Field efficacy of a 10 per cent pyriproxyfen spot-on for the prevention of flea infestations on cats

The clinical application of a new method for using the insect growth regulator, pyriproxyfen, for controlling flea populations in cat-owning homes is evaluated for the first time. In a multicentric, controlled and randomised trial, 107 flea-infested cats were treated with a minimum dose of 10 mg/kg bodyweight pyriproxyfen as a 10 per cent spot-on application on two occasions, with a three-month interval between doses. For comparison, 99 cats received lufenuron suspension orally, once a month, for six months. Flea counts decreased significantly over time in each group and were significantly lower in the pyriproxyfen group than in cats treated with the reference product. The percentage of 'zero-flea' cats increased from 49 per cent on day 30 to 88 per cent on day 180 in the pyriproxyfen group and from 30 to 71 per cent in the lufenuron group at the same time points ($P<0.05$). Appropriately timed topical applications of pyriproxyfen, therefore, offer a method of flea control in the domestic environment.

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INTRODUCTION

Fleas are the most common ectoparasites on dogs and cats. Traditional approaches to flea control rely on the application of adulticidal products to household pets with concurrent environmental application of chemicals targeted against developmental stages (Dryden and others 1989). In recent years, effective environmental control has been achieved with the use of insect growth regulators (IGRs). These can be delivered directly into the environment to prevent larval development (for example, using methoprene) or they can be given to the host to ensure that adult fleas are infertile (for example, using lufenuron).

Pyriproxyfen is a new photostable IGR that acts on insects as an imitator of juvenile hormone (Gortel 1997). Experimentally, topical treatments on cats will inhibit flea fertility and development (Jacobs and others 1996), but this concept has not previously been evaluated under field conditions. The present paper reports the results of a clinical trial designed to confirm that topically applied pyriproxyfen can control flea populations in cat-owning homes without the need for further environmental treatments. For this purpose, a new 10 per cent pyriproxyfen spot-on formulation (Cyclio;Virbac) was used, which has been shown in laboratory studies to exert an ovicidal and larvicidal effect in excess of 95 per cent persisting for 13 weeks following treatment (unpublished data).

MATERIALS AND METHODS

Study design

A multicentric, randomised and controlled clinical trial was performed in France and Germany from May 1997 to January 1998 to evaluate the field efficacy of a new IGR, pyriproxyfen, by comparison with a reference product, lufenuron (Program F; Novartis). Nineteen veterinary practices were selected; 15 in France and four in Germany. The study was conducted in compliance with 'good clinical practice' standards.

Animals

For inclusion in the trial, feline veterinary patients had to be carrying a natural flea infestation (*Ctenocephalides felis*) and had to be at least four weeks of age, although they could be either gender or any breed. Animals recently treated with a persistent anti-flea product or whose environment had been sprayed with an antiparasitic product within the previous month were excluded. The distribution of the cats into two groups was randomised within blocks of eight subjects so that four were treated with pyriproxyfen and four with lufenuron.

Treatment

Test animals

The test formulation was presented on 0·6 ml pipettes containing 60 mg of pyriproxyfen. Each cat was treated with the contents of one pipette irrespective of

Table 1. Characteristics of the cats in the two treatment groups on day 0

		Pyriproxyfen (n = 107)	Lufenuron (n = 99)	Significance
Gender ratio	Male	59	50	NS†
	Female	48	49	
Age (years) Mean ±SD		3·75 ±3·02	4·96 ±4·14	S†
Weight (kg) Mean ±SD		3·85 ±1·22	4·01 ±1·46	NS†
Hair length	Short	68	74	
	Mid-length	29	20	NS*
	Long	10	5	
Living conditions	Apartment	27	19	
	House/garden	58	55	NS†
	Cattery	22	25	
Number of fleas Mean ±SD		6·2 ±5·8	5·6 ±4·5	NS†
Pruritus	Yes	54	50	NS*
	No	53	49	
Dermatological lesions	Yes	12	15	NS*
	No	95	84	

*χ^2 test
†Student's *t* test
NS Not significant (P>0·05)
S Significant (P<0·05)

Table 2. Number (per cent) of zero-flea cats at each observation time in the two groups

Day	Pyriproxyfen	Lufenuron	
0	0 (0) (n = 107)	0 (0) (n = 99)	NS*
30	52 (48·6) (n = 107)	30 (30·3) (n = 99)	S*
60	80 (74·8) (n = 107)	39 (39·4) (n = 99)	S*
90	73 (69·5) (n = 105)	50 (51·5) (n = 97)	S*
120	90 (88·2) (n = 102)	54 (55·1) (n = 98)	S*
150	88 (88·0) (n = 100)	52 (61·9) (n = 84)	S*
180	42 (87·5) (n = 48)	27 (71·1) (n = 38)	NS*

