two treatments:

- (c) a combination of 10.0% (w/v) Imidacloprid and 1% (w/v) Moxidectin, administered topically at the skin surface at the base of the skull at a dose volume of 0.4 ml for cats less than 4 kg bodyweight and a dose volume of 1.0 ml for cats of more than 4 kg bodyweight.
- (d) a positive reference product (Drontal®, Bayer) containing 20 mg praziquantel and 230 mg pyrantel-embonat, administered orally at a dosage of 1/4 of a tablet for 1 kg bodyweight.

On days 7 – 1 before treatment and 7 to 13 days after treatment faecal samples were taken for faecal egg count examination. Efficacy was assessed by analysing the reduction in faecal egg counts of the first faecal sample (prior to treatment) in comparison to the second faecal sample taken post treatment.



## II. RESULTS (DOGS)

Efficacy was assessed for 74 dogs treated with the Imidacloprid and Moxidectin combination product and for 35 dogs treated with Drontal® Plus.

Treatment	Nematode species	Geo. mean of faecal egg count				Reduction (%)
		Period 0 Day -7 to -1, N	Period 1 Day 8 to 13, N	Period 0 Day -7 to -1, N	Period 1 Day 8 to 13, N	
Imidacloprid/ Moxidectin	<i>Toxocara canis</i>	462.9	46	5.5	46	98.81
	Ancylostomatidae	462.4	28	0.4	28	99.92
Drontal® Plus	<i>Toxocara canis</i>	413.9	18	1.4	18	99.66
	Ancylostomatidae	263.3	17	0.2	17	99.91

**Tab. 1** Geometric mean of faecal egg count and faecal egg count reduction in dogs

No adverse event was seen in any dog treated.

## III. CONCLUSION

It can be concluded that the 10% Imidacloprid / 2.5% Moxidectin spot-on combination is safe and highly efficacious against *Toxocara canis* and *Ancylostomatidae* in naturally infested dogs.



## II. RESULTS (CATS)

Efficacy was assessed for 78 cats treated with the Imidacloprid and Moxidectin combination product and for 40 cats treated with Drontal®.

In addition there were 4 cats showing a *Toxascaris leonina* infestation. Three cats were treated with Imidacloprid/Moxidectin and one was treated with Drontal®. Both treatments were highly efficacious (100%).

Treatment	Nematode species	Geo. mean of faecal egg count				Reduction (%)
		Period 0 Day -7 to -1, N	Period 1 Day 7 to 13, N	Period 0 Day -7 to -1, N	Period 1 Day 7 to 13, N	
Imidacloprid/ Moxidectin	<i>Toxocara cati</i>	1054.8	72	0.1	72	99.99
	Ancylostomatidae	259.9	6	0.94	6	99.64
Drontal® Plus	<i>Toxocara cati</i>	1149.8	38	0.4	38	99.96
	Ancylostomatidae	264.6	2	0.0	2	100

**Tab. 2** Geometric mean of faecal egg count and faecal egg count reduction in cats

No adverse event was seen in any cat treated with the Imidacloprid/Moxidectin combination.

## III. CONCLUSION

It can be concluded that the 10% Imidacloprid / 1% Moxidectin spot-on combination is safe and highly efficacious against *Toxocara cati* and *Ancylostomatidae* in naturally infested cats. There is also evidence for efficacy against *Toxascaris leonina*.



# The Efficacy of two Anthelmintics against Ascarids and Hookworms in naturally infected Cats

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## INTRODUCTION

There are numerous commercial cat anthelmintics available that contain pyrantel embonate or pamoate as the active ingredient which is effective against ascarids and hookworms. The dosage rate of this active ingredient when administered to dogs is considerably lower than that required in cats, but it remains a safe active. A new introduction in feline anthelmintics is Milbemax (a combination of milbemycin oxime plus praziquantel). Considering the private practitioner's desire to see comparative results of treatment options, a trial was conducted to compare the efficacy against natural ascarids and hookworm infections in cats, of Milbemax and Drontal Cat tablets.

## MATERIALS AND METHODS

The two commercially available formulations that were tested are: (i). Drontal Cat – a combination containing 230 mg pyrantel embonate and 20 mg praziquantel per tablet and used at a dosage rate of 1 tablet per 4 kg live weight; (ii) **Milbemax for Cats** containing 16 mg milbemycin oxime and 40 mg praziquantel per tablet and used at a dosage rate of ½ tablet per 2 – 4 kg or 1 tablet per 4 – 8 kg; OR **Milbemax for small cats and kittens** containing 4 mg milbemycin oxime and 10 mg praziquantel per tablet and used at a dosage rate of ½ a tablet per 0,5 – 1 kg or 1 tablet per 1 – 2 kg. An untreated control group was included to confirm the persistence of the infection throughout the trial period.

The cats used in the trial consisted of young cats of about 6 to 36 months of age which were obtained from feral colonies. Faecal samples were collected prior to treatment for worm egg counts using the McMaster method with a saturated sugar solution. All the faeces excreted for 7 days after treatment were collected for the recovery of excreted helminths. The actual treatment of individual cats was controlled by the authors, while all laboratory examinations were conducted by other investigators who were blinded to the treatment groups.

Cats were treated with the recommended dosage rate per kg except that with Drontal Cat, the dose was tailored to the exact weight of each animal by fractioning the tablets while with Milbemax, the recommended size of tablet was applied according to the live weight scale. The treatment (piece/s of



Mouth of a Toxascaris

tablet) was included in a small amount of a pure pilchard canned food, which was the only food presented to the cat on treatment day. (When this had been consumed, additional food was supplied).

All cats were euthanased and autopsied 7 days after treatment. The small intestines were removed and processed for worm recovery. Worm counts were conducted by dissecting microscope. At least 10% of all helminths recovered were identified at species level.

## RESULTS

The efficacy was determined for each animal by expressing the number of worms excreted in the faeces after treatment as a percentage of the sum of worms excreted and recovered at autopsy. Efficacy of a treatment was calculated as the average of efficacy in all individual animals in a group.

During the conduct of the trial, it was determined that a majority of cats were infected with hookworm alone and very few with both ascarids and hookworms. The trial thus had to be completed utilising some cats only infested with hookworms, others with both and some with ascarids alone. The percent efficacy calculated as a mean of eight cats per group, showed 100% efficacy of both products against *Toxocara* spp. The efficacy against *Ancylostoma* spp. differs, with Drontal Cat showing 99,1% efficacy, while Milbemax was lower at 93,5%.

## SOUTH AFRICAN PET ANTHELMINTICS

PRODUCT	PRESENTATION	DOSAGE	SCALE (PP)
<b>Canex 4</b> 143 mg PP + 543 mg OP + 50 mg PZQ	Tablets	C: 1/4 tablet = 1,5 kg – 2,5 kg	23,8 mg/kg (1,5 kg) 14,3 mg/kg (2,5 kg)
		C: 1/2 tablet = 2,6 kg – 5 kg	27,5 mg/kg (2,6 kg) 14,3 mg/kg (5,0 kg)
		C: 1 tablet = 5,1 kg – 10 kg	28,0 mg/kg (5,1 kg) 14,3 mg/kg (10kg)
<b>Anteazole Dog Tablets</b> 50 mg PZQ +140 mg PP + 545 mg OP	Tablets	C: 1 tablet / 10 kg (140 mg/10 kg PP)	140 mg/kg (1 kg) 14,0 mg/kg (10 kg)
<b>Drontal Dog Tabs</b> Small/Medium/Large Small 15 mg PZQ + 75 mg Febantel + 43,2 mg PP	Tablets	C: 1/4 tab / 0,75 kg	14,4 mg/kg (0,75 kg)
		1/2 tab / 1,5 kg	14,4 mg/kg (1,5 kg)
		1 tab / 3,0 kg	14,4 mg/kg (3 kg)
<b>Anteazole Liquid</b> 50 mg PP/ml	Liquid	C: 1 ml / 10 kg	50 mg/kg (1 kg) 5 mg/kg (10 kg)
		F: 1 ml / 2,5kg	100 mg/kg (0,5 kg) 20 mg/kg (2,5 kg)
<b>Nemex Liquid</b> 50 mg PP/ml	Liquid	C: 1 ml / 10 kg	50 mg/kg (1 kg) 5 mg/kg (10 kg)
		F: 1 ml / 2,5 kg	100 mg/kg (0,5 kg) 20 mg/kg (2,5 kg)
<b>Anteazole Cat Tablets</b> 20 mg PZQ +230 mg PP	Tablets	F: 1 tablet / 4 kg (230 mg/4 kg PP)	460 mg/kg (0,5 kg) 57,5 mg/kg (4 kg)
<b>Drontal Cat</b> 20 mg PZQ + 230 mg PP	Tablets	F: 1/2 tablet / 2 kg	230 mg/kg (0,5 kg)
		1 tablet / 4 kg	115 mg/kg (1 kg) 57,5 mg/kg (2 kg) 57,5 mg/kg (4 kg)
<b>Anteazole Cat Paste</b> 80 mg PP + 250 mg N/ml	Paste	F: 1,5 ml / 2 kg (120 mg/2 kg PP)	240 mg/kg (0,5 kg) 60 mg/kg (2 kg)

PP = Pyrantel pamoate/embonate  
C = Canine

PZQ = Praziquantel  
F = Feline

OP = Oxantel pamoate

N = Niclosamide

## DISCUSSION

The results of this trial confirm the high efficacy (> 99%) for Drontal against ascarids and hookworms, as reported by other researchers. It is well known that the pyrantel embonate and praziquantel combinations are very safe and have a high therapeutic index. The dosage rate of pyrantel embonate as tested in this trial was 57,5 mg/kg which is the minimum recommended dosage.

When new products are developed and the minimum effective dosage of the active ingredient is established, the next decision is in which dosage form to present the product. A dilemma is often encountered tailoring the dosage presentation to a particular live weight range. In Table 1 the variance in dosage rates is illustrated with particular reference to pyrantel pamoate in several of the South African registered products.

In this trial 2 presentations of Milbemax were used and are marketed commercially to cater for live weight ranges of 500 g to 8 kg. Most of the cats treated during this trial weighed between 2 to 2,5 kg. According to the Milbemax recommendations all cats above 2,0 kg should have received 1/2 a **Milbemax for Cats** tablet which in our trial resulted in a dose rate of 3,2 to 4,0 mg/kg for cats in this weight range.

However some cats were initially treated with **Milbemax for small cats and kittens** (Milbemax (s.cat +k)) and the dosage rate was 2,0 to 4,0 mg/kg.

Those cats treated with the **Milbemax for small cats and kittens** at or near the lower rate of 2,0 mg/kg showed efficacy results against hookworms below 90%.

During 2001 following completion of similar comparative trials in dogs, a series of presentations with discussions were delivered to small animal practitioners. During these meetings it was clear that these clinicians encounter problems which are then often misinterpreted as e.g. product resistance, product efficacy failure. Clinicians base their evaluation of the efficacy on the state of the animal presented or faecal egg examinations. In many cases a patient is treated and then returns to a highly infective environment, to again be presented 3 – 4 weeks later with a heavy hookworm infestation.

When the results of efficacy trials are presented to clinicians, it is strongly recommended that the epidemiology of the worm infestations be emphasized as well as relevant information on the pharmacology of the active ingredients, plus the variance in efficacy results that can occur on an individual basis.



# Recent Investigation on the Prevalence of Gastrointestinal Nematodes in Cats from France and Germany

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## STUDY OBJECTIVES/INTRODUCTION

Between 1997 and 2001 four multi-centre-studies evaluating the efficacy and safety of different pharmaceuticals have been conducted in France and Germany according to the guidelines of Good Clinical Practice. Collection of faecal samples in the course of the multi-centre-studies was aimed at detecting cats positive for nematodes. Additionally, all cat faecal samples that were sent to the Institute of Parasitology between 1998 and 2002 in the course of routine diagnostic examination were analysed and compared to the study samples. Both sets of data were not raised according to a preplanned epidemiological study with a representative sample calculation and distribution but nevertheless they give valuable information of the current prevalence situation due to the high number of samples that was examined and to the fact that samples were collected nearly from all over France and Germany.

