



Available online at www.sciencedirect.com



Veterinary Parasitology xxx (2007) xxx–xxx

veterinary
parasitology

www.elsevier.com/locate/vetpar

Dose determination of a novel formulation of metaflumizone plus amitraz for control of cat fleas (*Ctenocephalides felis felis*) and brown dog ticks (*Rhipicephalus sanguineus*) on dogs

D. Rugg^{a,*}, J.A. Hair^b

^a Fort Dodge Animal Health, P.O. Box 5366, Princeton, NJ 08543, USA

^b Nu-Era Farms, 320 N. Range Road, Stillwater, OK 74075, USA

Abstract

A novel spot-on formulation containing metaflumizone and amitraz (ProMeris[®]/ProMeris Duo[®] for Dogs, Fort Dodge Animal Health, Overland Park, KS) was evaluated in a laboratory study to determine the appropriate dose for efficacy against fleas and ticks on dogs for 1 month. Thirty-six Beagles were randomly allocated to six equal groups and individually housed. One group remained nontreated. Another was treated with a placebo formulation (solvents with no active ingredients). Three groups of dogs were treated topically with the metaflumizone plus amitraz formulation (150 mg of each of metaflumizone and amitraz/ml), at volumes providing doses of 10, 20 and 40 mg each active/kg. The final group was treated with a commercial spot-on providing 6.7 mg fipronil/kg. All treatments were applied to the skin at a single spot between the scapulae on Day 0. Dogs were infested with 50 adult brown dog ticks (*Rhipicephalus sanguineus*) on each of Days -2, 5, 12, 19, 26, 33 and 40, and with 100 cat fleas (*Ctenocephalides felis felis*) on Days -1, 6, 13, 20, 27, 34 and 41. Dogs were examined and parasites “finger counted” on Day 1 to estimate knock down efficacy, and all animals were comb counted to determine the numbers of viable fleas and ticks on Days 7, 14, 21, 28, 35 and 42. There were no significant differences in parasite counts between the nontreated control and the placebo-treated control groups for either fleas or ticks ($P > 0.05$) except for very slight reductions on Day 7 for fleas and Day 14 for ticks, demonstrating that the formulation excipients had no activity. The qualitative finger counts on Day 1 indicated that all of the insecticidal treatments resulted in a noticeable reduction in flea and tick numbers within 1 day of treatment. All of the metaflumizone and amitraz treatments and fipronil resulted in significantly lower flea and tick numbers relative to nontreated controls on all posttreatment count days ($P < 0.05$). For the metaflumizone plus amitraz treatments, mean flea and tick counts for the 10 mg/kg dose were significantly higher than those for the 20 mg/kg dose ($P < 0.05$) from Day 21 on. There was no significant advantage provided by the 40 mg/kg dose over the 20 mg dose throughout the entire study ($P > 0.05$). The two higher metaflumizone plus amitraz doses provided >95% control of fleas and >90% control of ticks for at least 35 days after treatment, and this level of control was similar to that of the commercial fipronil product. The 20 mg/kg dose was selected as the minimum commercial dose rate to provide effective flea and tick control for at least 1 month following a single treatment.

© 2007 Published by Elsevier B.V.

Keywords: ProMeris[®]; ProMeris Duo[®]; *Ctenocephalides felis felis*; *Rhipicephalus sanguineus*; Metaflumizone; Amitraz; Flea; Tick; Dog

1. Introduction

Permanent or semi-permanent ectoparasites occurring on dogs are mainly fleas, ticks and mites. Of these, the cat flea (*Ctenocephalides felis felis*) is the most

* Corresponding author. Tel.: +1 732 631 5860;
fax: +1 732 631 5832.
E-mail address: ruggd@pt.fdah.com (D. Rugg).

widespread, being endemic worldwide and considered the most important ectoparasite of dogs and cats (Rust and Dryden, 1997). Adult fleas are blood feeders, penetrating the skin with their sucking mouthparts and injecting salivary antigens as they feed. They are recognized as a major cause of allergic skin disease in dogs and are capable, when present in sufficient numbers, of causing anaemia (Krämer and Menke, 2001). They are intermediate hosts for the dog tapeworm, *Dipylidium caninum*, and can transmit a number of pathogens including *Bartonella henselae*, which causes cat scratch fever (Krämer and Menke, 2001).