*χ^2 test
n Number of cats at each examination time
NS Not significant (P>0·05), S Significant (P<0·05)

bodyweight, thereby providing a minimum of 10 mg pyriproxyfen per kg. The treatments were administered by the investigator as a 'spot-on' application directly on to the skin in the middle of the dorsal aspect of the neck. Two treatments were given, with a three-month interval in-between; that is, on day 0 (the day of inclusion) and again on day 90. Lufenuron was administered by the owner as an oral suspension at feeding time, once a month for six months, starting on day 0. One vial containing 133 mg lufenuron was given to cats weighing less than 4·5 kg and two vials to cats weighing more.

Treatment of other animals in the household

If there were two or more cats in the same household, all were treated with the same product. Each animal was observed separately and was considered to be a separate case. Dogs living with cats in the trial, irrespective of treatment group, were treated by the owner with a 1 per cent permethrin-based spray (Puce-Stop Direct; Virbac), according to label instructions. Therefore, for example, a medium-sized dog would be sprayed for about 20 seconds with the nozzle held at a distance of about 20 to 30 cm. Permethrin is a fast-acting adulticide and this treatment provided a 15-day protection period. Dogs were sprayed on day 0, but were only retreated when deemed necessary by the owner. No other ectoparasiticidal treatment, whether to treat the animal or the environment, was allowed.

Assessment

The animals were observed monthly over a six-month period (that is, on days 0, 30, 60, 90, 120, 150 and 180). An accurate assessment of the number of adult fleas on each cat on each observation day was obtained by treating the animal with 1 per cent permethrin foam (Defencat; Virbac) applied over the whole body at the recommended dose rate of 50 mg/kg bodyweight (in other words, a ball of foam with a diameter of about 8 cm/kg bodyweight, corresponding to 7 g of formulation). This treatment was chosen because of its rapid adulticidal effect and short persistency. The coat was meticulously combed for at least five minutes within 15 minutes of treatment. A flea comb was employed for this purpose, with particular attention being paid to the head, neck, loins, tail and thighs.

Statistical analysis

The main criterion for efficacy assessment was the number of fleas counted at each time point. A secondary efficacy criterion was the proportion of cats on which no fleas could be found ('zero-flea' animals) on each occasion. Data from animals dropping out of the study before day 60 were excluded. Statistical analysis was performed using NCSS97 software (Deltasoft) on the population of cases retained for data analysis. Between-group comparisons were made for gender, bodyweight, age, hair length, living condition and initial flea number using either a Chi-square test for qualitative variables or a Student's *t* test (or Mann-Whitney test) for quantitative variables.

A two-factor ANOVA test on repeated measures (two treatments and seven time points) was performed to compare the number of fleas between groups with time. The test was performed on log transformed data – $\log_{10}$ (number of fleas + 1) – if the variable 'fleas' was not normally distributed. Comparison of the frequencies of 'zero-flea' cats at each observation point was performed using the Chi-square test. A probability level of 0·05 was considered significant.

RESULTS

Two hundred and twenty-four cats (122 males and 102 females) of various breeds, 1·5 months to 20·8 years old and 0·6 to 11·2 kg bodyweight, were included in the trial. Records for 18 cats showed a major deviation from the protocol (for example, no fleas on day 0, application of another ectoparasiticide, not presented at observation times) and were excluded from data analysis. Therefore, 206 cases were considered as interpretable: 107 were treated with pyriproxyfen and 99 with lufenuron. The mean ages of cats in the pyriproxyfen and lufenuron groups were 3·75 and 4·96 years, respectively (P<0·05). This age difference between the test groups was fortuitous. No other statistically significant differences were detected between groups before treatment (Table 1).

According to the study protocol, cats were to be observed over a six-month period. However, approximately half of the cats were recruited over the summer and a proportion of these were not followed for a full six months.

