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Supplement

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Selamectin Plus Sarolaner:
A New Extended Spectrum, Topically Applied Parasiticide for Cats – Part 1

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Selamectin Plus Sarolaner: A New Extended Spectrum, Topically Applied Parasiticide for Cats – Part 1

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Amongst infectious agents, parasites are major players threatening the health and welfare of cats. This is well recognised by practitioners and pet owners and, for the potential role of some parasites as zoonotic agents, also by physicians working in Public Health, at all latitudes, from the poorest to the richest settings. Indeed, many parasites of cats are zoonotic agents infecting humans from pregnancy (toxoplasmosis), through childhood (Toxocara spp. and Ancylostoma spp.), and all through life (e.g., echinococcosis from accidental ingestion of embryonated eggs). Most importantly, cats share their environment with arthropod parasites (ticks, mites, fleas, and mosquitoes) that also feed on humans, thereby acting as potential vectors of viral, bacterial, protozoal, and helminth pathogens. Therefore, cats and their best friends, humans, are the main characters in this theatre piece (human and animal parasitology); they live together with their parasites. The stage for such a piece is the whole world from the Brazilian forests, to the Rocky Mountains, through the cosy historical Italian villages, to the savannah villages, the Romanian mountains, the freezing Kamchatka peninsula small towns, and in the south Asian tropical countries. Although there is much less information on parasitism in cats compared to dogs, parasites are rather common in cats, depending on their habits (stray or owned cats or individuals living in catteries), geographical areas or provenience, environment and, obviously, the use of parasiticides. The overall prevalence of cat endoparasites in Europe (from 20 to 40%) has been calculated in a number of studies based on coproscopic examination. Fleas are the main ectoparasites (up to 70% of cats suffer flea infestation in Austria, Spain and Germany) whereas the distribution of tick species in cats depends on where studies are carried out, with *Ixodes ricinus* and *Rhipicephalus* spp. (i.e., *R. turanicus* and *R. sanguineus* sensu lato), being the most prevalent in Europe. Until recently, detailed clinical efficacy and field studies have not been required in the European Union (EU) for the licensure of tick claims for cats if data were available for the product in dogs. The clinical field studies included in this volume provide up-to-date information on the actual incidence of ticks on cats in the EU from 270 cats enrolled with tick infestations in practices in France, Italy, Hungary and Germany.

Effective ectoparasiticides, alone or in combination, have been formulated in the last decades for cats. Products often target fleas; these include fipronil, which is also efficacious against ticks, imidacloprid, lufenuron, and spinosad, and the endectocide, selamectin, which has broad-spectrum activity against helminths, fleas, and mites but not ticks. Recently, a new class of ectoparasiticides has been introduced: the isoxazolines (e.g., fluralaner, afoxalaner and sarolaner), with efficacy against fleas and ticks. There are other less common licensed products for tick prevention in cats such as diazinon- and flumethrin-based collars.

Endo- and ectoparasites of cats are a rather frequent finding with co-infections occurring often. Thus effective broad-spectrum products with efficacy against the major endo- and ectoparasites of cats are needed to ensure the health and wellbeing of cats and their owners. In this supplement to Veterinary Parasitology a collection of selected papers illustrates the great potential of a new topical combination which merges tradition and innovation: selamectin plus sarolaner. This broad-spectrum antiparasitic drug is now available for the treatment and prevention of endo- and ectoparasites of cats. Selamectin (REVOLUTION® or STRONGHOLD®, Zoetis) has been in the market as a topical solution since 1999 for the treatment and prevention of fleas, prevention of heartworm disease, and the treatment of *Otodectes cynotis*, *Toxocara cati*, and *Ancylostoma tubaeforme* in cats. The efficacy spectrum of this well-known product has now been broadened to include ticks by the addition of a new isoxazoline, sarolaner.

Results of the studies included in this issue clearly indicate that a single spot-on application of this new product is efficacious for the treatment and prevention of flea infestations for 5 weeks, for the treatment and prevention of *I. ricinus* and *I. hexagonus* for 5 weeks, for the treatment and prevention of *Dermancotor reticulatus* and *R. sanguineus* for 4 weeks, for the treatment of ear mite infestations by *Otodectes cynotis*, and for the treatment of adult roundworms (*Toxocara cati*) and adult intestinal hookworms (*Ancylostoma tubaeforme*).

Laboratory studies have been corroborated by field studies in which the efficacy of treatments at monthly intervals of this new product against flea and tick infestations has been tested in cats presented as patients in European veterinary clinics. In the field studies, the new product was found to be safe and highly effective against natural infestations of fleas and ticks on cats. Rapid efficacy against fleas (within 24 h of infestation) makes the product useful as part of a treatment strategy for preventing environmental flea contamination and for the control of flea allergic dermatitis, as demonstrated by clinical data in the field study.

The ease of application of a spot-on product is a benefit that cannot be underestimated when treating feline patients. Spot-on products are often better tolerated by cats and hence easier for
owners to administer compared to oral products and to products that must be applied directly to an affected area (such as for ear mites). In addition, a spot-on product overcomes some of the issues with collars, such as intolerance by some cats and potential loss of the collar. The benefits of a spot-on product, especially one with a broad spectrum of activity and a low rate of adverse effects, increase owner compliance and improve protection of cats against endo- and ectoparasites.

The broad-spectrum efficacy provided by the combination of selamectin plus sarolaner, along with the ease of a monthly spot-on application, ensures the protection of cats against endo- and ectoparasites and provides pet owners and practitioners with a new tool to further improve cat health and welfare.