Infestations of ticks on dogs can be anything from an occasional nuisance to a continuous infestation, and range from having virtually no adverse effects on health to causing life-threatening disease. All stages of ticks feed by first slashing the host's skin with their chelicerae to gain entry, then piercing a blood vessel and inserting their mouthparts into, or close to, the blood vessel. Large amounts of blood are taken up by the tick, excess water is removed and returned to the host in the form of saliva, which contains a number of pharmacologically active substances, including anticoagulants and immunomodulators, the exact components varying between species. Some tick species excrete toxic substances within their saliva and tick-borne diseases are normally passed to their next host in saliva (Needham and Teel, 1991). Where tick populations are large, numbers may be high enough to cause anaemia. Ticks are responsible for the transmission of a number of diseases, some are dog-specific, some are zoonotic and some cause serious, even life-threatening, diseases (Dryden and Payne, 2004). *Babesia canis* and *Ehrlichia canis* are both dog-specific infections, the former primarily transmitted by *Dermacentor* spp. and the latter by the brown dog tick, *Rhipicephalus sanguineus*. Zoonotic infections include Lyme disease caused by *Borrelia burgdorferi*, which is transmitted by *Ixodes* spp., and Rocky Mountain Spotted Fever, caused by *Rickettsia rickettsii*, which is transmitted primarily by ticks in the genera *Amblyomma*, *Dermacentor* and *Ixodes* (Dryden and Payne, 2004).

Control of fleas and ticks is primarily based on chemical means and recently, convenient topical treatments have become the standard accepted method (Dryden and Payne, 2004; Rust, 2005). The most widely used products are generally applied as spot-on applications on a monthly schedule and include compounds with efficacy against both fleas and ticks, as well as specific insecticides or acaricides. A number of chemical classes are used for flea and/or tick control

and include: the phenyl pyrazole, fipronil; the neonicotinic, imidacloprid; the synthetic pyrethroids, permethrin and phenothrin; and the avermectin, selamectin (Rust, 2005). Of these imidacloprid is a specific insecticide with little efficacy against ticks (Krämer and Menke, 2001) while the others are effective against both fleas and ticks. These all have direct insecticidal/acaricidal activity and control the parasites on the animal. In addition, there are products such as insect growth regulators that may be fed (lufenuron) or applied (s-methoprene) to the pet but control fleas by disrupting the off-host life stages (eggs and larvae), and others (e.g. pyriproxyfen) that may be used for environmental applications. Despite the variety of available products and different application methods, both fleas and ticks remain an ongoing problem for many pet owners. Also, the susceptibility of populations may decrease with prolonged exposure to individual products. Fleas have potentially developed resistance to a number of compounds and pest management strategies to reduce the development of resistance are needed (Bossard et al., 1998; Ross et al., 1998; Rust, 2005).

Metaflumizone is a novel insecticide in the semicarbazone class of chemistry with no known cross-resistance to other chemistries (Salgado and Hayashi, this volume). This insecticide was combined with the formamidine acaricide amitraz in a novel spot-on formulation to develop a product for flea and tick control on dogs. This study was conducted to determine the appropriate dose rates of a formulation of metaflumizone and amitraz (ProMeris[®]/ProMeris Duo[®] for Dogs, Fort Dodge Animal Health, Overland Park, KS) applied as a single spot application to dogs to provide at least 1 month of control of fleas and ticks.

2. Materials and methods

The study was conducted at Nu-Era Farms, OK. The study was conducted according to Good Laboratory Practices as outlined in US EPA 40CFR160, and followed the basic methodology of US EPA Guideline 810.3300.

2.1. Animals

Eighteen male and 18 female adult Beagle dogs, 3–7 years of age from the Nu-Era Farms colony were used in the study. Each dog was individually identified by numbered or lettered ear tattoos. The dogs had not been treated with an ectoparasiticide for at least 60 days and were in good health when enrolled in the study and at treatment. The animals weighed from 9.3 to 16.6 kg on

Day –2. These animals were selected from a group of 20 male and 20 female dogs based on pretreatment flea and tick counts.

Dogs were housed individually in indoor runs that conformed to accepted animal welfare guidelines. Each run was approximately 3 m × 1 m with wire mesh walls on epoxy-coated concrete flooring and contained a raised mesh rest. Dog runs were grouped by treatment. Each individual run was labeled with the dog identification number only and was not identified by treatment. Dogs from different treatment groups were physically separated by space equivalent to at least one empty run.