Flea counts are presented in Fig 1. Mean values for both groups decreased regularly and significantly from day 0 to day 180 (P<0·001), the only exception being a small increase on day 90 in the

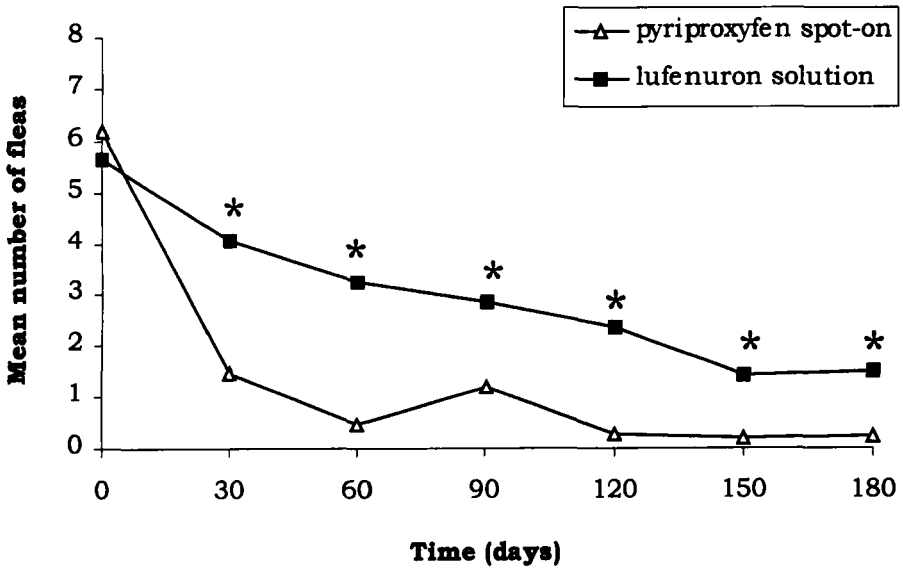

FIG 1. Time course of the number of fleas in the two treatment groups. *Statistically significant difference between the groups (P<0·05)

pyriproxyfen group (although there was still a highly significant difference [P<0·0001] between the groups at this time). Mean flea counts for the pyriproxyfen group at each post-treatment observation point (that is, days 30, 60, 90, 120 150 and 180) were significantly lower than those of the lufenuron group.

The percentage of 'zero-flea' cats in each group increased with time from 49 per cent on day 30 to 88 per cent on day 180 in the pyriproxyfen group and from 30 per cent on day 30 to 71 per cent on day 180 in the lufenuron group (Table 2). Figures for the pyriproxyfen group were statistically higher (P<0·05) than for the lufenuron group at all post-treatment time-points, except day 180.

Abnormal reactions after application of the pyriproxyfen formulation were restricted to salivation in three cats and inappetence in one. In each case, the reaction was slight and disappeared rapidly without any specific treatment. One cat in the lufenuron group displayed signs of nausea.

DISCUSSION

This study was designed to test the hypothesis that strategic pyriproxyfen spot-on treatments can protect cats from subsequent flea infestation in the home by eliminating or reducing environmental challenge. This effect is principally achieved by preventing fleas from laying eggs but, as discussed below, pyriproxyfen also has direct effects on adult fleas and on eggs and larvae on surfaces that have been in close contact with a treated animal.

The cooperation of veterinary practices was solicited from a large geographical area with wide climate variation. Cats of both sexes and of a wide range of breeds, ages, weights and hair lengths were recruited in the spring and early summer to ensure that observations continued throughout the flea season. All had fleas when first presented and were assumed therefore to originate from infested environments. Once animals were included in the study there were no restrictions on their exposure to rain, bathing or movement outdoors.

Efficacy was judged by performing a carefully controlled study in a large number of households at a time when flea population would normally be high. As the trial involved veterinary patients, an untreated control group would not have been ethical. Results for pyriproxyfen-treated cats were therefore compared with those from cats in other households treated with an established reference product (lufenuron).

The purpose of the permethrin treatment given at each monthly observation point was to provide an accurate method of assessing the net number of fleas acquired by each cat over the preceding 30 days. These data provide a practical indicator of the level of environmental challenge during that period. The use of a short-acting adulticidal formulation ensured minimal impact on the dynamics of the flea population.

The results show that, even though the trial was conducted in infested homes during the flea season, environmental challenge was maintained at very low levels in both treatment groups, indicating a high level of efficacy in both treatment groups. In fact, flea counts were significantly lower for the pyriproxyfen group than for the lufenuron-treated cats. Therefore, two spot-on applications with pyriproxyfen at a 90-day interval provided better control than monthly treatment with the reference product.

A number of factors could contribute to the observed differences in potency between the two treatments. For example, the mode of action is different in each case. Lufenuron is an oral systemic medication that is taken up by adult fleas when they suck blood and subsequently prevents egg hatching by killing the enclosed larva (Meola 1999a). Its lethal effect on immature, developmental life-cycle stages is due to an interruption in normal chitin production (Shipstone and Mason 1995). Close examination of eggs and unhatched larvae shows that the cuticle, epidermal cells, chorion and vitelline membrane are all affected (Meola 1999b). The viability of adult fleas on lufenuron-treated cats is not affected (Hink and others 1991, Blagburn and others 1994), although Stansfield (1997) fed female adult fleas in vitro on an artificial membrane system with blood containing lufenuron and found 28 per cent mortality after eight days compared with 6 per cent in controls. These results were confirmed by Dean and others (1999) who found 23·9 per cent mortality at the highest blood concentration (4 ppm) after 10 days compared with 7 per cent in untreated fleas.