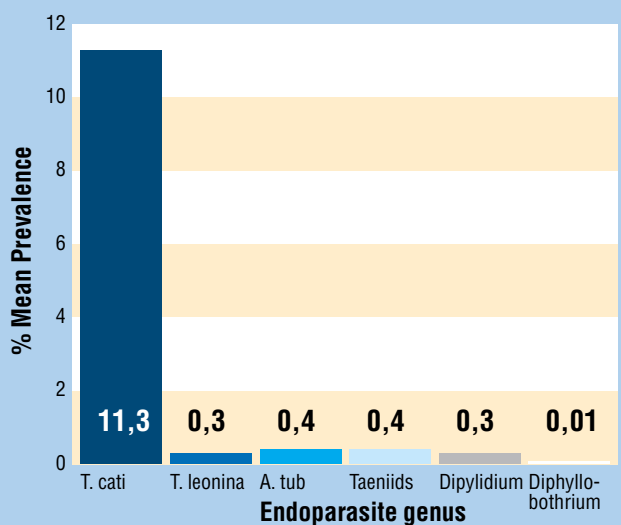
## MATERIAL AND METHODS

44 veterinary practices and two laboratories in France and Germany participated in the four multi-centre-studies during which approximately altogether 3500 cat faecal samples were examined. For coproscopical examination a modified McMaster-Technique was used. Only cats, that were tested positive for endoparasites were included in the studies.

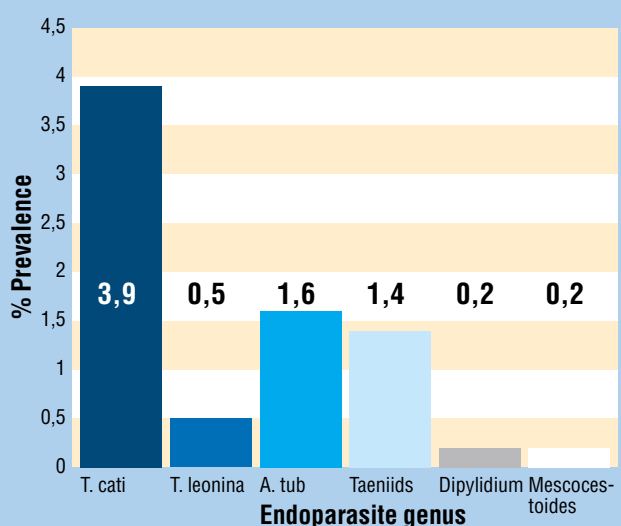
441 cat faecal samples sent to the Institute of Parasitology between 1998 and 2002 were examined. Routinely, the Institute's lab uses a combined sedimentation-flotation method with ZnSO<sub>4</sub> (see Rommel et al., 2000). Due to these differences concerning methods and motivation of these two data sets, protozoa remained disregarded and tapeworm stages were not included in the cumulative interpretation of the data.

For more detailed information about the cats tested positive for the multi-centre-studies a questionnaire was sent to the participating veterinarians subsequently. Practitioners were asked to answer questions about origin, environment, husbandry and frequency of anthelmintic treatment of the endoparasite positive animals.

Tab. 1 Overall Prevalences of Multi-Centre Studies



Tab. 2 Overall Prevalences of Cat Samples in the Institute



Dipylidium caninum



## RESULTS

The samples of the multi-centre-studies contained in 11,3% of the positive samples stages of *Toxocara cati*, 0,4% of *Ancylostoma tubaeformae*, 0,4% of taeniids, 0,3% of *Toxascaris leonina*, 0,3% of *Dipylidium* spp. and 0,1% of *Capillaria* sp. (Tab.1).

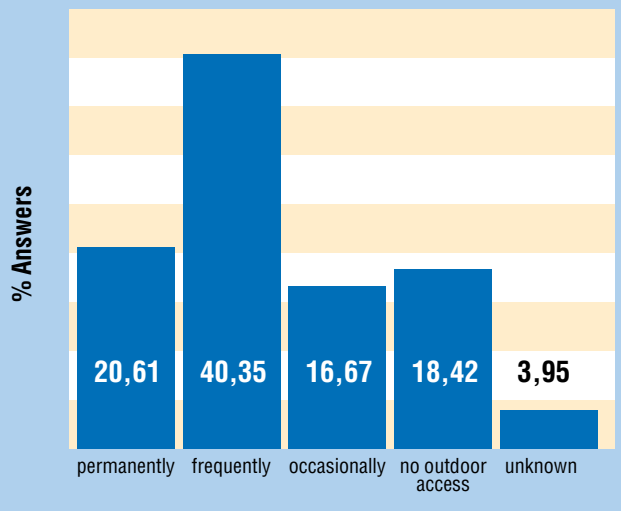
In 3,9% of the samples examined in the Institute of Parasitology *T. cati* eggs were found, 1,6% contained stages of *A. tubaeformae*, 1,4% of taeniids, 0,5% of *T. leonina* and 0,2% of *Dipylidium* spp. (Tab.2).

The questionnaire evaluation revealed that 59% of the cats for which the questionnaire was answered were of private households, 21% were farmcats, 11% belonged to boarding kennels and in 9% the origin was stated unknown. About 60% had daily or permanent access to outdoor environment, 17% went outdoors occasionally and approximately 20% of the helminth positive animals were housed indoors and did never have any access to the outdoor environment according to owners and veterinarians information (Tab. 3). About 80% of the cats, that were included in the multi-centre-studies were treated anthelmintically twice a year or rarer, 11% were dewormed 3 – 4 times per year and 2% more frequently (Tab. 4).

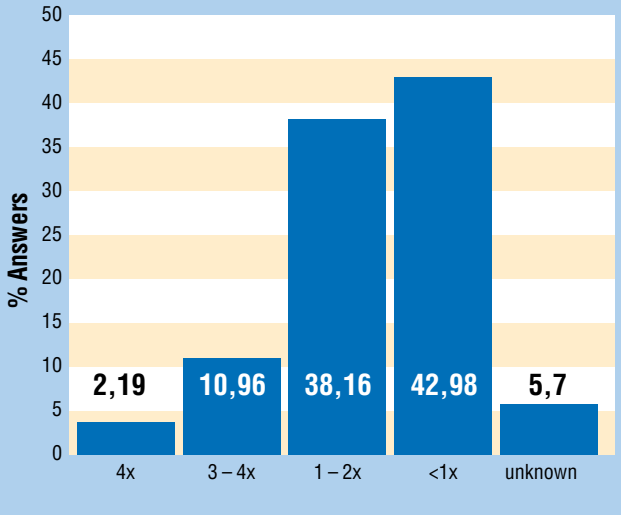
## CONCLUSION AND DISCUSSION

Surprisingly, about 20% of the cats, that had been tested positive for endoparasites and therefore took part in the multi-centre-studie never had any access to the outdoor environment. The etiology of these infections remains speculative – neither a mechanical transmission (by owners) of infective stages to the indoor cats as source of the infection nor difficulties in the

Tab. 3 Outdoor Access of Cats



Tab. 4 Anthelmintic Treatment of Cats



definition of “no outdoor access” in the questionnaire, meaning that owners or practitioners did not designate their private property (garden etc.) as “outdoors” could be excluded. However, such a high percentage is definitely surprising and a re-thinking about the current practice of excluding indoor cats automatically from routine anthelmintic treatment is strongly suggested.

Approximately 80% of cats tested positive and included in the study were treated 1 – 2 times a year or rarer. Since we can notice indirectly in these data that only anthelmintic treatment more than 3 times a year influenced the parasite population, a more frequent anthelmintic treatment regimen is advisable for exclusion of any contamination of the environment, especially for exclusion of any zoonotic infection. ●



# Endoparasites in Dogs and Cats in Germany 1999 – 2002

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## SUMMARY

Infections with endoparasites in dogs and cats were determined by analysing the results of faecal examinations (Flotation, MIFC, sedimentation, Baermann, smear, ProSpecT *Giardia* Microplate Assay). Samples of 8438 dogs and 3167 cats from the years 1999 until 2002 have been included in the investigation. Evaluation of the samples indicated that 2717 dogs (32.2%) and 771 cats (24.3%) were infected with endoparasites.

In the infected dogs the following parasites have been identified: Class Nematodea: *Toxocara canis*: 22.4%; *Toxascaris leonina*: 1.8%; Ancylostomatidae: 8.6%; *Trichuris vulpis*: 4.0%; *Capillaria* spp.: 2.3%; *Crenosoma vulpis*: 0.9%; *Angiostrongylus vasorum*: 0.3%; Class Cestodea: Taeniidae: 1.2%; *Dipylidium caninum*: 0.4%; *Diplopylidium/Joyeuxiella*: 0.1%; *Mesocestoides*: 0.2%; *Diphyllobothrium latum*: <0.1%; Class Sporozoea: *Sarcocystis* spp.: 9.0%; *Cystoisospora* spp.: 22.3%; *C. canis*: 8.0%; *C. ohioensis*: 17.0%; *Hammondia/Neospora*: 1.7%; Class Zoomastigophorea: *Giardia* spp.: 51.6%.

In the 771 infected cats the following prevalences of parasites were found:

Class Nematodea: *Toxocara mystax*: 26.2%; *Ancylostoma tubaeforme*: 0.3%; *Capillaria* spp.: 7.0%; *Aelurostrongylus abstrusus*: 2.7%; Class Cestodea: Taeniidae: 2.6%; *Dipylidium caninum*: 0.1%; Class Sporozoea: *Sarcocystis* spp.: 2.2%; *Cystoisospora* spp.: 21.9%; *C. felis*: 15.3%; *C. rivolta*: 7.9%; *Toxoplasma/Hammondia*: 4.5%; Class Zoomastigophorea: *Giardia* spp.: 51.6%.

## INTRODUCTION

Infections with endoparasites cause very different clinical symptoms depending on the parasite species and abundance. This may vary from mild gastrointestinal disorders with anorexia and low weight gain to weight loss, anaemia and dehydration. In severe cases developmental disorders or even mortality may occur. The development of safe broad spectrum anthelmintics has led to a more regular deworming of especially young animals, commonly without previous diagnosis. Whether or not this has led to a decrease in infection rates is

not known. The target of this study was to gain actual data on endoparasite infections (helminths and protozoa) in dogs and cats in Germany by analysing faecal checks from a diagnostic laboratory.

## MATERIAL AND METHODS

The results of parasitological examinations from the Veterinary Laboratory Freiburg from 8438 dogs and 3167 cats have been included in the analysis (Tab. 1).

Reason for submission of the samples were:

- routine check for annual vaccination,
- general health check,
- gastrointestinal disorders,
- not specified.

All samples have been investigated with following methods:

- Flotation (spec. grav. 1.3),
- MIFC (Merthiolate-Iodine-Formaldehyde-Concentration),
- Sedimentation and Baermann (only if sufficient material),
- Smear (in case of fluid faeces),
- Giardia* AG-ELISA (ProSpecT *Giardia* Microplate Assay) (most of the samples).

To examine the age dependence of the infection rates with endoparasites the data were statistically proved by the chi-square-test.

**Tab. 1** Endoparasite infections in dogs and cats

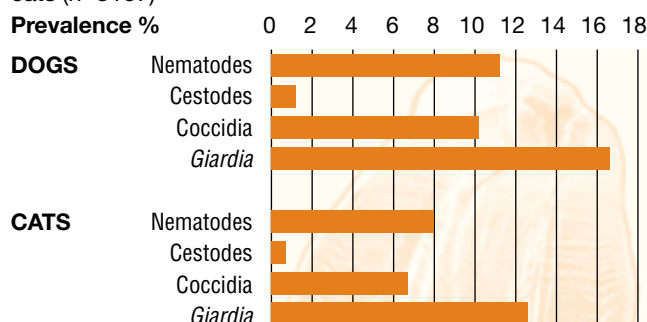
Year	No. of examined dogs		No. / share infected dogs		No. of examined cats		No. / share infected cats	
	n	n	n	%	n	n	%	
1999	2129	649	30.5		764	152	19.9	
2000	2014	702	34.9		758	197	26.0	
2001	2063	663	32.1		819	218	26.6	
2002	2232	703	31.5		826	204	24.7	
1999-2002	8438	2717	32.2		3167	771	24.3	

## RESULTS AND DISCUSSION

In dogs the most prevalent endoparasite (Fig. 1) was *Giardia* spp. (16.6% of 8438 dogs), followed by nematodes (11.2%), coccidia (10.4%) and cestodes (0.6%). Infections in cats showed a similar pattern (*Giardia* spp. in 12.6%, nematodes in 8.0%, coccidia in 6.7% and cestodes in 0.7% of 3167 cats).