Dogs were fed an appropriate maintenance ration of a commercial dry canine feed (27% Hi-Protein Complete Ration, A + M Feeds, Stillwater, OK 74074) for the duration of the study. Water was available *ad libitum*.

2.2. Experimental design and methods

Day 0 was the day that treatments were applied to the dogs. Twenty dogs were acclimated to the study conditions for 14 days prior to treatment. The dogs were observed for general health once daily during the preconditioning period. All dogs were bathed with Allergroom[®] shampoo (Virbac) on Day –12. On Day –11, a physical exam was performed on each dog by a veterinarian to determine health and suitability for inclusion in the trial. On Day –7, each dog was infested with 50 adult brown dog ticks. On Day –6, each dog was infested with 100 unfed cat fleas. On Day –5, each dog was thoroughly examined and combed to remove and count fleas and ticks.

The study was conducted as a randomized complete block design. For each sex there were three blocks of six animals, to provide a total of six test animals (three of each sex) in each of six treatment groups. Using the Day –5 flea and tick counts, 18 dogs of each sex with the highest flea and tick counts were selected for inclusion in the study. For each sex, these dogs were ranked in descending order primarily by flea count then secondarily by tick count. Any dogs with equal flea and tick counts were ranked by ascending identification number. The six dogs within each block were randomly allocated to treatment groups (A, B, C, D, E and F).

On Day –2, the dogs were each infested with 50 adult brown dog ticks. On Day –1, the dogs were infested with 100 adult fleas each. On Day 0, each animal was treated as follows: (A) Negative control, animals were not treated; (B) Placebo control, vehicle (formulation solvents with no active ingredients) was

applied at the middle dose (0.134 ml/kg); (C) Metaflumizone plus amitraz at a dose (0.067 ml/kg) providing 10 mg/kg of each of metaflumizone and amitraz; (D) Metaflumizone plus amitraz at a dose (0.134 ml/kg) providing 20 mg/kg of each of metaflumizone and amitraz; (E) Metaflumizone plus amitraz at a dose (0.268 ml/kg) providing 40 mg/kg of each of metaflumizone and amitraz; (F) The minimum commercial dose of fipronil (6.7 mg fipronil/kg, 0.067 ml/kg, Frontline[®] Top Spot, Merial, Duluth, GA). Dogs were observed for general health and any reaction to treatment approximately 1, 2, 3 and 4 h after treatment on Day 0, then once daily for the remainder of the study.

On Day 1 (approximately 24 h after treatment), all dogs were examined and a “finger count” conducted to qualitatively assess the parasite burden; the dogs were examined using a flea comb and the hands to part the hair to expose the skin and a qualitative assessment of the numbers of live ticks and fleas observed during a 5 min period was recorded. Numbers of fleas were assessed as 0, <10 or >10, and numbers of ticks as 0, <5 or >5. On Day 2, each dog was examined and combed to remove and count fleas and ticks. Subsequently, the animals were reinfested with ticks on Days 5, 12, 19, 26, 33 and 40, and with fleas on Days 6, 13, 20, 27, 34 and 41. The dogs were examined, combed and parasite counted on Days 7, 14, 21, 28, 35 and 42. For the counts, all dogs were first examined visually, and any ticks detected were removed using forceps. Ticks were examined to determine their viability. Any tick able to move in a coordinated manner was considered live. The dogs were then thoroughly flea combed to count and remove fleas and any remaining ticks. Commercial fine-toothed flea combs (Twinco) were used. Dogs were systemically combed using repeated strokes initially while standing starting from the head, then posteriorly along the dorsum and sides. The dog was then restrained on each side and then on its back for combing of the sides and ventral surface. Dogs were repeatedly combed until no fleas were recovered for about 5 min. Each animal was examined for a minimum of 10 min. Fleas able to stand up right and/or move in a coordinated manner were considered live. Any live fleas were killed by immersion in alcohol.

Flea and tick counts were performed by personnel trained in the standard procedures in use at the test facility. Protective gloves and clothing were changed before handling dogs from different groups. Dogs from different groups were identified by a color code and personnel conducting parasite or general health exams were unaware of treatment assignments.