Conflict of interest

Zoetis supported the editorial assistance provided by Dr. Domenico Otranto and Dr. Susan Little.
Research paper

Efficacy of a new spot-on formulation of selamectin plus sarolaner against four common tick species infesting cats in Europe

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Tick
Cat
Dose confirmation

A B S T R A C T

A single application of a new spot-on formulation of selamectin plus sarolaner (Stronghold® Plus, Zoetis) was evaluated for efficacy against the most common tick species infesting cats in Europe. In each of the seven laboratory studies, 16 adult and purpose-bred cats were randomly allocated to one of two treatment groups based on pre-treatment tick counts. Weekly infestations with 50 uned adult Ixodes ricinus (2 studies), Ixodes hexagonus (1 study), Dermacentor reticulatus (2 studies), or Rhipicephalus sanguineus (2 studies) were scheduled on Days −2, 5, 12, 19, 26 and 33. Cats were treated on Day 0 with the spot-on formulation at the minimum recommended label dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight or with a placebo. Ticks were counted 48 h after treatment and after each re-infestation. No treatment-related adverse reactions were recorded in any of the studies. Geometric mean live tick counts were significantly (P < 0.0012) lower in the selamectin/sarolaner-treated group compared to the placebo-treated group at all time-points. Against I. ricinus and I. hexagonus, efficacy was >97.2% against existing infestations and >97.4% against weekly re-infestations for at least 5 weeks. Treatment was 100% effective against existing R. sanguineus infestations and >95.8% for at least 4 weeks. Against D. reticulatus treatment resulted in >94.4% efficacy for at least 4 weeks. Thus, a single application of the new spot-on formulation of selamectin plus sarolaner at the minimum dose provides rapid treatment of existing infestations and is at least one month effective against re-infestation by all relevant European tick species in cats.

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1. Introduction

Ticks are common ectoparasites in cats. In the EU, Ixodes ricinus (castor bean tick or sheep tick), I. hexagonus (hedgehog tick), Rhipicephalus sanguineus (brown dog tick) and Dermacentor reticulatus (ornate dog tick or marsh tick) have all been reported to infest cats, although with marked regional differences (Ogden et al., 2000; Stanneck et al., 2012; Claerebout et al., 2013; Geurden et al., 2017). Apart from the direct effects of infestation, such as skin irritation and alopecia (Dryden and Payne, 2004), ticks are known to transmit several vector-borne diseases (Beugnet and Marié, 2009). Some pathogens transmitted by ticks can cause serious disease and are known to be zoonotic, such as Lyme disease caused by Borrelia burgdorferi and transmitted by I. ricinus and I. hexagonus. Although less frequently described, tick-borne pathogens do cause specific infections in cats, such as Ehrlichia spp. and Anaplasma phagocytophilum (Beugnet and Marié, 2009).

Tick prevention in cats is based on the use of acaricidal compounds. Prior to the development of selamectin/sarolaner, very few products were available for the treatment and control of ticks on cats (Appendix A), and the compounds that were available either claim efficacy for a short duration and/or have an efficacy claim against only a limited number of the tick species of greatest relevance to cats in the EU. Selamectin is a well-known parasiteic with efficacy against fleas, ear mites, lice, heartworm, hookworms and roundworms. Sarolaner is an isoxazoline with excellent activity against ectoparasites (McTier et al., 2016) and broadens the efficacy spectrum in the new spot-on formulation to include ticks. The objective of the seven laboratory studies reported here was to confirm the efficacy of a new spot-on formulation of selamectin plus sarolaner against existing infestations and re-infestations with the four tick species of relevance to cats in Europe.

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Table 1
Arithmetic (geometric) mean live tick counts for *Ixodes ricinus* (castor bean tick) and *Ixodes hexagonus* (hedgehog tick); with ranges for placebo and selamectin/sarolaner-treated cats. Percent efficacy based on arithmetic (geometric) mean tick counts for cats treated at 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight in three laboratory studies.

<table>
<thead>
<tr>
<th>Tick and tick strain origin</th>
<th>Day</th>
<th>Placebo Mean</th>
<th>Placebo Range</th>
<th>Selamectin/Sarolaner Mean</th>
<th>Selamectin/Sarolaner Range</th>
<th>% Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ixodes ricinus</em> from Germany (Slovakia and Ireland)</td>
<td>2</td>
<td>16.3 (15.6)</td>
<td>9–22</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>17.5 (17.3)</td>
<td>13–23</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>20.6 (20.5)</td>
<td>17–25</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>20.5 (20.1)</td>
<td>14–25</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>22.4 (20.3)</td>
<td>8–38</td>
<td>0.1 (0.1) *</td>
<td>0–1</td>
<td>99.4 (99.6)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>19.9 (19.3)</td>
<td>13–30</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td><em>Ixodes ricinus</em> from Germany</td>
<td>2</td>
<td>9.0 (7.1)</td>
<td>0–14</td>
<td>0.3 (0.2) *</td>
<td>0–1</td>
<td>97.2 (97.3)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>23.8 (23.3)</td>
<td>16–32</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>22.4 (21.4)</td>
<td>12–33</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>17.4 (15.3)</td>
<td>3–27</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>18.6 (17.1)</td>
<td>6–30</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>22.4 (22.3)</td>
<td>18–25</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td><em>Ixodes hexagonus from The Netherlands</em></td>
<td>2</td>
<td>14.0 (10.9)</td>
<td>2–27</td>
<td>0.4 (0.3) *</td>
<td>0–2</td>
<td>97.3 (97.7)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>18.6 (18.4)</td>
<td>14–23</td>
<td>0.4 (0.3) *</td>
<td>0–1</td>
<td>98.0 (98.4)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>19.0 (18.3)</td>
<td>11–28</td>
<td>0.5 (0.4) *</td>
<td>0–1</td>
<td>97.4 (97.7)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>17.9 (17.2)</td>
<td>8–24</td>
<td>0.3 (0.2) *</td>
<td>0–1</td>
<td>98.6 (98.9)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>21.0 (20.7)</td>
<td>15–29</td>
<td>0.1 (0.1) *</td>
<td>0–1</td>
<td>99.4 (99.6)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>14.1 (13.7)</td>
<td>10–20</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
</tbody>
</table>

*Geometric mean live tick count significantly lower than placebo (*P*<0.0001).
1 Country where the ticks were originally isolated, and in brackets country from where ticks were isolated to rejuvenate the colony.
2 Range = minimum and maximum tick count among animals.