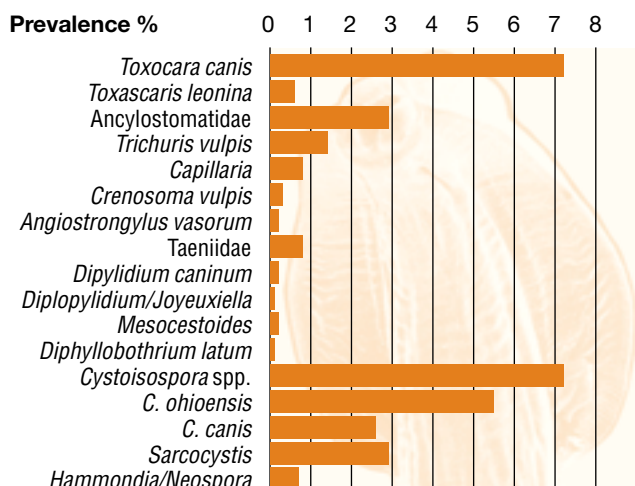
In the 2717 endoparasite positive (32.2%) of 8438 examined dogs eggs from *Toxocara canis*, *Toxascaris leonina*, Ancylostomatidae, *Trichuris vulpis*, *Capillaria* spp., Taeniidae, *Dipylidium caninum*, *Diplopylidium/Joyeuxiella*, *Mesocestoides* spp. and *Diphyllbothrium latum*, larvae from *Crenosoma vulpis* and *Angiostrongylus vasorum* as well as oocysts from *Cystoisospora ohioensis*, *C. canis*, *Hammondia heydorni/Neospora caninum* and *Sarcocystis* spp. have been identified (Fig. 2).

**Fig. 1** Infections with endoparasites in dogs (n=8438) and cats (n=3167)



To analyse the age dependence of the determined infection rates, all dogs with known age (n = 7113) have been grouped into four age groups (Tab. 2, 3). Dogs up to one year of age showed significantly higher infections rates with *Cystoisospora* spp. ( $p < 0.0001$ ), *Toxocara canis* ( $p < 0.0001$ ), *Trichuris vulpis* ( $p < 0.001$ ) and *Ancylostomatidae* ( $p < 0.0001$ ) compared to older dogs, whereas infections with *Sarcocystis* spp. and cestodes seemed to be equally distributed amongst all age groups. >>

**Fig. 2** Prevalence (%) of helminths and coccidia in dogs (n=8438)



**Tab. 2** Prevalence of *Cystoisospora* spp. and *Sarcocystis* spp. per age group of examined dogs

Age (years)	Examined dogs with known age(n)	<i>Cystoisospora</i> positive dogs		<i>Sarcocystis</i> positive dogs	
		No. (n)	Share (%)	n	%
up to 1	2933	435	14.8	92	3.1
>1 – 5	2286	43	1.9	68	3.0
>5 – 10	1365	14	1.0	31	2.3
>10	529	7	1.3	13	2.5
total	7113	499	7.0	204	2.9

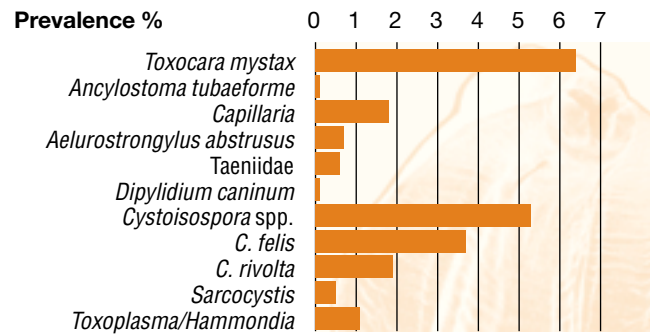
**Tab. 3** Prevalence of *Toxocara canis*, *Trichuris vulpis* and hookworms per age group of examined dogs

Age (years)	Examined dogs with known age(n)	<i>Toxocara</i> positive dogs		<i>Trichuris</i> positive dogs		Ancylostomatidae positive dogs	
		No. (n)	Share (%)	n	%	n	%
up to 1	2933	389	13.3	52	1.8	111	3.8
>1 – 5	2286	81	3.5	26	1.1	55	2.4
>5 – 10	1365	34	2.5	8	0.6	20	1.5
>10	529	10	1.9	0	0	12	2.3
total	7113	514	7.2	86	1.2	198	2.8

In the 771 endoparasite positive (24.3%) of 3167 examined cats eggs of *Toxocara mystax*, *Ancylostoma tubaeforme*, *Capillaria* spp., *Taeniidae* and *Dipylidium caninum*, larvae from *Aelurostrongylus abstrusus* as well as oocysts from *Cystoisospora felis*, *C. rivolta*, *Toxoplasma gondii*/*Hammondia hammondi* and *Sarcocystis* spp. have been found (Fig. 3).

In order to analyse the age dependence of the infection rates, all cats with known age have been grouped into four age groups (Tab. 4). Cats up to one year old showed significantly higher infection rates with *Cystoisospora* spp. ( $p < 0.0001$ ) and *Toxocara mystax* ( $p < 0.0001$ ) compared to older cats, whereas infections with *Toxoplasma gondii*/*Hammondia hammondi* and *Sarcocystis* spp. seemed to be equally distributed in all age groups.

**Fig. 3** Prevalence (%) of helminths and coccidia in cats (n=3167)



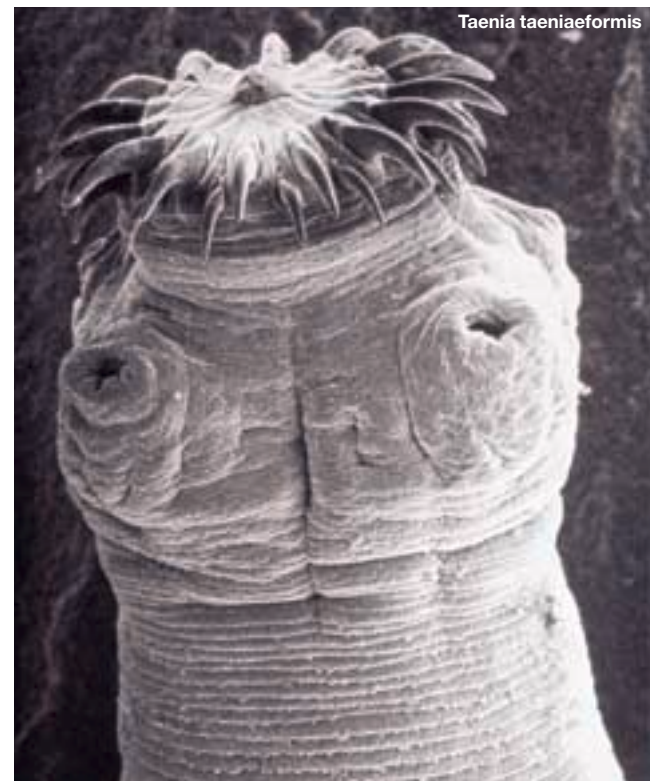
**Tab. 4** Prevalence of *Cystoisospora* spp., *Sarcocystis* spp., *Toxoplasma gondii* and *Toxocara mystax* per age group of examined cats

Age (years)	Examined cats with known age (n)	<i>Cystoisospora</i> positive cats		<i>Sarcocystis</i> positive cats		<i>Toxoplasma</i> positive cats		<i>Toxocara</i> positive cats	
		No. (n)	Share (%)	n	%	n	%	n	%
up to 1	1229	101	8.2	9	0.7	12	1.0	117	9.5
>1 – 5	495	20	4.0	5	1.0	5	1.0	30	6.1
>5 – 10	375	7	1.9	0	0	2	0.5	7	1.9
>10	374	6	1.6	1	0.3	3	0.8	4	1.1
total	2473	134	5.4	15	0.6	22	0.9	158	6.4

Although safe broad spectrum anthelmintics are used more or less routinely today, helminth infections – especially with ascarids in young dogs and cats up to one year – are still prevalent. In addition, infections with *Giardia* and protozoa are found even more often, both diseases which have only little awareness amongst pet owners. The relatively low percentage of infection rates with tapeworms must be questioned as the flotation method is not sensitive enough to give a realistic figure about the prevalence of cestodes.

## CONCLUSIONS

- The results demonstrate high prevalences of endoparasites in dogs and cats in Germany.
- Both young and adult dogs and cats should be routinely checked by faecal examination for endoparasite infections.
- In case of gastrointestinal disorders protozoan infections with *Giardia* and coccidia should be considered.
- A coproantigen-test for *Giardia* is recommended.
- Dogs and cats which are at risk for cestode infections based on their lifestyle should be dewormed on a regular basis even without positive faecal diagnosis. ●



# Synergistic Effects of Pyrantel and the Febantel Metabolite Fenbendazole on Adult *Toxocara canis*



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## SUMMARY

Pyrantel embonate and febantel are both constituents of Drontal® Plus and Drontal® Puppy broad spectrum anthelmintics for dogs. The effects of pyrantel and the febantel metabolite fenbendazole were investigated against *Toxocara canis* in-vitro by studying changes in worm motility and tissue damage. Pyrantel and fenbendazole were added to worms incubated in media for 8 h at the following concentrations: pyrantel: 12,2 µg, 25 µg, or 50 µg; fenbendazole: 50 µg, 100 µg or 200 µg; mixture of pyrantel and fenbendazole: 12,2 µg p + 50 µg f, 25 µg p + 100 µg f or 50 µg p + 200 µg f. Following this 8 h incubation period, one group of the worms was immediately fixed and studied by light- and electron microscopical examination. Other groups have been observed for further 8 h periods up to 56 hours and then studied in the same way.

## RESULTS

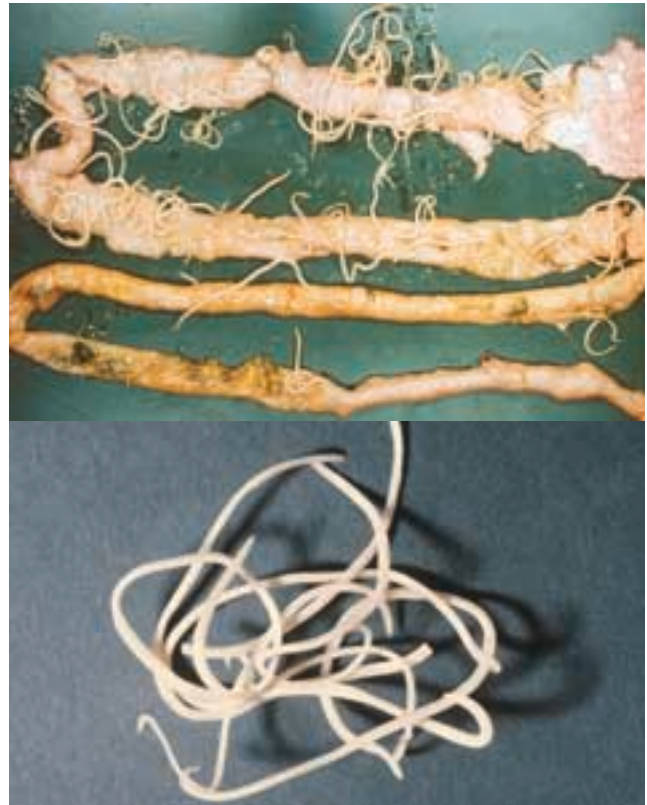
There were no significant differences observable regarding motility changes between pyrantel or fenbendazole given as individual compounds and the pyrantel/fenbendazole combination. By contrast if the worms had been incubated with the combination, severe irreversible tissue damages could be observed after shortest periods and they were more severe even in lowest concentration as compared to the single components in highest concentration (Tab 1 – 4). Also, the number of damaged organs was higher in worms kept in the combinations as compared to the single compounds. In this *in-vitro*-analysis the concentrations chosen matched the *in-vivo*-situation, so it can be concluded, that similar synergistic effects can be seen after Drontal® Plus or Drontal® Puppy treatment in dogs with similar lethal effects on the worms.