2.3. Parasites

Cat fleas were from the Nu-Era farms colony, which was initiated with fleas originally obtained from stray animals at the Stillwater Animal Shelter, Payne County, OK. The strain had been maintained for six generations in the laboratory. Brown dog ticks were from the Nu-Era Farms colony, which was initiated with ticks originally obtained from stray animals at the Stillwater Animal Shelter, Payne County, OK. The strain had been maintained for two generations in the laboratory.

At each infestation, either 50 unfed adult ticks or approximately 100 unfed adult fleas were applied to each dog. The fleas or ticks were applied to the lateral midline of each dog, which was restrained by hand for sufficient time to allow the parasites to penetrate the hair coat. For tick infestations, dogs were held in tick infestation chambers (solid plastic dog crates with a wire gate) to minimize the movement of the dogs for about 8 h after ticks were applied to increase the chances of successful tick attachment.

2.4. Treatment

Dogs were treated with three different amounts of a metaflumizone plus amitraz in a commercial formulation containing 150 mg metaflumizone and 150 mg amitraz/ml. The placebo-treated dogs were treated with 0.134 ml/kg of the same solvent formulation with out the active ingredients. Group F dogs were treated with a commercial product containing fipronil. All applications were administered using disposable syringes. Doses were calculated using Day –2 body weights and were rounded to the nearest 0.1 ml. The dose was applied to the skin at a single spot between the shoulder blades.

2.5. Data analysis

Statistical analyses were performed separately for flea and tick counts and for each examination day. Flea and tick counts were transformed by the $\log_{10}(\text{count} + 1)$ transformation prior to analysis in order to stabilize the variance and normalize the data. Using the PROC MIXED procedure (SAS 8.2, Cary, NC), transformed counts were analyzed by an Analysis of Variance (ANOVA) with a model that considered treatment as a fixed effect and replicate as a random effect. Treatment was tested against the residual error at the 5% level of significance. Each treatment groups' least square means were compared using the two-sided Student's *t*-test at the 5% level of significance. Percent efficacy, relative to the nontreated control group and based on geometric means (gm), was calculated as follows:

$$\text{efficacy (\%)} = \frac{\text{gm control} - \text{gm control}}{\text{gm control}} \times 100$$

3. Results

3.1. Fleas

All dogs included in the study demonstrated good pretreatment flea holding ability. Day –5 flea counts ranged from 56 to 86. Nontreated animals maintained good flea infestations through out the study, except on Day 2 when flea counts were relatively low for nontreated and placebo-treated dogs (Table 1). This was due to the fact that because of the hot weather on Day –1 (flea infestation), portable fans were being used to keep the animals cool. These were inadvertently left on during the infestation process on this day and the increased air movement could have stimulated flea

Table 1

Geometric mean flea counts for nontreated and placebo control dogs, and counts and percent efficacy^a relative to nontreated controls for Beagles treated with a metaflumizone plus amitraz formulation or fipronil

Count day	Nontreated control	Placebo control	Metaflumizone plus amitraz (mg/kg)			Fipronil (6.7 mg/kg)
			10	20	40	
2	24.9 a	17.2 a	0.0 b (100)	0.0 b (100)	0.0 b (100)	0.0 b (100)
7	78.5 a	69.9 b	0.0 c (100)	0.0 c (100)	0.0 c (100)	0.0 c (100)
14	84.7 a	79.0 a	0.3 b (99.6)	0.2 b (99.8)	0.0 b (100)	0.0 b (100)
21	84.4 a	72.5 a	4.0 b (95.3)	0.2 c (99.8)	0.5 c (99.4)	0.0 c (100)
28	82.3 a	79.6 a	1.6 b (98.1)	0.1 c (99.9)	1.6 b (98.1)	0.0 c (100)
35	84.1 a	71.8 a	8.6 b (89.7)	1.6 c (98.1)	3.9 bc (95.3)	0.0 d (100)
42	72.3 a	77.7 a	17.9 b (75.3)	1.8 c (97.5)	22.8 b (68.5)	0.1 d (99.8)

^a Geometric mean counts with the same letter grouping within rows were not significantly different; $\alpha = 0.05$. Percent efficacy is given in brackets.

jumping response resulting in fleas leaving the animals rather than crawling down onto the skin.