2. Materials and methods

Seven laboratory studies were conducted to confirm the efficacy of selamectin/sarolaner against *I. ricinus* (two studies), *I. hexagonus* (one study), *D. reticulatus* (two studies) and *R. sanguineus* (two studies). All studies complied with Good Clinical Practices (VICH guideline GL9, 2000) and with the relevant WAAPV (World Association for the Advancement of Veterinary Parasitology) guidelines (Marchiondo et al., 2013). All studies were reviewed by an appropriate Ethical Review Committee.

2.1. Animals

An adequate wash-out period (minimum three months) was respected for all enrolled cats so that no residual ectoparasiticide efficacy remained from any previous treatments. All 16 cats were purpose-bred, received a unique identification and were examined prior to the start of the study to evaluate suitability for inclusion. Cats were between 8 months and 9 years old, and their bodyweight ranged from 2.0 to 5.8 kg. Both male and female cats were enrolled, and female cats were confirmed not to be pregnant or lactating. The cats were acclimatized to the housing facilities at latest 7 days prior to treatment. Cats were housed individually in indoor pens to avoid physical contact and tick transfer. Cats received an appropriate commercial diet during the study. Water was provided ad libitum. From the start of the acclimation period until the end of the study, general health observations were performed at least once a day.

2.2. Study design

On Day –7, cats were examined to confirm the absence of ticks. Cats were infested on Day –7 to evaluate host suitability, on Day –2 to evaluate efficacy against existing infestations, and on Days 5, 12, 19, 26 and 33 to evaluate persistent efficacy. At each tick infestation, approximately 50 adult and unfed ticks were used at an approximate 1:1 sex ratio, except for *I. ricinus* and *I. hexagonus* with a 3:2 female to male ratio. Ticks were sourced from differ-

Table 2
Arithmetic (geometric) mean live tick counts for *Dermacentor reticulatus* (ornate dog tick) with ranges for placebo and selamectin/sarolaner-treated cats. Percent efficacy based on arithmetic (geometric) mean tick counts for cats treated at 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight in two laboratory studies.

<table>
<thead>
<tr>
<th>Tick Strain Origin</th>
<th>Day</th>
<th>Placebo Mean</th>
<th>Placebo Range</th>
<th>Selamectin/Sarolaner Mean</th>
<th>Selamectin/Sarolaner Range</th>
<th>% Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany (The Netherlands)</td>
<td>2</td>
<td>27.3 (25.2)</td>
<td>9–45</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>26.9 (22.1)</td>
<td>4–44</td>
<td>0.1 (0.1) *</td>
<td>0–1</td>
<td>99.5 (99.6)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>19.5 (16.6)</td>
<td>3–33</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>24.4 (16.4)</td>
<td>0–43</td>
<td>1.4 (0.8) *</td>
<td>0–6</td>
<td>94.4 (95.1)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>19.5 (17.0)</td>
<td>6–32</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>27.8 (21.5)</td>
<td>2–46</td>
<td>3.5 (1.7) *</td>
<td>0–16</td>
<td>87.4 (91.9)</td>
</tr>
<tr>
<td>UK (The Netherlands and Slovakia)</td>
<td>2</td>
<td>21.1 (17.7)</td>
<td>7–42</td>
<td>0.9 (0.3) *</td>
<td>0–7</td>
<td>95.9 (98.3)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>30.6 (29.5)</td>
<td>19–45</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>15.3 (11.4)</td>
<td>0–24</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>20.1 (18.5)</td>
<td>8–34</td>
<td>0.0 (0.0) *</td>
<td>0–0</td>
<td>100 (100)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>17.6 (16.9)</td>
<td>9–25</td>
<td>0.1 (0.1) *</td>
<td>0–1</td>
<td>99.3 (99.5)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>32.3 (31.1)</td>
<td>18–45</td>
<td>9.0 (5.8) *</td>
<td>1–23</td>
<td>72.1 (81.2)</td>
</tr>
</tbody>
</table>

*Geometric mean live tick count significantly lower than placebo (*P*<0.0012).
1 Country where the ticks were originally isolated, and in brackets country from where ticks were isolated to rejuvenate the colony.
2 Range = minimum and maximum tick count among animals.
ent laboratory colonies and were originally isolated from the field in Europe, US and South Africa (see also Tables 1–3) with introduction of field-collected ticks within the previous ten years. To inhibit grooming and enhance tick retention, cats were fitted with Elizabethan collars during all infestation periods (Day –7 to –5, Day –2 to 0, Day 5–7, Day 12–14, Day 19–21, Day 26–28 and Day 33–35), except for Day 0 (when collars were removed prior to treatment administration so as not to interfere with the application site) to Day 2.

The number of ticks was enumerated by adequately trained staff at 48 (±2) hours after infestation or treatment, as follows: the hair was pushed against its natural nap, exposing the ticks which were counted and removed. Subsequent to the manual inspection, cats were combed to remove any residual ticks. The entire body of each cat was examined for at least 10 min, and the examination was continued in 1 min increments until no ticks were encountered in the last minute of the examination. The viability (live-dead), attachment (free-attached) and engorgement status (engorged-non engorged) of the ticks was assessed and recorded. The staff performing the tick counts wore protective clothing that was changed between cats to avoid cross-contamination. The staff conducting tick counts or health observations was blinded to the treatment.