Scanning electron micrograph of the anterior end of a *T. canis* worm



Intestine with *T. canis* worms (top); Bundle of *T. canis* worms (bottom)



**Table 1** Damage level of control worms following light and electron microscopical examination

Group/concentration	Time (h)	Hypo-dermis	Longitudi- nal muscles	Intestine	Nerve axons	Sexual system
A1 0 µg/ml	8	0	0	0	0	0
	32	0	0	0	0	0
	56	0	0	0	0	0
A2 DMSO	8	0	0	0	0	0
	56	0	0	0	0	0

**Table 2** Damage level of worms after incubation in medium containing pyrantel

Group/concentration	Time (h)	Hypo-dermis	Longitudi- nal muscles	Intestine	Nerve axons	Sexual system
B1 12,2 µg/ml	8	0	0	0	0	0
	32	0-1	0-1	1	1-2	0
B2 25 µg/ml	8	0	1	0-1	1	0
	56	0	1	1-2	1-2	0
B2 50 µg/ml	8	0	1	0-1	1	0
	32	0-1	1	1	1-2	0

**Table 3** Damage level of worms after incubation in medium containing fenbendazole

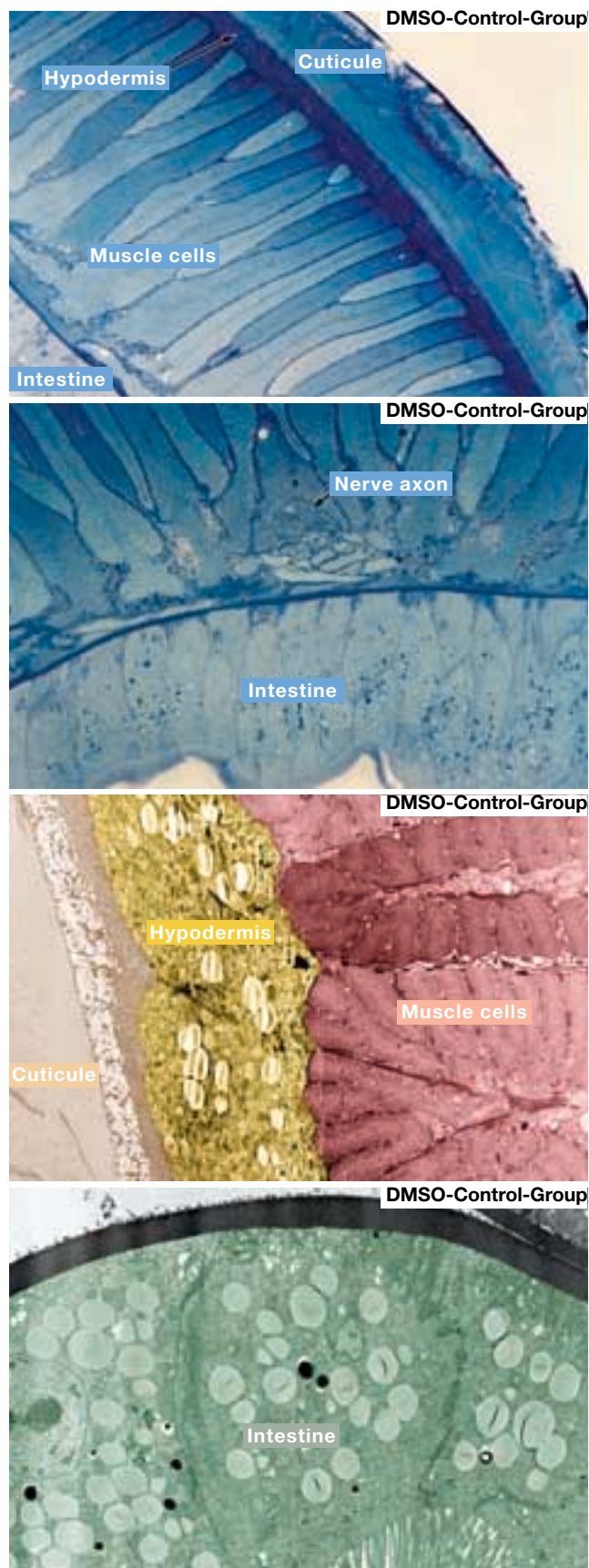
Group/concentration	Time (h)	Hypo-dermis	Longitudi- nal muscles	Intestine	Nerve axons	Sexual system
B4 50 µg/ml	8	0	0	0	0	0
	32	1	0-2	1-2	1	0
B5 100 µg/ml	8	0	0	0-1	0-1	0
	56	1	1	1-2	1	0
B6 200 µg/ml	8	0	0	1-2	1	0
	32	1-2	1-2	2-3	2-3	1

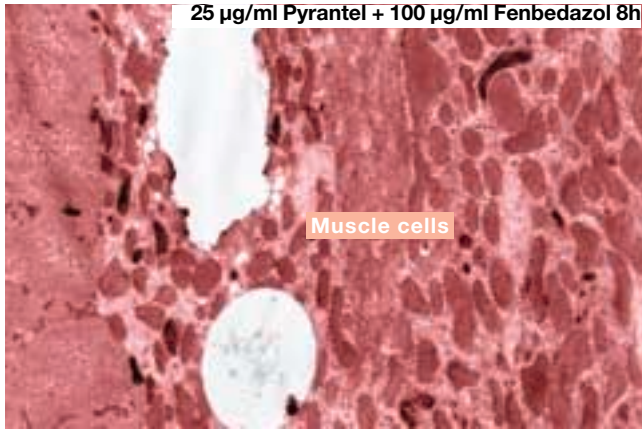
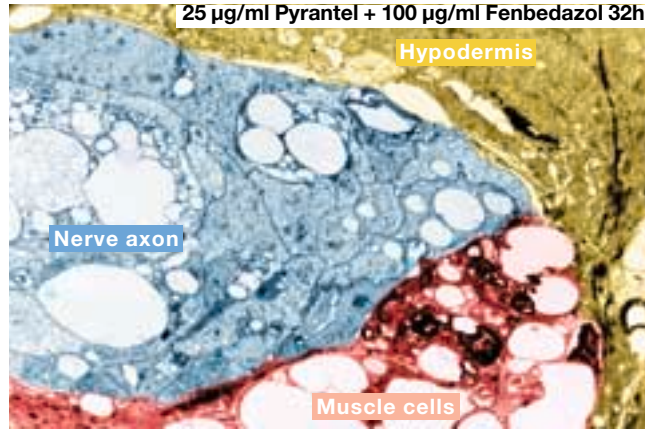
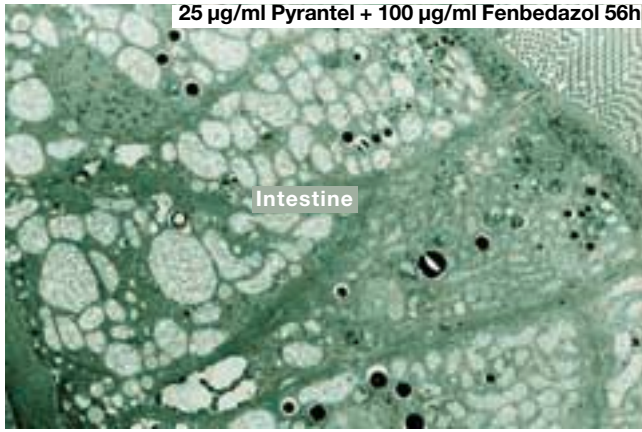
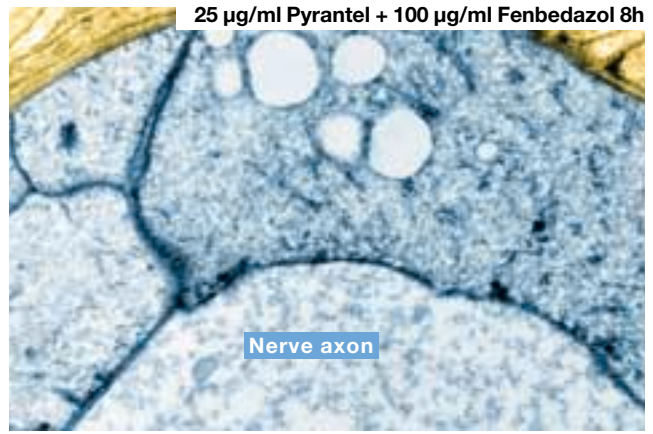
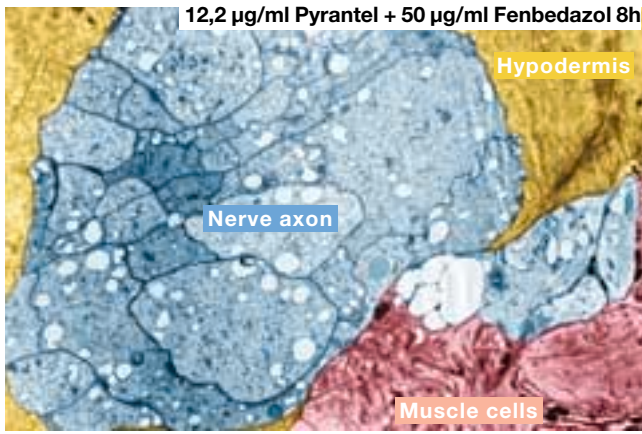
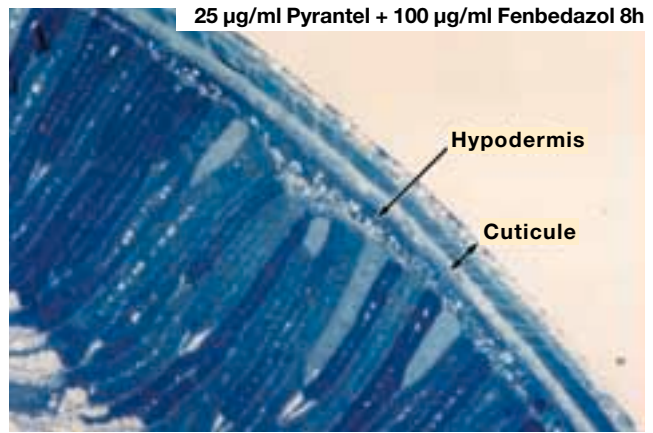
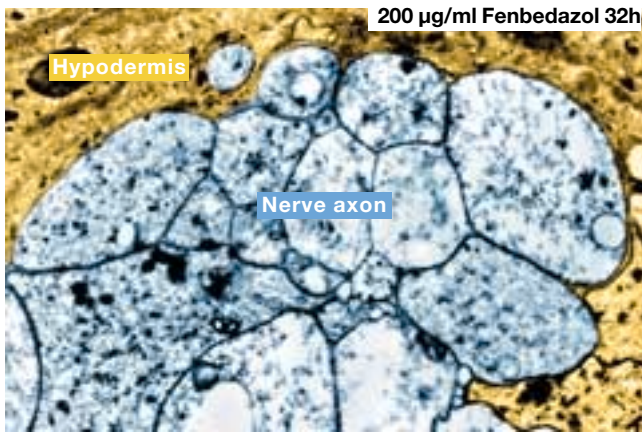
**Table 4** Damage level of worms after incubation in medium containing a **combination** of pyrantel and fenbendazole

Group/concentration	Time (h)	Hypo-dermis	Longitudi- nal muscles	Intestine	Nerve axons	Sexual system
B7 12,2 µg/ml Pyrantel + 50 µg/ml Fenbendazole	8	0	0	0	0-1	0
	32	1	1	1-2	1-2	0-1
	56	0	1-2	2	2-3	1
B8 25 µg/ml Pyrantel + 100 µg/ml Fenbendazole	8	0	0	1-2	2	1
	32	1-2	1-2	2-3	2	1
	56	2-3	2-3	3	3	2-3
B9 50 µg/ml Pyrantel + 200 µg/ml Fenbendazole	8	0	1	1-2	1-2	1
	32	1	2-3	3	2-3	2
	56	2-3	2-3	3	3	2-3

0 = no damage, 1 = slight damage, 2 = increasing damage (irreversible), 3 = severe damage

**Bold numbers:** Synergistic effect by combining the two compounds





## CONCLUSION

If *Toxocara canis*-worms are incubated in media containing a combination of pyrantel and fenbendazole, lethal damage is achieved faster and at lower doses as compared to incubation in pyrantel or fenbendazole alone in higher doses. ●



## Prevalence of *Giardia* spp. in Dogs and Humans in Northern and Central Italy

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**G***iardia* spp. is a cosmopolitan flagellate which infects the small intestine of several animals and also humans. During the last decade, *Giardia* has gained momentum both as a dog and a human pathogen and giardiasis is nowadays considered as a re-emerging infectious disease. The prevalence in dogs ranges from 5 to 80% according to the age of dogs, management system and colony size (Traldi and Castiglione, 1993; Nolan and Smith, 1995; Mochizuki et al., 2001). In humans, children, HIV-positive subjects or persons living in poor hygienic conditions (Kotler and Orestein, 1998; Ballone et al., 2001) are frequently infected. According to the most recent research, some *Giardia* genotypes may be shared by human and

*Giardia* cysts

dogs, particularly those belonging to the Assemblage A, Cluster A-I, revealing the possibility of an *interspeciem* transmission mainly in urban areas (Graczyk et al., 1999; Thompson et al., 2000; van Keulen et al., 2002). Despite the public health importance and the possible zoonotic role of *Giardia*, epidemiological surveys on the prevalence of giardiasis in Italy are lacking and fragmentary, compared to other countries.