The placebo treatment had little if any effect on flea numbers (Table 1). There was no significant difference between nontreated and placebo-treated mean counts on any day except Day 7, when significantly fewer fleas were recovered from the placebo-treated animals ($P < 0.05$). However, efficacy due to treatment with placebo was only 11% on Day 7. The maximum reduction seen in placebo-treated animals was 30.6% on Day 2. The qualitative finger counts on Day 1 indicated that all of the insecticidal treatments resulted in a noticeable reduction in flea numbers within 1 day of treatment; a score >10 was recorded for all nontreated dogs and three of six placebo-treated dogs, the other three were scored as <10 , while most treated animals were scored 0 or <10 . All of the metaflumizone and amitraz treatments and fipronil resulted in significantly lower flea counts relative to nontreated controls on all posttreatment count days ($P < 0.05$, Table 1). The combination of metaflumizone plus amitraz at 10 mg/kg provided $>95\%$ control of fleas from Days 2 to 28 and the 40 mg/kg dose resulted in $>95\%$ control from Days 2 to 35. Metaflumizone plus amitraz at 20 mg/kg and fipronil both provided $>95\%$ control from Days 2 to 42. For fleas, there were no significant differences among the three metaflumizone plus amitraz dose rates to 14 days posttreatment ($P > 0.05$, Table 1). From Day 21 on, the mean counts for the 10 mg/kg dose were significantly higher than those for the 20 mg/kg dose ($P < 0.05$). There was no significant advantage provided by the 40 mg/kg dose over the 20 mg dose through the entire study ($P > 0.05$). Flea counts for the 20 mg/kg dose were not significantly different to those of the fipronil-treated dogs to 28 days after treatment ($P < 0.05$) and were numerically similar there after.

3.2. Ticks

All dogs demonstrated good pretreatment tick holding ability. Day -5 tick counts ranged from 12 to 31. Nontreated animals maintained tick infestations through out the study (Table 2). The placebo treatment appeared to have little if any effect on brown dog tick numbers (Table 2). There was no significant difference between nontreated and placebo-treated mean counts on any day except Day 14, when significantly fewer ticks were recovered from the placebo-treated animals ($P < 0.05$); tick numbers on nontreated animals were relatively high on this count day. However, efficacy due to treatment with placebo on this day was only 23.1%. The maximum reduction seen in placebo-treated animals was 35.6% on Day 2. The qualitative finger counts on Day 1 indicated that all of the insecticidal treatments resulted in a noticeable reduction in tick numbers soon after treatment; a score >5 was recorded for five of six nontreated dogs and five of six placebo-treated dogs, the final dog in each group had a score of <5 , all six dogs in the metaflumizone plus amitraz 20 mg/kg dose had <5 ticks, and in all other treated groups five of six dogs had <5 ticks and the remaining dog in each group scored >5 .

All of the metaflumizone plus amitraz treatments and fipronil resulted in significantly lower tick counts relative to nontreated controls on all posttreatment count days ($P < 0.05$, Table 2). Metaflumizone plus amitraz at 10 mg/kg provided $>90\%$ control of ticks from Days 2 to 21. Treatment with metaflumizone plus amitraz at 20 and 40 mg/kg, and Frontline resulted in $>90\%$ control of ticks from Days 2 to 35. On Day 2, there was no significant difference in the tick counts between the metaflumizone plus amitraz 10 and 20 mg/kg dose groups or the 20 and 40 mg/kg groups ($P > 0.05$, Table 2). However, the metaflumizone plus amitraz 40 mg/kg treatment provided a significant

Table 2

Geometric mean *Rhipicephalus sanguineus* (brown dog tick) counts for nontreated and placebo control dogs, and counts and percent efficacy^a relative to nontreated controls for Beagles treated with a metaflumizone plus amitraz formulation or fipronil