On Day –5, tick counts were performed to evaluate the host suitability, and the 16 cats with the highest tick counts were ranked in two blocks by decreasing tick counts. Treatment (placebo or selamectin/sarolaner) was randomly allocated within block. At latest by Day –2, cats were moved into their randomly allocated pens. On Day –2, cats were infested again with ticks and the bodyweight was recorded. On Day 0 cats were treated with either the placebo or the new spot-on formulation at the minimum dose of 6.0 mg selamexitin and 1.0 mg sarolaner per kg bodyweight. The treatments were applied at the base of the neck cranial to the scapulae at a dose volume of 0.1 ml per kg bodyweight. Each cat was observed immediately after dosing for potential adverse events. Approximately 1, 3, 6 and 24 h after treatment, general health and any reaction to treatment was observed. Evaluations of the application site were performed at approximately 1, 3, 6 and 24 h after treatment and again at 3 and 5 days after treatment.

2.3. Data analysis

The experimental unit in the study was defined as the individual cat. Prior to analysis, the live tick counts (primary variable) were transformed by log$_e$(count + 1), and analyzed (two-sided at the significance level α = 0.05) using a mixed linear model for repeated measures (PROC MIXED procedure, SAS 9.3, Cary NC) with fixed effects of treatment, day of study and the interaction between treatment and day of study. Block, the interaction between treatment (animal term) and block, and error were included into the model as random effects. The efficacy (percent reduction) was calculated using the Abbott’s formula:

$$\text{% reduction} = \frac{100 \times \text{mean count (placebo)} - \text{XPSndash; mean count (treated)}}{\text{mean count (placebo)}}$$

3. Results

3.1. Efficacy

Cats in the placebo-treated group were adequately infested throughout the studies (Tables 1–3), although the attachment rates varied between studies. Against existing infestations by *L. ricinus*, the arithmetic mean efficacy was 97.2% and 100% after treatment. The reduction in live tick counts was >99.4% for at least 5 weeks post treatment (Table 1). Against *L. hexagonus*, a 97.3% treatment efficacy was observed as well as a ≥97.4% persistent efficacy for at least 5 weeks (Table 1). For *D. reticulatus*, treatment efficacy was 95.9% and 100%, and persistent efficacy was >94.4% for at least 4 weeks (Table 2). Efficacy against existing infestations of *R. sanguineus* was 100% and persistent efficacy was ≥95.8% for at least 4 weeks (Table 3). At all time-points, the mean live tick counts in the selamectin/sarolaner-treated animals were significantly lower compared to placebo-treated animals (P values between 0.0001 and 0.0012).

3.2. Health observations and application site reactions

No adverse events were recorded that were related to selamectin/sarolaner treatment. A number of cats in both treatment groups had transient cosmetic observations at the application site, including greasiness, matting, spiking of the hair and white deposits, all of which are typical of a spot-on application. These application site reactions were generally observed between 1 and 24 h after application but not afterwards. In one cat treated with selamectin/sarolaner, white deposits were recorded on Day 3 but not on Day 5 after treatment.
4. Discussion

Sarolaner is an isoxazoline class compound and is known to be highly efficacious against ticks and other ectoparasites (McTier et al., 2016). In the present studies, the new spot-on formulation of selamectin plus sarolaner for cats (Stronghold® Plus, Zoetis) was evaluated for efficacy against the four most prevalent tick species reported on cats in Europe. The results confirmed the efficacy of a single treatment with selamectin/sarolaner at the minimum recommended dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight against existing infestations as well as the persistent efficacy against *I. ricinus* and *I. hexagonus* for 5 weeks and against *R. sanguineus* and *D. reticulatus* for 4 weeks.

Sarolaner is the only isoxazoline shown to be highly efficacious against *I. hexagonus*, both in dogs (Geurden et al., 2016) and in cats (current study). Although less prevalent compared to *I. ricinus*, the hedgehog tick is found in 8.4% to 59.7% of examined cats (Ogden et al., 2000; Stanneck et al., 2012; Claerebout et al., 2013). Furthermore, *I. hexagonus* is frequently infected with tick-borne diseases, including Lyme disease (Schreiber et al., 2014; Claerebout et al., 2013), warranting the high and persistent efficacy against this tick species. In general, the new spot-on formulation of selamectin plus sarolaner has a broader tick efficacy spectrum compared to any other acaricidal product authorized for use in cats. The broad efficacy ensures protection against the relevant tick species in all EU regions, and may as such decrease the transmission of tick-borne pathogens. Furthermore, the expansion of tick species, such as *D. reticulatus*, to regions where they were previously not reported emphasizes the need to have products effective against all tick species (Abdullah et al., 2016; Olivieri et al., 2016).

Persistent efficacy of at least 4–5 weeks enables the new spot-on formulation to be used in a monthly treatment routine. Furthermore, a spot-on is generally considered the most convenient application method for cats. A number of the fipronil-based tick products have either an undefined or shorter persistent efficacy against *I. ricinus* (Fourie et al., 2015; see also Appendix A). Other therapeutic alternatives, such as fluralaner or the imidacloprid plus flumethrin collar provide a longer period of persistency, but do not have documented efficacy against all tick species relevant in the EU or a confirmed rapid speed of kill in cats (Becskéi and Lin, 2017). The importance of a broad, rapid and persistent efficacy is not only relevant due to the direct beneficial effect on the clinical symptoms associated with tick infestations, but is also in light of tick-borne disease ecology and transmission.

5. Conclusions

The high and persistent efficacy for at least 4–5 weeks of a single topical application of new spot-on formulation of selamectin plus sarolaner (Stronghold® Plus, Zoetis) at the minimum recommended dosage of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight was confirmed against the four most common tick species infecting cats in Europe.

**Conflict of interest**

The studies reported here were funded by Zoetis. TG, CB, AV, VK, NS, and DL are employees of Zoetis. MR and DR are former employees of Zoetis. All authors assisted with the design of the studies, interpretation of the data and manuscript review. The studies were conducted by Contract Research Organisations. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

**Acknowledgements**

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**Appendix A.**

Overview of the efficacy and persistent efficacy against ticks of those compounds currently authorized for use in cats in the EU. Information as described on the Summary of Product Characteristics.