The objective of this study was to determine the prevalence of *Giardia* in dogs and humans in Abruzzo and Veneto regions, (central and northern Italy, respectively) and, when possible, to identify risk factors (habitat, age, infection evolution, sex, breeding) associated with dog's infection.



A total of 916 faecal samples were examined, 616 from dogs (436 from central Italy, 180 from northern Italy) and 300 from humans. Epidemiological data of dogs concerning life style (owned, kennel or stray dogs), sex, breed, age, presence of gastrointestinal symptoms, were collected. Faecal samples of humans were kindly obtained from the local Hospital of Abruzzo region. Two coprological tests were performed in order to maximize the chance of finding *Giardia* cysts: a centrifugation and flotation technique, combined with a sedimentation technique in Abruzzo region, and with MIF procedure (Meridian Diagnostic Inc.) in the Veneto region. The prevalence differences in relation to epidemiological data were tested by  $\chi^2$  test. The strength of the relation between giardiasis and potential risk factors was estimated by the Prevalence Ratio (PR), and its importance for the population was expressed by the Attributable Proportion for the exposed dogs (APe).

The overall prevalence was 21.3% in dogs and 2% in humans. No differences were noted between the prevalence in dogs of central and northern Italy (22.2% and 19.4%, respectively). The major risk factors for giardiasis in dogs were represented by density of dogs and age, i.e. increased prevalence was significantly associated with kennel dogs (PR=2.13, APe=53%) and young dogs (PR=1.99, APe=50%). Higher prevalence was noted also in dogs with gastrointestinal symptoms (PR=1.86, APe=46%). The findings of this study reveals that the prevalence of *Giardia* in dogs in northern and central Italy is high, and probably underestimated. The prevalence in dogs overlaps the rates obtained in other countries (Oliveira-Sequeira et al., 2002) and seems to be higher than those registered in Umbria (Piergili Fioretti e Moretti, 1989) and Lazio regions (Rosso et al., 1989).

The high prevalence of *Giardia* in kennel dogs confirms that confinement in a limited area is an important risk factor: contamination of such environment readily occurs through infected animals, thus providing a continuous source of infection to susceptible animals. Other contributing factors may be the stress and the unbalanced diet. The age is also confirmed as an important risk factor: dogs less than 1 year old were statistically more infected than adults.

The relationship between symptoms and presence of *Giardia* seems to be strict and the dogs may be considered, in analogy with humans, very sensitive to its pathological effects.

Prevalence in humans overlaps the survey carried out in other industrialized countries (Fontaine et al., 1984) including Italy (Ballone et al., 2001).

The high level of giardiasis in dogs in the Abruzzo and Veneto regions demonstrates that they may be considered a significant potential reservoir of infection for humans even though, in our

conditions, such a role for dogs has hitherto been unknown. Molecular techniques will have a major impact for understanding the epidemiology of *Giardia* infections, and the molecular characterization of canine and human genotypes of *Giardia* – which is in progress in this study – may add more information on the zoonotic risk of animal origin. Looking forward to obtain more information in this respect, screening of dogs for giardiasis, particularly puppies, and their treatment to prevent eventual human infection, is highly recommended. ●

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# Humans, Dogs and Parasitic Zoonoses – Unravelling the Relationships in a Remote Endemic Community in Northeast India using Molecular Tools

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## ABSTRACT

Canine parasitic zoonoses pose a continuing public health problem, especially in developing countries and communities that are socio-economically disadvantaged. Our study combined the use of conventional and molecular epidemiological tools to determine the role

of dogs in transmission of gastrointestinal (GI) parasites such as hookworms, *Giardia* and *Ascaris* in a parasite endemic teagrowing community in northeast India. A highly sensitive and specific molecular tool was developed to detect and differentiate the zoonotic species of canine hookworm eggs directly from faeces. This allowed epidemiological screening of canine hookworm species in this community to be conducted with ease and accuracy. The zoonotic potential of canine *Giardia* was also investigated by characterising *Giardia duodenalis* recovered from humans and dogs living in the same locality and households at three different loci. Phylogenetic and epidemiological analysis provided compelling evidence to support the zoonotic transmission of canine *Giardia*. Molecular tools were also used to identify the species of *Ascaris* egg present in over 30% of dog faecal samples. The results demonstrated the role of dogs as a significant disseminator and environmental contaminator of *Ascaris lumbricoides* in communities where promiscuous defecation practices exist. Our study demonstrated the usefulness of combining conventional and molecular parasitological and epidemiological tools to help solve unresolved relationships with regards to parasitic zoonoses.

## BACKGROUND

Molecular epidemiological tools have formed an integral part of most epidemiological studies. It adds another dimension to studies of disease aetiology and provides information that classical epidemiology cannot (Thompson, 2000). In this study we developed and applied molecular tools in combination with classical parasitological and epidemiological methods to detect, diagnose and genetically characterise infectious agents, such as *Ascaris*, *Giardia* and canine hookworms. Molecular tools were utilised to accurately determine parasite prevalence,

increase the understanding of transmission dynamics and provided important information on the zoonotic potential of these parasites in this community.

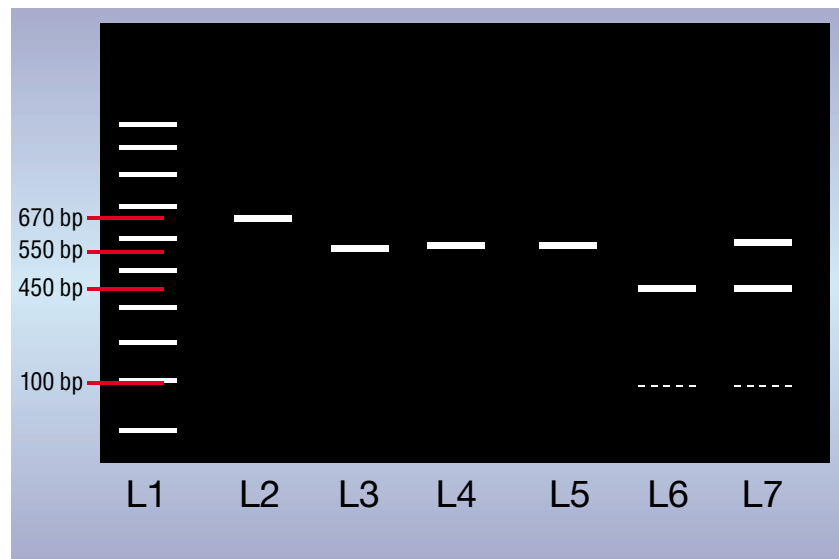
Faecal samples were collected from a total of 101 dogs and 328 humans from three separate tea estates in Assam, India. The majority of the population live in poor, overcrowded conditions, are uneducated and practice poor standards of hygiene. The close relationship shared with dogs coupled with a lack of veterinary attention places this community at a high risk of acquiring zoonotic diseases. Humans were found heavily infected with geohelminths (*Ascaris*, *Trichuris* and hookworms). Ninety-nine percent of dogs were found positive for at least one zoonotic GI parasite, with hookworms being the most common. Parasites presumed to be host specific for humans such as *Ascaris* were also encountered in over 30% of dog faeces.

## MOLECULAR RESULTS AND DISCUSSION

96% of dogs were positive for hookworms using conventional microscopy. A PCR technique based on nucleotide differences in the ITS regions of canine *Ancylostoma* was developed and applied to detect and distinguish hookworm eggs of zoonotic significance, directly from canine faeces. Two separate PCRs were utilised to distinguish between *A. caninum* and *A. braziliense* from *A. ceylanicum* based on the difference in size of amplified products (Fig 1). Since the PCR for *A. caninum* and *A. braziliense* cross-reacted and the amplified products were identical in size, an RFLP was utilised to distinguish between the two species using restriction enzyme BStN1 (Fig 1). The PCRs showed 97% sensitivity and proved highly specific. Out of the 101 dogs sampled 72% were found positive for *A. caninum*, 60% positive for *A. braziliense* and 37% had mixed infections. No *A. ceylanicum* was found in the dogs. The overall prevalence of canine hookworms using a combination of microscopy and PCR was 98%, proving dogs to pose a high zoonotic risk with regards to hookworms in this community. This molecular technique allowed epidemiological screening of canine hookworm species to be conducted with ease and accuracy (Traub *et al.*, In preparation).

A nested PCR was utilised to amplify a 130 bp region of the SSU-rDNA gene of *Giardia* (Read *et al.*, 2002) and to screen canine faeces in this community. Twenty percent of dogs were found to be positive for *Giardia* by PCR compared to 3% using conventional microscopy. In order to determine the zoonotic potential of canine *Giardia* in this community, *Giardia* isolates from positive dogs and humans were further amplified at two additional genetic loci, the elongation factor 1- $\alpha$  (ef1- $\alpha$ ) and triose phosphate isomerase (tpi) genes using a simple direct PCR method. Phylogenetic analysis of the tpi gene provided better genetic resolution over the SSU-rDNA and ef1- $\alpha$  genes and clearly placed canine *Giardia* isolates within the genetic groupings of human isolates (Assemblages A and B). Further evidence for zoonotic transmission was supported by recovery of genetically similar genotypes of *Giardia* isolates from dog and humans living in the same household. Humans belonging to a household in which at least one dog was infected with *Giardia* were also significantly more likely to be infected with *Giardia* (odds ratio 3.01, 95% CI, 1.11, 8.39) than individuals that did not reside with infected dogs. We therefore provide strong molecular and epidemiological evidence to support the zoonotic potential of canine *Giardia* in this remote tea-growing community in Assam (Traub *et al.*, Submitted March 2003).

Finally, a PCR-RFLP technique based on a four base pair nucleotide difference in the first and second ITS region of *A. lumbricoides* and *A. suum* was utilised to determine the species of *Ascaris* eggs in dog faeces. *Ascaris* eggs in dog faeces were identified as human derived *A. lumbricoides*. The frequent finding of these eggs in high intensities in dog faeces suggested that dogs were acting as significant mechanical transmitters of *Ascaris* for humans. Multifactorial analyses showed that *Ascaris*-positive dogs were more likely to belong to a household where at least one member defecated outdoors. Moreover, most dogs were allowed to roam freely. This indicated that dogs were ingesting their owners' faeces and disseminating *Ascaris* infection by increasing the net exposure of infective stages in contact with the human population. (Traub *et al.*, 2002)



**Figure 1** Determination of the species of hookworm eggs in canine faeces using PCR-RFLP analysis

L2, L3 and L4 display undigested PCR amplified products of ITS regions of *A. ceylanicum* (670 bp), *A. caninum* (550 bp) and *A. braziliense* (550 bp) respectively. L5, L6 and L7 display the PCR products of *A. caninum* (uncut), *A. braziliense* (cut) and a mixed infection with *A. caninum* and *A. braziliense* after digestion with BStN1, respectively.

Our study demonstrates the invaluable information molecular tools can provide when combined with classical parasitological and epidemiological methods to help unravel yet unresolved relationships with regard to parasitic zoonoses. ●

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# Population Biology Studies on *Isospora suis* in Piglets

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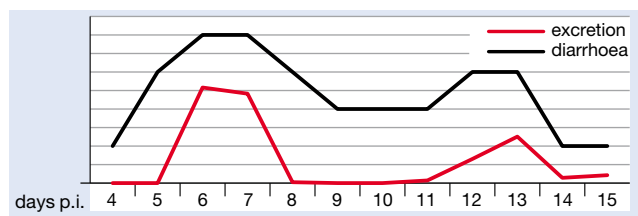
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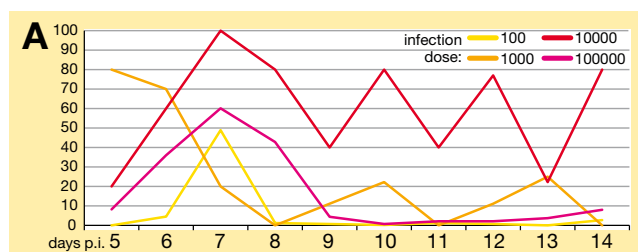
The protozoan *Isospora suis* is one of the most common enteropathogens in intensive piglet production worldwide (as reviewed by Mundt and Dauschies 2000). Around four to five days after the ingestion of infectious oocysts and the subsequent invasion of epithelial cells in the small intestines, affected animals frequently show pasty to watery diarrhoea for 3 – 7 days. Although animals of all age groups are susceptible to infection only suckling piglets in the first three weeks of life develop clinical signs of disease (for refs., see Robinson et al., 1983, Martineau and de Castillo 2000 and others). However, from field observations it is obvious that not all animals in one litter are equally affected, although it can be assumed that the infection pressure should be equal for all piglets of the same litter (Meyer et al.; 1999, Martineau and de Castillo, 2000).