Count day	Nontreated control	Placebo control	Metaflumizone plus amitraz (mg/kg)			Fipronil (6.7 mg/kg)
			10	20	40	
2	23.6 a	15.2 a	1.5 b (93.5)	0.8 bc (96.5)	0.0 c (100)	0.8 bc (96.7)
7	25.5 a	19.9 a	0.0 b (100)	0.0 b (100)	0.0 b (100)	0.0 b (100)
14	33.0 a	25.4 b	0.0 c (100)	0.0 c (100)	0.0 c (100)	0.0 c (100)
21	27.1 a	24.8 a	1.7 b (93.8)	0.0 c (100)	0.0 c (100)	0.0 c (100)
28	27.3 a	25.1 a	3.0 b (89.0)	0.0 c (100)	0.0 c (100)	0.0 c (100)
35	24.8 a	23.4 a	9.4 b (62.1)	0.8 cd (96.7)	1.5 c (93.9)	0.1 d (99.5)
42	23.2 a	28.3 a	11.1 b (52.1)	2.8 c (87.9)	7.4 b (68.2)	2.5 c (89.2)

^a Geometric mean counts with the same letter grouping within rows were not significantly different; $\alpha = 0.05$. Percent efficacy is given in brackets.

advantage over the 10 mg/kg group ($P < 0.05$). The three metaflumizone plus amitraz groups had equivalent efficacy (100%) on Days 7 and 14. From Day 21 on, the mean tick counts for the 10 mg/kg dose were significantly higher than those for the 20 mg/kg dose ($P < 0.05$). There was no significant advantage provided by the 40 mg/kg dose over the 20 mg/kg dose throughout the study ($P > 0.05$). Tick counts for the metaflumizone plus amitraz 20 mg/kg dose were not significantly different to those of the fipronil-treated group throughout the study ($P < 0.05$).

3.3. Health observations

One nontreated dog was noted to have vomited when all animals were inspected at about 24 h after treatment on Day 1. The dog otherwise appeared normal in behavior. There were no other adverse health observations observed during the study and there were no apparent adverse reactions to treatment.

4. Discussion

There were no significant differences in parasite counts between the nontreated control and the placebo-treated control groups for either fleas or ticks except for very slight reductions on Day 7 for fleas and Day 14 for ticks. These results demonstrated that the formulation excipients had no antiparasitic or repellent activity.

All dose levels of the metaflumizone plus amitraz formulation provided effective parasite control within 48 h posttreatment. The 20 mg/kg dose provided significantly better flea and tick control than the 10 mg/kg dose ($P < 0.05$) from Day 21 on. There was no significant advantage provided by the 40 mg/kg dose over the 20 mg/kg dose ($P > 0.05$). Both of these treatments controlled subsequent flea infestations for up to 5–6 weeks after treatment and tick infestations for up to 5 weeks posttreatment. Optimal control throughout

the study was provided by 20 mg metaflumizone plus 20 mg amitraz/kg dose and efficacy of this treatment was similar to the commercial product, fipronil. Thus, this dose rate was proposed as the minimum commercial dose rate to provide effective flea and tick control for at least 1 month following a single treatment.

5. Conclusions

This study demonstrated that the minimum dose rate to provide the optimal treatment and control of fleas and ticks on dogs for up to 1 month after treatment was 20 mg metaflumizone and 20 mg/amitraz/kg.

Acknowledgments

We thank Deborah M. Amodie for the statistical analyses. The excellent technical assistance of Connie Beagle, Jan Jones and Vanessa McClain is gratefully acknowledged.

References

- Bossard, R.L., Hinckle, N.C., Rust, M.K., 1998. Review of insecticide resistance in cat fleas (Siphonaptera: Pulicidae). *J. Med. Entomol.* 35, 415–422.
- Dryden, M.W., Payne, P.A., 2004. Biology and control of ticks infesting dogs and cats in North America. *Vet. Ther.* 26, 2–16.
- Krämer, F., Menke, N., 2001. *Flea Biology and Control*. Springer, Berlin, 192 pp.
- Needham, G.R., Teel, P.D., 1991. Off-host physiological ecology of ticks. *Annu. Rev. Entomol.* 36, 659–681.
- Ross, D.H., Young, D.R., Young, R., Pennington, R.G., 1998. Topical pyriproxyfen for control of the cat flea and management of insecticide resistance. *Feline Pract.* 26, 16–22.
- Rust, M.K., 2005. Advances in the control of *Ctenocephalides felis felis* (cat flea) on cats and dogs. *Trends Parasitol.* 21, 232–236.
- Rust, M.K., Dryden, M.W., 1997. The biology, ecology, and management of the cat flea. *Annu. Rev. Entomol.* 42, 451–473.
- Salgado, V.L., Hayashi, J.H., this volume. Mode of action of metaflumizone. *Vet. Parasitol.*