In comparison, topically applied pyriproxyfen is absorbed through the cuticle of the adult flea to enter the developing eggs, where it interferes with maturation by limiting vitelline reserves and impeding blastoderm development (Meola 1999a). Affected fleas produce premature, dull-coloured eggs, which rapidly die (Meola and others 1993b). Normal eggs from unexposed fleas are also affected if they come into contact with pyriproxyfen. For example, freshly laid eggs exposed to filter papers impregnated with pyriproxyfen for two hours failed to hatch. After shorter exposures, hatching was possible, but most of the larvae died while in the first larval stage (Meola and others 1993a).

Under experimental conditions, sufficient pyriproxyfen can transfer from cats to their immediate surroundings to pre-

vent flea development (Jacobs and others 1996). When the pyriproxyfen spot-on as used in the present study was applied to beagles in laboratory tests, more than 90 per cent ovicidal efficacy was detected as early as 24 hours post-treatment on samples of carpet from the kennels. Efficacy reached 100 per cent at day 2 and remained at this level for the entire study duration until day 71; that is, 10 weeks after the animal had been in contact with the carpet (Houffschmitt and Cruthers 1999). This effect probably contributed to the relatively rapid initial decline in environmental challenge illustrated by the flea burdens of pyriproxyfen-treated cats in the present study (see Fig 1).

As well as having ovicidal and larvicidal activity, pyriproxyfen also has delayed adulticide effects (Kunkle 1997). In another study, whether fed or unfed, fleas experienced 96 per cent mortality after pyriproxyfen contact for eight days, compared with 10 to 20 per cent mortality in a control group (Meola and others 1993a). This pattern was further demonstrated in vitro by Meola and others (1996), with fed adult females maintained on hair treated with pyriproxyfen at various concentrations. In the intermediate group (125 µg/g hair), mortality was 80 and 100 per cent after eight and 10 days respectively, compared with 20 and 27 per cent in the control animals. As well as yolk damage in female fleas, lysis of malpighian tubules and male accessory gland cells was observed, plus cell death and degeneration of internal tissues, with destruction of epithelial cells surrounding glands.

A direct lethal effect on adult fleas is of advantage in the initial phase of a control strategy reliant on animal treatment. At this time, treated cats are exposed to reinfestation from fleas derived from eggs dropped before the start of the control programme. Therefore, there is a lag period before the environmental benefits of IGR therapy become fully realised. Shipstone and Mason (1995) reported a lag phase of approximately eight weeks between initiation of lufenuron therapy and elimination of adult fleas on the animal. In Japan, 94 per cent of 190 cats were clear of fleas two months after initiation of lufenuron treatment (Stansfield 1997). Clinical field trials in the USA involving 114 cats showed 55 per cent reduction after four weeks using a similar counting technique to that employed in the present study (FOI Summary 1995).

A test using area count methodology (thumb-counting on five areas for one minute each) on adulticide plus lufenuron-treated cats and dogs showed a 91 per cent reduction in flea burden after four weeks (Dryden and others 1999). In an experimental model in which groups of cats were kept in carpeted pens, it took 112 days before mean flea counts of lufenuron-treated cats fell below 10 (Fisher and others 1996). This lag time may be diminished in the case of compounds that transfer in ovicidal or larvicidal amounts from the coat or skin of the treated animal to frequently contacted surfaces (Jacobs and others 2000).

Conclusions

A carefully designed field trial was conducted to demonstrate that flea populations in initially infested cat-owning households can be controlled by on-animal applications of pyriproxyfen (an insect growth regulator). Environmental challenge was estimated at monthly intervals throughout the summer by combing the cats resident in each home. This procedure was facilitated by application of a rapidly effective but short-acting insecticidal treatment containing permethrin. One topical dose of a 10 per cent pyriproxyfen spot-on formulation effectively and safely controlled fleas in the household for three months. This pyriproxyfen control strategy gave significantly better reduction in flea numbers ($P<0.0001$) than monthly usage of an established reference product (oral lufenuron). The use of on-animal treatments for preventing the accumulation of off-host flea life-cycle stages removes the need to apply large quantities of chemicals directly into the domestic environment.

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