Under standardised, experimental conditions the qualitative infection model (faecal consistency/oocyst excretion) is highly reproducible. Starting from a standard model (10,000 oocysts/piglet on the third day of life) several modifications and their influence on clinical and parasitological parameters were investigated. Individual excretion rates are highly variable between animals, although the course of infection in individuals usually follows the course of diarrhoea starting on day 5 – 7 followed by excretion of oocysts 1 – 2 days later. Both curves are usually double-peaked (Fig. 1).

**Fig. 1** Example of the course of isosporosis in individual animals in a standard model



**Fig. 2** Influence of the different infection doses on the percentage of piglets excreting oocysts (A) and showing clinical signs (B). Data compiled from Wüstenberg 2003 (100 and 100,000 oocysts) and Joachim et al. 2003 (1000 and 10,000 oocysts).



## 1. INFLUENCE OF INFECTION DOSE

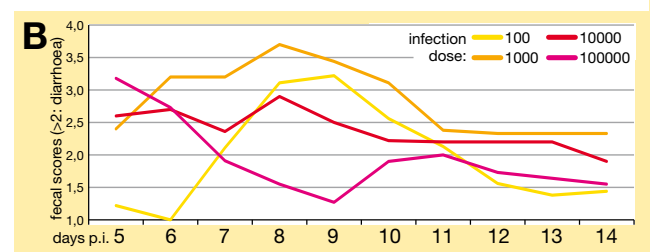
Although the manifestation of coccidial infections is usually dose-dependent, different trials conducted under the same standardised conditions with the same *I. suis*-strain revealed no clear correlation between infection dose and qualitative excretion rates or diarrhoea (Fig. 2). This is supported by Christensen and Henriksen (1994) who demonstrated that the excretion rates of litter infected with 100 – 10,000 oocysts did not differ significantly.

## 2. INFLUENCE OF AGE

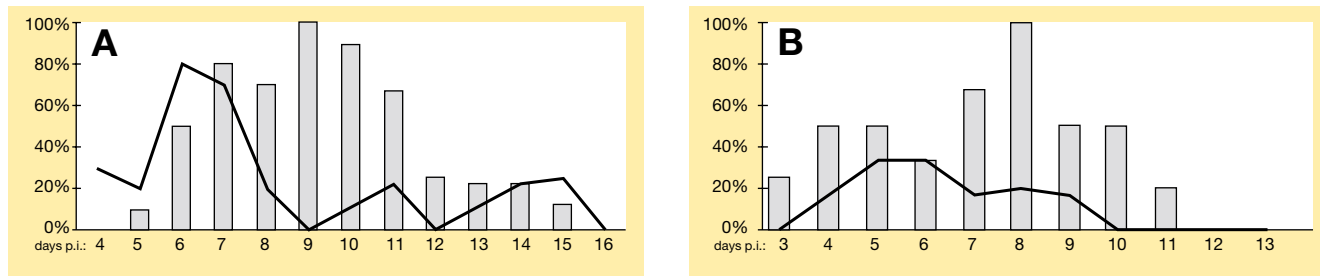
In contrast to that, age seems to be an important factor for the development of the disease; piglets infected on day three of life were less affected than those infected on day 11 (Fig. 3). Similarly, when piglets are infected on day 3 vs. day 7, the cumulative excretion and diarrhoea rates indicate a slower onset of infection for older animals (Fig. 4). Although these data are limited they support the findings of Stuart et al. (1982) and Koudela and Kucerová (1999) that piglets develop an age-related resistance to the infection which results in lower excretion rates and less pronounced diarrhoea in older animals.

## 3. INFLUENCE OF INITIAL INFECTION

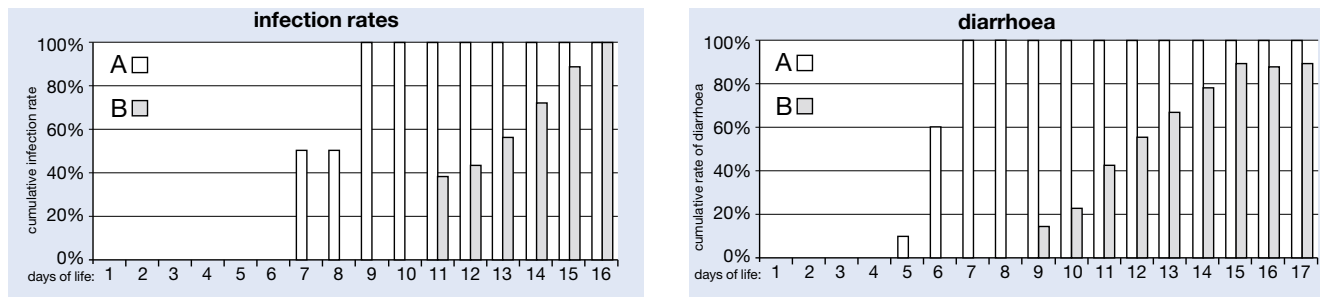
Since under natural conditions litter mates appear to become infected at different time points, a low initial infection (2/11 piglets infected with 100 oocysts each on day 3 of life) and a high initial infection (10/20 piglets infected with 1000 oocysts each on the third day of life) were applied to mimic this situation and it could be demonstrated that a higher initial infection results in a fast and complete distribution of the infection within the litter, whereas a lower infection pressure initiates a slower and incomplete spread of the disease (Fig. 5).



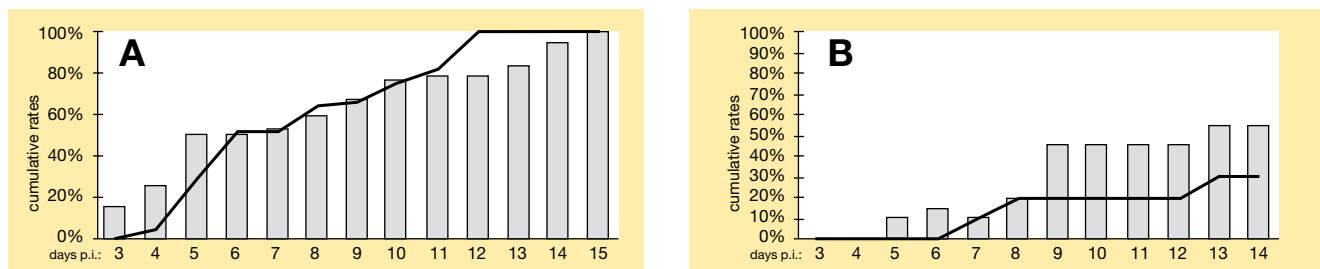
**Fig. 3** Influence of the age on the course of isosporosis: Piglets infected with 1000 oocysts on the third day of life (A) excreted more oocysts than those infected on day 11 (B). Diarrhoea was less prevalent in group B. lines: oocyst excretion, bars: diarrhoea



**Fig. 4** Influence of age on the cumulative rates of infection and diarrhoea. Group A (infection on day 3 of life) developed complete patent infections by day 6 post infection, while in group B (infection on day 7 of life) the complete patent infection established only on day 9 post infection. Similarly, diarrhoea was present in all animals by day 13 post infection in group A, while in group B only 87.5% of the animals developed clinical signs during the time of examination.



**Fig. 5** Effect of high (A) and low (B) initial infection rates in different litter. In litter where half of the piglets were infected with 1000 oocysts all piglets developed diarrhoea by day 12 post infection (p.i.) and started to shed oocysts by day 15 p.i. In contrast, when only 18% of the piglets were infected with 100 oocysts each the infection did not fully spread through the litter. bars: excretion, line: diarrhoea



These studies support the assumption that minimising the initial infection pressure is a key measure to control clinical manifestation of isosporosis. This can be achieved by strict disinfection regimen and/or metaphylactic treatment of the piglets with anticoccidials that effectively suppress the excretion of oocysts. Utilising these measures the spread of the infection can be decelerated until the piglets have developed sufficient resistance to the infection and clinical signs are minimal.

The efficacy of disinfection or treatment or a combination of both under the aspect of the parasite's population biology will require further investigations. ●

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# Studies on the Efficacy of Toltrazuril, Diclazuril and Sulphadimidine against Artificial Infection with *Isospora suis* in Piglets

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## INTRODUCTION

*Isospora suis*, the causal agent of piglet coccidiosis, is a prevalent and economically important parasite. This study was undertaken to examine the therapeutic potential of various substances.

obtained from a piglet-rearing farm in northern Germany. The animals were treated orally with either toltrazuril (Baycox® 5%), diclazuril (0.25% suspension and 3% suspension), sulphadimidine or remained as infected untreated controls (table 1).

## MATERIALS AND METHODS

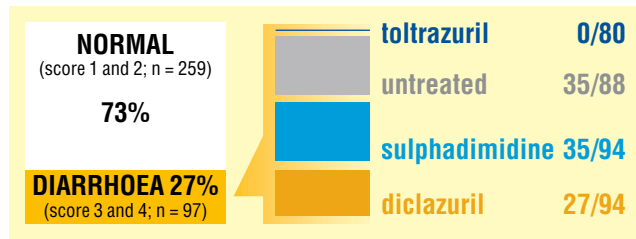
The investigations were carried out with piglets infected artificially with *I. suis* in three blinded studies. The sows were housed conventionally. In each study 8 to 12 piglets per group were allocated to four treatment groups on a random basis. At the age of 3 days all the piglets were infected orally with 10<sup>4</sup> sporulated oocysts of a pathogenic field isolate of *I. suis*

Faecal consistency (score: 1 firm and formed, 2 pasty, 3 semi-liquid, 4 liquid) and oocyst excretion (opg: oocysts per gram of faeces) were determined from individual rectal faecal samples taken once daily in the morning on the day of infection (day 3) and from days 7 to 14 of life. Oocyst determination was carried out by flotation in saturated sodium chloride solution with glucose and counting in a McMaster chamber. Liveweight development was measured on days 0, 7, 14, 21, 28. Measurement of villi length was performed histologically.

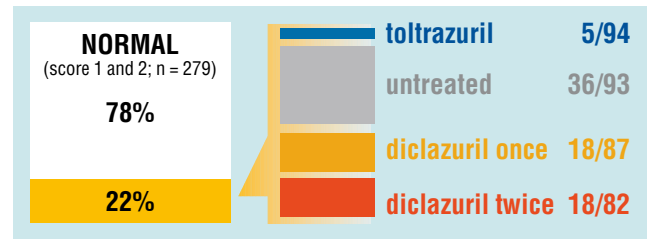
**Table 1** Experimental design

Study	Group	Treatment	Dose	Frequency / age (days of life)	Parameters
1	A	tap water	1 ml	5	Clinical picture, faecal consistency, oocyst excretion, body weight.
	B	toltrazuril	20 mg / kg	5	
	C	diclazuril	2 mg / kg	5 and 6	
	D	sulphadimidine	200 mg / kg	5, 6 and 7	
2	A	tap water	1 ml	5	Study 2 primarily: villi length, patho-morphological alterations
	B	toltrazuril	20 mg / kg	5	
	C	diclazuril	2 mg / kg	5 and 6	
	D	sulphadimidine	200 mg / kg	5, 6 and 7	
3	A	tap water	1 ml	5	
	B	toltrazuril	20 mg / kg	5	
	C1	diclazuril	15 mg / kg	5	
	C2	diclazuril	15 mg / kg	5 and 12	

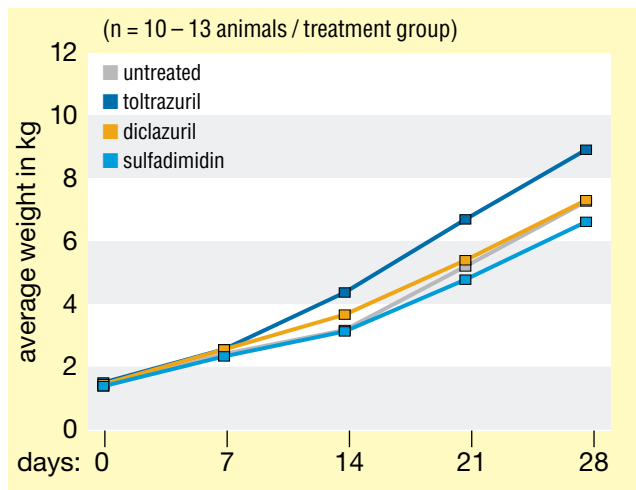
**Fig. 1** Faecal consistency days 7 to 14 (study 1; n = 356 samples)