<table>
<thead>
<tr>
<th>Product</th>
<th>Application Route</th>
<th>Active substance(s)*</th>
<th><em>I. ricinus</em></th>
<th><em>I. hexagonus</em></th>
<th><em>Rhipicephalus sanguineus</em></th>
<th><em>Dermacentor reticulatus</em></th>
<th><em>Rhipicephalus turanicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stronghold® Plus (Zoetis)</td>
<td>Topical</td>
<td>selamectin/sarolaner</td>
<td>5 weeks</td>
<td>5 weeks</td>
<td>4 weeks</td>
<td>4 weeks</td>
<td>NR</td>
</tr>
<tr>
<td>Bravecto® Topical (Merial)</td>
<td>Topical</td>
<td>fipronil, s-methoprene, praziquantel, eprinomectin</td>
<td>Up to 3 weeks</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Broadline® (Merial)</td>
<td>Topical</td>
<td>fipronil</td>
<td>T + P ¹</td>
<td>NR</td>
<td>T + P ²</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Frontline® (Merial)</td>
<td>Spray</td>
<td>fipronil</td>
<td>T + P ¹</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Frontline® Combo (Merial)</td>
<td>Topical</td>
<td>fipronil, s-methoprene</td>
<td>Up to 2 weeks²</td>
<td>NR</td>
<td>Up to 2 weeks²</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Seresto® for cats (Bayer)</td>
<td>Collar</td>
<td>imidacloprid, flumethrin</td>
<td>8 months</td>
<td>NR</td>
<td>NR</td>
<td>8 months</td>
<td>NR</td>
</tr>
</tbody>
</table>

*active providing efficacy against ticks is underlined.

¹Summary of Product Characteristics states treatment and prevention (T + P), but does not specify the duration of persistent efficacy.

²Based on experimental data only.

NR = not reported.
References


Research paper

Speed of kill of a new spot-on formulation of selamectin plus sarolaner for cats against induced infestations with *Ixodes ricinus*

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  * Sarolaner
  * Stronghold
  * Cat

**Abstract**

The speed of kill of a new spot-on formulation containing selamectin plus sarolaner (Stronghold® Plus, Zoetis) for cats was evaluated against *Ixodes ricinus* ticks in a placebo-controlled, blinded study. Sixteen (16) cats were blocked by pre-treatment tick counts and randomly allocated to the placebo-treated group or the selamectin/sarolaner-treated group. Cats either received a single topical treatment at the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight or a placebo formulation on Day 0. On Days -2, 7, 14, 21, 28 and 35, cats were infested with approximately 50 unfed, viable and adult *I. ricinus* ticks. Tick counts were performed in situ 8 and 12 h after treatment or re-infestation. Ticks were removed from the cats and counted at the 24 h tick count. Acaricidal efficacy at each time point was calculated based on the reduction of mean live tick counts in the selamectin/sarolaner-treated group versus the placebo-treated group.

There were no treatment-related adverse reactions during the study. Placebo-treated cats maintained infestations with mean tick counts ranging from 10.3 to 21.9 throughout the study. The new spot-on formulation of selamectin plus sarolaner demonstrated 99.3% efficacy (P < 0.0001) within 24 h after treatment against pre-existing infestations. For subsequent re-infestations, efficacy was >97.9% for at least 3 weeks and was 89.0% after the re-infestation on Day 28. Mean live tick counts were significantly reduced by 12 h after re-infestation for at least 28 days (P < 0.0338). Thus, a single application of the new spot-on formulation of selamectin plus sarolaner at the minimum label dose started killing ticks within 24 h after treatment and within 12 h after re-infestations for 4 weeks. High acaricidal efficacy was achieved within 24 h after treatment and this persisted following subsequent re-infestations for a month.

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1. Introduction

Tick infestations in cats are perceived not be as prevalent as in dogs by both pet owners and veterinarians (Shaw et al., 2001). This perception of lower exposure to ticks might be due to differences in behaviour, such as frequent grooming, a more urban or indoor lifestyle in cats, because cats do not accompany owners to outdoor activities where the risk of tick infestation is perceived to be the highest (Ogden et al., 2000; Mendes-de-Almeida et al., 2011). Nevertheless, cats are exposed to ticks even in urban environments, when in parks, cemeteries or back yards. Furthermore, cats often frequent those environments where the tick life cycle is maintained by reservoir hosts including feral pets, rodents, lizards, birds and insect eaters such as hedgehogs (Dautel and Kahl, 1999; Rizzoli et al., 2014). Cats are thus an important host to ticks in all epidemiological settings and as a close companion might serve as a source of infected ticks for humans (Lempereur et al., 2011). Ticks collected from cats harbour a wide range of tick-borne pathogens. Specifically in *Ixodes ricinus* ticks, *Anaplasma phagocytophylum and Borrelia burgdorferi* s.l. are commonly found (Situ et al., 2013; Heyman et al., 2010; Claerebout et al., 2013; Geurden et al., 2016). Consistent and rapid killing of *I. ricinus* ticks is thus required to reduce both the spread of clinical disease in the cat population and the infestation pressure for the cat owners and other family pets.

Selamectin is a macrocyclic lactone effective against flea, louse, ear mite and gastrointestinal nematode infestations and in the prevention of *Dirofilaria immitis* infection in cats (European Medicines Agency, Stronghold: Product information). It is however a weaker acaricidal (Jernigan et al., 2000). To widen the spectrum to include ticks, it was combined with sarolaner, a potent isoxazoline (McTier et al., 2016). The new topical formulation of selamectin plus sarolaner was shown to be highly effective against four European...
tick species, including *I. ricinus* (Geurden et al., 2017b). This study was conducted to evaluate the speed of kill of the new topical formulation at the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight against *I. ricinus* for 5 weeks in cats.