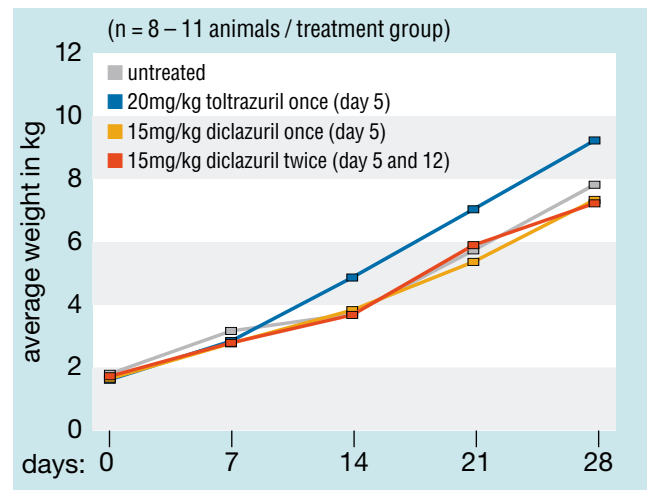
**Fig. 2** Faecal consistency days 7 to 14 (study 3; n = 356 samples)



**Fig. 3** Average body weight gain of piglets (study 1)



**Fig. 4** Average body weight gain of piglets (study 3)



## RESULTS

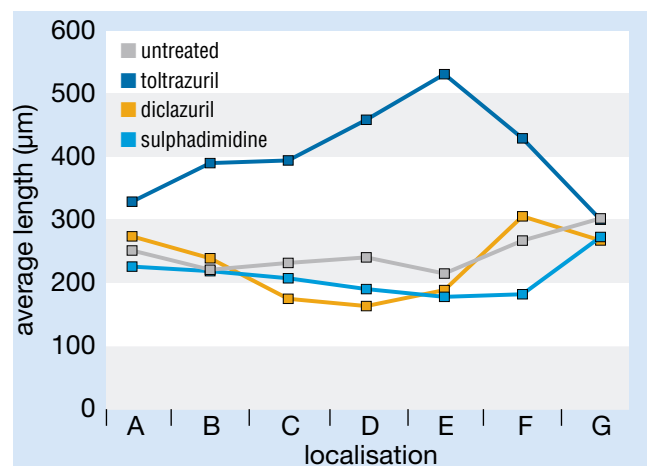
The untreated control animals developed the typical picture of severe isosporosis as a result of the infection, namely diarrhoea. The clinical picture of the treated groups was different. Signs of isosporosis were manifest in groups C (diclazuril) and D (sulphadimidine), while the animals of group B (toltrazuril) remained normal.

In studies 1, 2 and 3, there was a significant difference ( $p \leq 0.05$ ) in the occurrence of diarrhoea between the toltrazuril-treated piglets (no diarrhoea) and groups A, C and D. The diclazuril and sulphadimidine groups did not differ significantly from the untreated control animals. The results of the individual daily assessment of faecal consistency scores of studies 1 and 3 are summarised in figures 1 and 2.

Similar results were obtained for oocyst excretion. The diclazuril and sulphadimidine groups did not differ significantly from the untreated control animals. In the toltrazuril groups, oocyst excretion was substantially reduced and remained low.

The weight-gain profile (figure 3 and 4) from day 7 to day 28 for the toltrazuril treatment groups revealed consistently higher values than for the other treatment groups.

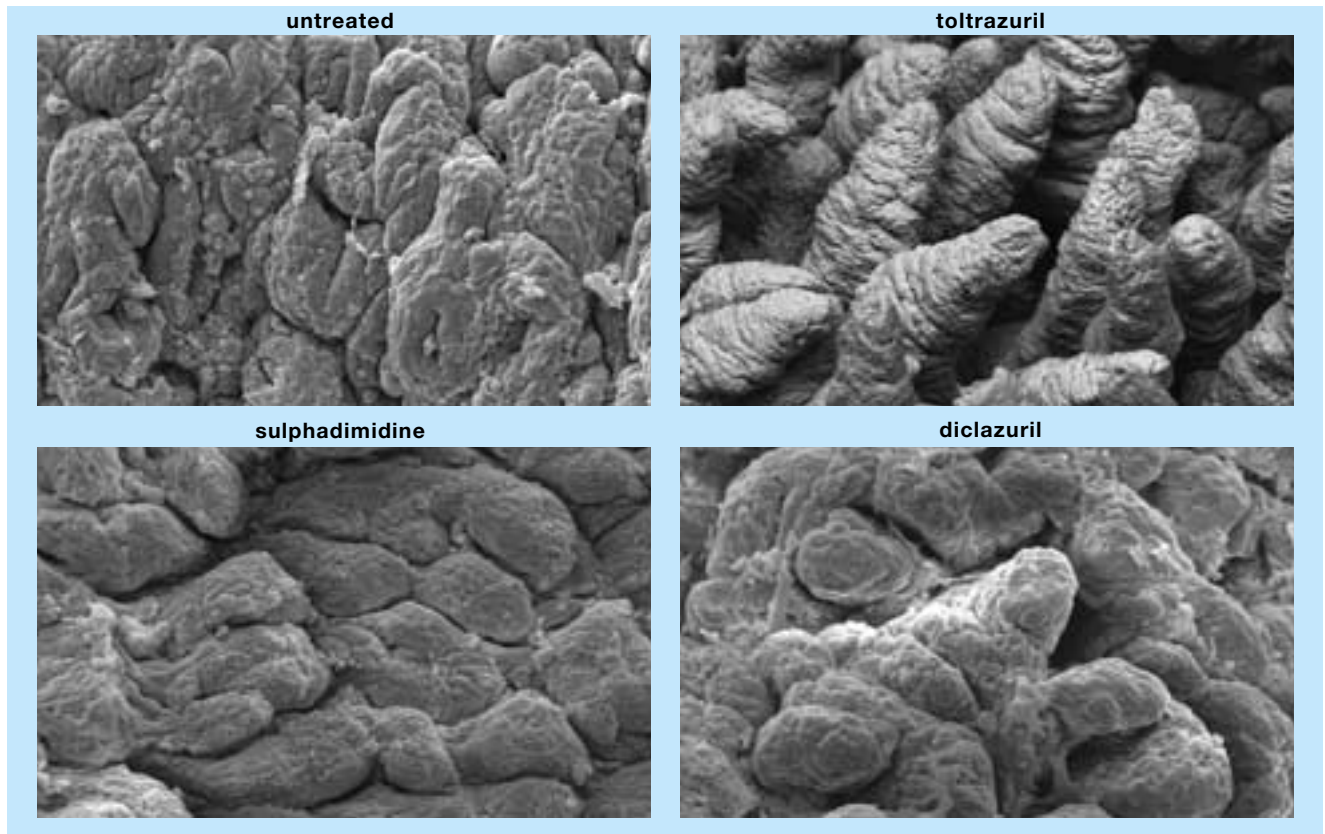
**Fig. 6** Average villi length day 11 p.i. (average,  $\mu\text{m}$ )



In the second study, the gut villi were considerably longer on average in the animals treated with toltrazuril than in the untreated control animals and the other treatment groups on days 10 and 14 (day 7 and 11 p.i.). Differences to the toltrazuril group could still be determined 14 days p.i. despite rapid regeneration of the villi in the affected groups (figures 5 and 6).



Fig. 5 Villous structure 11 days p. i.



## DISCUSSION

Of the treatment regimens applied, only toltrazuril showed satisfactory efficacy in this standardised infection model. A single treatment with 20 mg toltrazuril / kg bodyweight given at an early stage of infection (2 dpi) controlled a massive artificial infection with *I. suis* in suckling pigs.

Treatment with diclazuril produced no pronounced differences to the results in the untreated groups in the tested treatment regimes (2 or 15 mg/kg, repeated treatment in some cases). A similar picture was seen in the group treated with sulphadimidine. ●

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# The Role of *Isospora suis* in the Etiology of Diarrhoea in Suckling Piglets



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*Isospora suis* has been described as an important cause of suckling piglet diarrhoea in a number of European countries (Denmark, Germany) (Henriksen et al., 1989; Nierstrath et al., 2002), in Canada (Bergeland, 1977) and in the U.S.A. (Sanford et al., 1981). In Italy, recent epidemiological investigations (Kramer et al., 2002; Vezzoli et al., 2002) confirmed the remarkable spread of *Isospora suis* in our farms too (68.2% positive farms in Northern-Central Italy and 85.2% in Sardinia). In spite of its remarkable spread, the role of this coccidia as a primary pathogen has been often questioned due to the absence of a specific clinical picture and mainly to the difficulties in getting a laboratory diagnostic confirmation. The animals infected by *Isospora suis* develop diarrhoea with a varying appearance (liquid to pasty) and accompanied by sensory depression and dehydration, mostly in the second week of age. Notwithstanding the mortality is very low or completely absent, economic losses can be remarkable and attributable to the decrease in daily weight gain and to the impaired performances of infected animals in the subsequent production phases (Dreisen et al., 1993; Del Castillo et al., 1996; Gherpelli e Barbieri, 1997).

The objective of the present study was to evaluate the real role of *Isospora suis* in the etiology of suckling piglet diarrhoea.

## MATERIALS AND METHODS

Two problem farms were selected, positive for *Isospora suis*, with a good hygienic standard and no ongoing treatment. Positivity was confirmed through parasitological examination of fecal pools collected from litter with symptoms consistent with coccidiosis. We selected the litter with ongoing characteristic symptoms and from different delivery rooms. Concomitantly, at least 1 litter with no enteric symptoms from each delivery room (for a total of 18 litter), as negative controls. A fecal pool was collected from each litter at the onset of the characteristic diarrhoea (yellow to grayish-yellow feces with liquid to pasty appearance) by direct sampling from the rectum (T<sub>0</sub>). The same litter were then sampled 2 days (T<sub>1</sub>) and 4 days (T<sub>2</sub>) after the onset of symptoms and a last time about 1 week after weaning (T<sub>3</sub>). At each sampling time, the number of involved piglets per litter, the appearance and consistency of feces, and the number of possibly dead animals were recorded.



Diagnostic investigations were carried out on all pools sampled at T<sub>0</sub> (litter with characteristic symptoms and control litter) as follows:

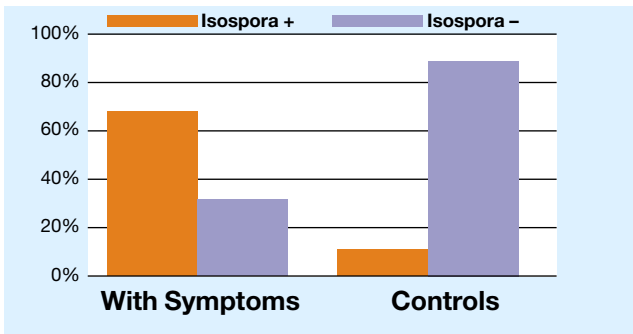
fecal microscopic examination, bacteriological examination, test for *Salmonella* spp., test for *Cryptosporidium* spp., virological examination.

The fecal pools sampled at T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were instead tested only to detect and count oocysts attributable to *Isospora suis*.

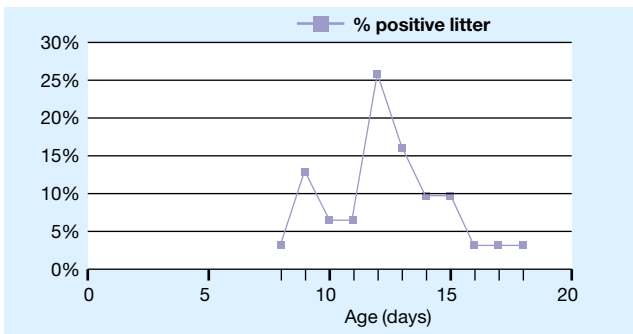
Aimed at verifying the presence of lesions at the enteric level, 3 piglets (T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub>) with ongoing characteristic symptoms and positive at the coproexamination were sacrificed. Besides the above examinations, a direct smear was performed for each animal, along with the parasitological examination of content of duodenum, jejunum, cecum and colon. Smears of each intestinal portion were stained (Giemsa) and observed at the optical microscope (×1,000) to detect type I merozoites inside the cells of intestinal epithelium. On sections of duodenum (descending portion), jejunum (medium portion), ileum, cecum (caudal third) and colon (central flexure) were carried out histological examinations.



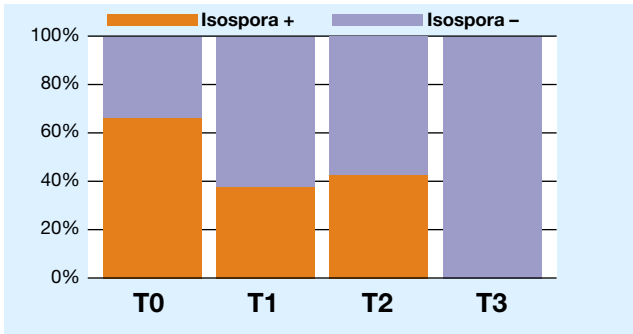
**Fig. 1** Relation between symptomatic litter and positivity for *Isoospora suis*



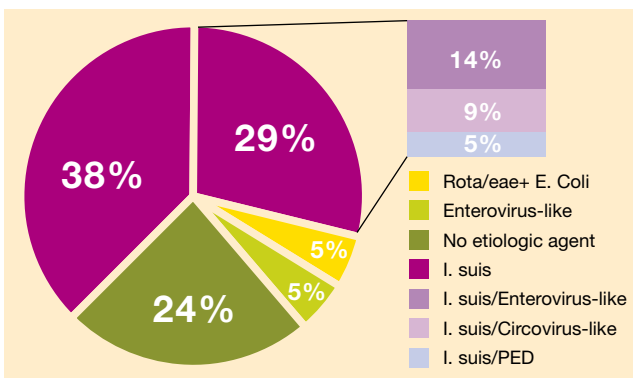
**Fig. 2** Trend of litter positivity (%) versus time (age expressed in days)



**Fig. 3** Positivity for *Isoospora suis* of fecal pools in the 4 samplings



**Fig. 4** Co-infections in the 21 litter with characteristic diarrhoea



## RESULTS

Overall, 15 litter (68.2%) among those presenting characteristic diarrhoea were positive for *Isoospora suis* versus only 2 control litter out of 18 (11.1%) (Fig. 1). In the litter with diarrhoea, the exposure to *Isoospora suis* was 17 times greater than in litter without symptoms (OR = 17.13; 95% CI = 2.56 to 147.66). Within litter, the number of affected animals ranged between 3 and 5 and the whole litter was never involved.

The onset of diarrhoea occurred during the second week of life in all litter but one (95%); the percentage of litter positive for *I. suis* versus time (age expressed in days) is illustrated by Fig. 2. Maximum positive percentage was observed at day 12 (25.8%).

As far as appearance and consistency of feces are concerned, 24 out of 31 litter with yellow liquid feces (77.4%) were positive for the presence of the protozoan, in comparison with just 7 out of 25 litter with yellow pasty feces (21.9%).

Among the 15 positive litter, just 1 became positive at T1, while the remaining 14 (93.3%) were positive right from the first sampling (T0). Among these 14 litter, 6 (42.9%) kept positive at all samplings times, while 5 (35.7%) were positive only at T0.

Concerning the positive percentage of pools collected during the 4 samplings, 14 out of 21 pools (66.67%) were positive at T0 (diarrhoea onset), 8 out of 21 (38.1%) at T1 (2 days later) and 9 out of 21 (42.86%) at T2 (4 days later). No litter showed diarrhoea and no fecal pools were positive for *Isoospora suis* at T3 (7 days after weaning) (Fig. 3). All fecal pools sampled at T0 (litter with symptoms and controls) were screened for enteric pathogens characteristic of the suckling period. No litter was positive for *Salmonella* spp., *Clostridium perfringens* and *Cryptosporidium* spp. Figure 4 reports in detail the findings relevant to symptomatic litter.

At the post-mortem examination, bacteriological examination of individual tracts was negative for *Salmonella* spp. and *Cl. perfringens*; the isolated strains of *E. coli* did not exhibit any pathogenic factor of those searched for; virological examination was negative; *I. suis* was found out in the colon of all 3 animals and in the cecum of the animal sacrificed at T0. Histological examination disclosed hyperemia, generalized in the first animal and mostly involving the small intestine (jejunum and ileum) in both other animals. At the level of jejunum, 3 animals showed de-epithelization and 1 animal showed fusion of villi, with the presence of eosinophilic material within the crypt lumens of the animal sacrificed at T2.

## CONCLUSIONS

The importance of *Isoospora suis* as a primary pathogen in the onset of suckling piglet diarrhoea was shown in several trials (Stuart et al., 1980; Robinson et al., 1983; Harleman et al., 1984; Vitovec et al., 1990) and it was confirmed by this field experience of ours. The results of performed fecal-microscopic, bacterio-

logical and virological examinations gave a clear evidence of the correlation between diarrhea and presence of *Isoospora suis*. Though a high prevalence of *Isoospora suis* was also demonstrated on our territory (Vezzoli et al., 2002), this condition does not yet draw the proper attention from both veterinarians and farmers, because of both difficulties in diagnostic confirmation and underestimate of the associated economic damage. In spite of the low mortality, economic losses can be in fact noteworthy and attributable to prolonged and unsuccessful antibiotic therapies, but mostly to growth retardation (up to 20% weight loss and decrease in daily weight gain) continuing in the post-weaning phase too (Dreisen et al., 1993; Del Castillo et al., 1996; GherPELLI and Barbieri, 1997). The microclimate of delivery rooms offers optimal conditions of temperature and humidity for the development of oocysts which are highly resistant to physical and chemical agents. Therefore, once confirmed a suspected coccidiosis, even based only on the clinical picture and differential diagnosis, an intervention with effective preventive strategies aimed at limiting the economic damage is mandatory. ●

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# Efficacy of Toltrazuril against Artificial Infections with *Eimeria bovis* in Calves

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## INTRODUCTION

*Eimeria bovis* is a pathogen that causes clinical coccidiosis in calves and can lead to bloody diarrhoea and, in some cases, death in affected animals.

The objective of the study was to establish a model for infection with *Eimeria bovis* in calves and to determine the efficacy of toltrazuril at various doses and at various treatment times.

## MATERIAL AND METHODS

Two infection trials were carried out in calves.

The animals in both trials were kept under comparable conditions commensurate with their age at Bayer AG's Large Animal Centre in Monheim.

All the animals were observed clinically every day; the consistency of their faeces and oocyst excretion were determined three times a week in the prepatent period and daily from day 16 post infection (p.i.). Oocyst excretion was determined using flotation with saturated NaCl solution followed by counting in McMaster chambers.

In the first trial, 53 bull calves between about two and four weeks of age were infected orally with  $5 \times 10^4$  sporulated *Eimeria bovis* oocysts each. On day 14 p.i. the calves were randomised and treated once. On the randomisation day, animals which did not fulfil the inclusion criteria were excluded. The remaining animals were divided into four treatment groups (Table 1).

**Table 1** Treatment groups; Trial 1

GROUP (NUMBER OF ANIMALS)	DOSAGE
A (8)	15 ml water
B (8)	5 mg toltrazuril/kg live weight
C (9)	15 mg toltrazuril/kg live weight
D (9)	25 mg toltrazuril/kg live weight

In the second trial, 24 calves between about two and four weeks of age were infected with  $10^5$  sporulated *Eimeria bovis* oocysts each. On day 12 p.i. the calves were randomised and treated on days 12 and 18 p.i. (Table 2).

**Table 2** Treatment groups; Trial 2

GROUP (NUMBER OF ANIMALS)	DOSAGE	TREATMENT TIME
A (8)	15 ml water	Day 12 p.i.
E (8)	15 mg toltrazuril / kg live weight	Day 12 p.i.
F (8)	15 mg toltrazuril / kg live weight	After clinical manifestation of coccidiosis

The animals were slaughtered at various times during the study and examined pathologically. The slaughtering times were during the prepatent period (day 16 p.i., animals in groups A and E), in the patent period (day 20 p.i., animals in groups A, E and F) and in the late patent period or after it (day 28 p.i., animals in groups A, E and F; day 35 p.i., animals in group F).

**Figure 1** bloody diarrhoea



## RESULTS

In both trials, artificial infection led to the clinical picture of coccidiosis associated with diarrhoea and excretion of *Eimeria bovis* oocysts.

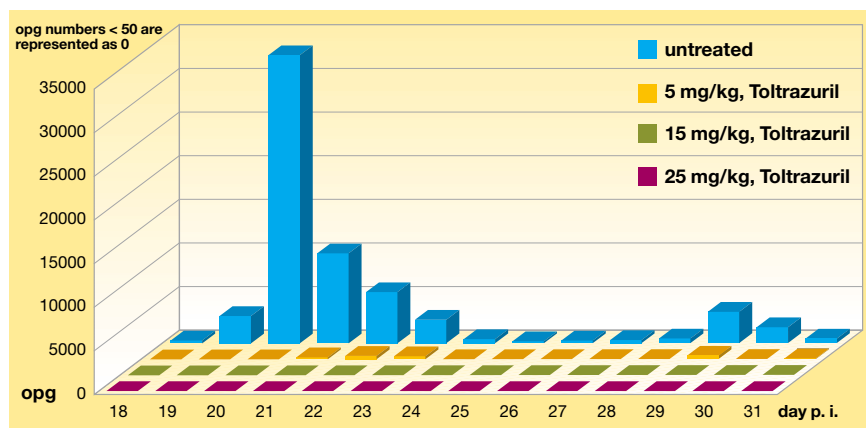
In the first trial, considerably more animals in group A had diarrhoea and it was considerably more severe than in groups B, C and D. There was no significant difference between the treatment groups. Severe, bloody diarrhoea only occurred in untreated animals.

In the second trial, bloody diarrhoea in part with tissue occurred in the untreated animals (Figure 1) and in those that received treatment at a late stage; it was not observed in the group given early treatment.

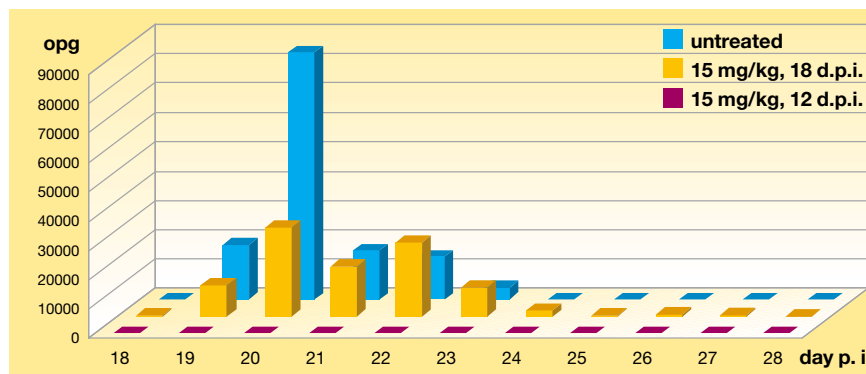
In the first trial, all the animals in the untreated group excreted oocysts for between four and 12 days; the highest opg value was 147,700 opg recorded on day 20 p.i. All the animals in group B also excreted oocysts, although this lasted only for one to seven days and the highest opg value recorded was 2,450 opg on day 29 p.i. In group C one calf excreted oocysts on day 22 p.i. (100 opg); in group D four animals excreted oocysts on one or two days (50 opg in each case). Overall there was a distinct difference in oocyst excretion between the untreated control and the treatment groups, with untreated animals excreting considerably more oocysts than treated animals. The results obtained in group B also differed markedly from those in groups C and D; the animals in group B excreted more oocysts than those in groups C and D. There was no statistically significant difference between groups C and D (Figure 2).

In the second trial, all the animals in the untreated group and the group that received treatment at a late stage excreted oocysts (group A up to 302,000 opg; group F up to 150,200 opg). In group E (early treatment) only two animals excreted oocysts; they excreted on one day each (day 22 and 24 p.i. respectively) and the number of oocysts was low (50 opg) (Figure 3).

**Figure 2** Average oocyst excretion following artificial infection with  $5 \times 10^4$  sporulated oocysts of *E. bovis*/calf and treatment with various doses of toltrazuril on day 14 p.i. (n = 8–9 animals per group)



**Figure 3** Average oocyst excretion following artificial infection with  $5 \times 10^5$  sporulated oocysts of *E. bovis*/calf and treatment with toltrazuril on day 12 or 18 p.i. (n = 2–8 animals per group)



## DISCUSSION

A model of infection with *Eimeria bovis* in calves was established. Infection intensities of  $5 \times 10^4$  and  $1 \times 10^5$  oocysts led to clinical coccidiosis. More severe clinical signs developed at higher infectious doses.

Toltrazuril controlled the infection in a positive dose-dependent manner (trial 1). A dosage of 15 mg/kg live weight proved to be fully effective in controlling both infection intensities (trial 1 and 2). As expected, treatment after the clinical signs had appeared was too late and was not sufficiently effective. ●

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