2. Materials and methods

The study was conducted in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats (Marchiondo et al., 2012) and complied with Good Clinical Practice and VICH guideline GL9 (EMA, 2000). Study protocols were reviewed and approved by the local and Zoetis Institutional Animal Care and Use Committees.

2.1. Animals and treatment

Sixteen purpose bred cats (9 male and 7 female, aged 12 to 115 months, and weighing 2.3 to 5.0 kg) originating from the colony of the study site were selected for the study. Cats were acclimatized to the study conditions for 7 days before treatment. Cats were housed in indoor individual pens, so that no physical contact was possible between cats. A commercial cat food provided a maintenance diet. Fresh water was available ad libitum. Cross-contamination between cats was avoided by using separate equipment and by changing the protective clothing of the personnel handling cats. The health of the cats was monitored twice a day by clinical inspection by suitably qualified personal.

Cats were ranked in a descending order based upon the host-suitability tick counts on Day -5 and the cats with the highest tick counts were allocated to one of two treatment groups of eight cats each using a randomized complete block design. On Day 0, treatments were administered topically to the skin in a single spot at the base of the neck. Placebo and the selamectin plus sarolaner combination product (Stronghold<sup>®</sup> Plus) was administered at a dose volume of 0.1 ml/kg. This volume provided the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight. Clinical observations were conducted pre-treatment and at 1, 3, 6 and 24 h after treatment.

2.2. Tick infestations and tick counts

On Days –7, –2, 7, 14, 21, 28, and 35, each cat was infested with 50 (±4) adult *I. ricinus*, at a ratio of 30 (±2) female ticks and 20 (±2) male ticks. The ticks originated from natural populations collected in Slovakia, Germany, and Ireland and have been maintained in the laboratory. Cats were sedated for the infestations using 0.08 ml/kg medetomidine hydrochloride (1 mg/ml) by intramuscular injection. Ticks were applied directly onto the cats’ hair coat at a site away from the treatment site, approximately 2–3 inches behind the shoulder blades. Cats were fitted with Elizabethan collars to restrict grooming during all infestation periods except on Days 0 and 1, when these could interfere with the treatment application site. Host-suitability tick counts were conducted 48 h after the infestation on Day –7. Post-treatment tick counts were conducted 8, 12 and 24 h after treatment on Day 0 and after the subsequent infestations on Days 7, 14, 21, 28, and 35. At the 8 and 12 h counts, ticks were counted in situ without removal. Tick counts with removal were conducted at the 24 h counts. The order of the tick counts was determined by randomization at each count. The counts were conducted by systematically examining the entire body manually. Specific care was taken that at the 8 and 12 h counts to ensure all body areas were only examined once. After the manual inspection at the 24 h counts, an extra-fine tooth comb was used to comb the animal to remove any otherwise missed ticks. Each cat was examined for at least 10 min. If ticks were encountered in the last minute, manual examination (at the 8 and 12 h counts) or combing (at the 24 h counts) was continued in 1 min increments until no ticks were encountered. Masking was accomplished by ensuring that all persons who made observations, conducted tick infestations and tick counts and/or categorizations, or performed general care for the cats were not involved in the treatment administrations and were not aware of the treatment group assignments.

2.3. Data analysis

Prior to data analysis, the tick counts of individual cats (sum of the free and attached live ticks) were transformed by using the formula ln(count + 1) in order to stabilize the variance and normalize the data. Transformed counts were analyzed using a general linear mixed model for repeated measures. The model included the fixed effects of treatment, time point and treatment by time point interaction and random effects of block, animal within block, and error. Testing was two-sided at the significance level of 0.05. Percent efficacy relative to the control group was calculated using the Abbott formula: [(C – T)/C] × 100, where C = arithmetic mean live tick count for the control group and T = arithmetic mean live tick count for the treated group (Marchiondo et al., 2013).

3. Results

3.1. Speed of kill

The mean tick counts in the two treatment groups and the percent reduction in the treated vs the placebo group are summarized in Table 1 for all time points.

Placebo-treated cats maintained tick infestations throughout the study with mean tick counts ranging from 10.3 to 21.9. Against existing infestations, selamectin/sarolaner achieved 99.3% reduction in tick counts within 24 h after treatment administration (*P* < 0.0001). After re-infestation, a significant reduction in tick

Table 1

<table>
<thead>
<tr>
<th>Count time</th>
<th>Treatment group</th>
<th>Day after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>8h</td>
<td>Placebo</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>16.1 (4.4%)</td>
</tr>
<tr>
<td>12h</td>
<td>Placebo</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>14.6 (13.3%)</td>
</tr>
<tr>
<td>24h</td>
<td>Placebo</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>0.1 (99.3%)</td>
</tr>
</tbody>
</table>

* Mean live tick counts significantly *(P* < 0.05) lower in the treated group than the placebo group. Cats were infested with 50 *I. ricinus* ticks on study days –2, 7, 14, 21, 28, and 35.
counts was achieved within 8 h after infestation on days 7, 14 and 28 and within 12 h on day 21 ($P < 0.0338$). A reduction in tick counts $\geq 99.2\%$ was achieved within 24 h after re-infestation until Day 21 and was 89.0% on Day 28.

### 3.2. Health observations

Three placebo-treated cats and two selamectin/sarolaner-treated cats had local dermatological signs due to tick bites. Two cats in the placebo-treated group required systemic antibiotic treatment. In the selamectin/sarolaner-treated group loose faeces was observed in the pen of one cat before treatment and in another cat 1 h after treatment on Day 0. Both conditions resolved without treatment. None of these conditions were considered to be related to treatment.

### 4. Discussion

This study demonstrated that the new spot-on formulation of selamectin plus sarolaner (Stronghold® Plus, Zoetis) has a rapid speed of kill against existing *I. ricinus* infestations in cats with a reduction in mean tick counts of 99.3% within 24 h after treatment. Further, the single topical treatment at the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight significantly reduced the number of ticks on cats within 12 h for at least 28 days. High efficacy ($\geq 99.2\%$) was achieved within 24 h after re-infestation until Day 21 and was >89.0% for the entire month.

*I. ricinus* is a widespread tick species that feeds on over 300 different vertebrates including cats (Bowmann and Nuttall, 2008). In Northern, Western and Central Europe, *I. ricinus* is the most common tick species found on cats (Ogden et al., 2000; Beracková and Kočišová, 2008; Stanneck et al., 2012a; Capári et al., 2013; Geurden et al., 2017b). Furthermore, *I. ricinus* is known as an important vector of tick-borne pathogens including *Borrelia* spp. and *A. phagocytophilum* causing disease in humans and dogs and in dogs and cats respectively (Shaw et al., 2001; Beugnet and Marié, 2009; Geurden et al., 2016).

The new spot-on formulation of selamectin plus sarolaner demonstrated high efficacy against *I. ricinus* for 5 weeks under experimental conditions after a single administration (Geurden et al., 2017a) and after multiple monthly administrations under field conditions (Geurden et al., 2017b). These reports with the results of the current study, on the rapid speed of kill, demonstrate that selamectin plus sarolaner help reduce the spread of vector-borne pathogens directly by rapidly eliminating tick infestations on the cats and thereby indirectly breaking the epidemiological cycle of these pathogens (Geurden et al., 2017a,b).

Despite the relevance of tick infestations in cats, there are surprisingly few data on efficacy of acaricides in cats, particularly on their speed of kill (Stanneck et al., 2012a,b, Kužner et al., 2013; Tielemans et al., 2014; Fourie et al., 2015; Geurden et al., 2017a,b). A spot-on formulation containing fipronil/(S)-methoprene/eprinomectin/praziquantel (Broadline®, Merial) was reported to significantly reduce *I. ricinus* tick counts at 24 h with a peak in efficacy of 81.5% 2 weeks after treatment administration and further declining towards the end of the month (Fourie et al., 2015). While an imidacloprid/flumethrin impregnated collar was reported to kill 100% of *I. ricinus* within 6 h after infestations for 34 weeks on cats, its efficacy at the same time points against *Rhipicephalus turanicus* varied considerably between 54.8 and 85.4% during the 34 weeks of study duration (Stanneck et al., 2012b; Fourie et al., 2015). Additionally, it has suboptimal efficacy against ticks that are present on the cats at the time of collar application (Stanneck et al., 2012b).

A major disadvantage is that cats wearing a collar are at risk of strangulation by getting caught on objects while roaming freely in the scrubs and passing through tight passages while hunting and exploring (Stanneck et al., 2012b). A monthly spot-on with persistently high efficacy against four relevant tick species (*Ixodes ricinus*, *Ixodes hexagonus*, Dermacentor reticulatus, and *Rhipicephalus sanguineus*) in Europe (Geurden et al., 2017a,b) and with fast speed of kill therefore offers an attractive alternative. An additional advantage is that sarolaner is absorbed through the skin after topical administration and is distributed systemically. The systemic distribution via the blood circulation provides a uniform exposure to the ticks in all body areas including the extremities, whereas with cutaneous acaricides, such as fipronil and flumethrin, this may not be the case (Pfister and Armstrong, 2016). It has been reported that 82.42% and 70.84% of the ticks found on imidacloprid 10%/permethrin 50% spot-on and fipronil 10%/methoprene 12% spot-on treated dogs were recovered from the legs including interdigital spaces, respectively, while in the untreated dogs ticks were homogeneously distributed on the legs, head and the body (Otranto et al., 2005).

### 5. Conclusion

The new spot-on formulation at the minimal label dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight has a rapid onset of activity against *I. ricinus*, resulting in a fast reduction of existing infestations and subsequent re-infestations for a month.

### Conflict of interest

The study reported here was funded by Zoetis. CB, DL, DR and TG are current employees of Zoetis. All authors assisted with the design and/or conduct of the studies, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

### References


Geurden, T., Becskei, C., Faraks, R., Lin, D., Rugg, D., 2017b. Efficacy and safety of a new spot-on formulation of selamectin plus sarolaner in the treatment of naturally occurring flea and tick infestations in cats presented as veterinary


Lemperere, L., De Cat, A., Caron, Y., Madder, M., Claerebout, E., Saegerman, C., Losson, B., 2011. First molecular evidence of potentially zoonotic Babesia microti and Babesia sp, EU1 in Ixodes ricinus ticks in Belgium. Vector-Borne Zoonotic Dis. 11, 125–130.


Research paper

Efficacy and safety of a new spot-on formulation of selamectin plus sarolaner in the treatment of naturally occurring flea and tick infestations in cats presented as veterinary patients in Europe

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A B S T R A C T

Two randomised, blinded, multi-centre field studies were conducted in Europe (Germany, Italy, France, Hungary) to demonstrate the efficacy and safety of three monthly applications of a new spot-on formulation of selamectin plus sarolaner (Stronghold® Plus, Zoetis) against natural flea and tick infestations in cats presented as veterinary patients. The spot formulation was administered at the commercial dose range of 6.0–12.0 mg selamectin and 1.0–2.0 mg sarolaner per kg bodyweight. In the flea study, the improvement in clinical signs associated with flea allergy dermatitis (FAD) was also monitored. Imdacloprid plus moxidectin (Advocate® for Cats, Bayer) and fipronil (Frontline® Spot on, Merial) were used as positive control products in the flea and tick studies, respectively. Treatments were administered on Days 0, 30 and 60. Efficacy was calculated based on the mean percent reduction of live parasite counts on post-treatment days 14, 30, 60 and 90 versus the pre-treatment count on Day 0. Non-inferiority of selamectin/sarolaner to the control products was assessed at each time-point using a non-inferiority margin of 15% at the one-sided 0.025 significance level. Cats were enrolled in a 2:1 ratio (selamectin/sarolaner: comparator).

In the flea study, 277 cats were assessed for efficacy and safety, and an additional 170 cats were assessed for safety only. On days 14, 30, 60 and 90, efficacy against fleas was 97.4%, 97.3%, 98.8% and 99.4% in the selamectin/sarolaner-treated group and was 90.0%, 83.6%, 87.7% and 96.3% in the imdacioprid/moxidectin-treated group, respectively. Selamectin/sarolaner was non-inferior to imdacioprid/moxidectin at all time-points. For the 16 cats identified as having FAD at enrolment, clinical signs related to FAD improved following treatment administration. In the tick study, 200 cats were assessed for efficacy and safety, and a further 70 cats were assessed for safety only. Four tick species were identified. Overall efficacy against ticks was 96.7%, 92.6%, 98.8% and 99.5% in the selamectin/sarolaner-treated group and was 90.2%, 74.6%, 83.0% and 93.4% in the fipronil-treated group on Days 14, 30, 60 and 90, respectively. Selamectin/sarolaner was non-inferior to fipronil at all time-points, and was superior on Days 30 and 60. There were no serious treatment-related adverse events in any study. Thus, the new spot-on formulation of selamectin plus sarolaner administered at monthly intervals was safe and highly effective against natural infestations of fleas and ticks on cats, and improved clinical signs of FAD.

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1. Introduction

Treatment and/or prevention of ecto-and endoparasites in cats is an integral part of general veterinary practice. Domestic cats can be plagued by a range of parasite species, all causing distinct clinical signs. In a recent European study it was found that 50.7% of the cats had at least one internal or external parasite and that 11.9% were both infested with ectoparasites and gastro-intestinal helminths (Beugnet et al., 2014). Cat fleas (Ctenocephalides felis) are known to frequently infest cats with prevalence rates reported up to 70% (Beck et al., 2006; Bond et al., 2007; Farkas et al., 2009; Capári et al., 2013; Knaus et al., 2014; Lefkaditis et al., 2015). The annual expenditures to control fleas on companion animals exceed US$1 billion in the USA and €1.1 billion in Western Europe (Krämer and Mencke, 2001). Although tick infestations are less frequently reported in cats than dogs, recent studies indicate that mainly four...
tick species (Ixodes ricinus, I. hexagonus, Rhipicephalus sanguineus s.l. and Dermacentor reticulatus) are found to infest cats in the EU (Ogden et al., 2000; Otranto and Dantas-Torres, 2010; Stanneck et al., 2012; Capári et al., 2013; Claerbeaut et al., 2013; Pennisi et al., 2015). Both fleas and ticks are important vectors of disease agents caused by pathogenic protozoa (e.g. Babesia spp.), viruses (e.g. tick-borne encephalitis virus), rickettsias, and bacteria (e.g. Bartonella henselae; Borrelia burgdorferi s.l., Ehrlichia spp., Francisella tularensis, Anaplasmata spp.), many of which are zoonotic. Although cats are not always directly affected, acaricidal or insecticidal treatment can be important in managing the spread of these vector-borne pathogens to more susceptible hosts, including humans.

Due to the high prevalence of both endo- and ectoparasites there is a need for safe and efficacious products to treat these parasites in cats. Selamectin is a well-known parasiticide with efficacy against fleas, ear mite, lice, heartworm and gastro-intestinal nematodes. Sarolaner is a novel isoxazolone with potent activity against a wide range of ectoparasites (McTier et al., 2016). In the new spot-on formulation of selamectin plus sarolaner (Stronghold® Plus), sarolaner broadens the efficacy spectrum to include ticks. In laboratory studies, new spot-on formulation of selamectin plus sarolaner was found to be highly effective against fleas and ticks (Becskei et al., 2017; Geurden et al., 2017). Two clinical field studies were conducted in France, Italy, Hungary and Germany to evaluate the efficacy and safety of the new spot-on formulation of selamectin plus sarolaner against natural flea and tick infestations in cats presented as veterinary patients.

2. Materials and methods

Two randomised, blinded, positive-controlled clinical field studies enrolling cats presenting with flea or tick infestations were conducted at veterinary clinics in France, Hungary, Italy and Germany. All personnel (e.g. the examining veterinarian) involved with the collection of efficacy and safety data were blinded to treatment. All treatments were dispensed by separate study personnel (dispenser), who were not involved in any other study activity. The studies were conducted in compliance with Good Clinical Practice, (VICH guideline GL5, EMEA, 2000) and the study protocols were reviewed and approved by the Zoetics Ethics Review Assessment team.

2.1. Animals

Enrolment was limited to households with three or fewer cats. One cat in each household was enrolled as the primary patient and only that cat received efficacy evaluations. Other cats living in the same household as the primary cat were enrolled as supplementary patients and received the same treatment but were only evaluated for safety. The primary patient had to harbour ≥5 live fleas or ≥3 live attached ticks at enrolment. Within each clinic the primary cats were randomly allocated to the two treatment groups (separately in each study) in a ratio of 2:1, so that for every two patients that received new spot-on formulation of selamectin plus sarolaner, one patient received the positive control product (imidacloprid/moxidectin (Advocate® for Cats, Bayer) in the flea study and fipronil (Frontline® Spot on, Merial) in the tick study). Supplementary cats in the flea study had to be enrolled and treated, while in the tick study, the enrolment and treatment of the supplementary cats was optional. Cats that were pregnant, lactating or intended for breeding were not enrolled in the studies. All cats received a physical examination by a veterinarian at study inclusion. Each animal was enrolled with the written informed consent of its owner.

2.2. Treatment administration

Cats received three consecutive monthly treatments; first treatment was designated on Day 0. Follow-up treatments and evaluations on Days 30 and 60 could be conducted ±3 days of the target date, but are reported as Days 30 and 60. All treatments were dispensed according to a randomization plan that was provided for each clinic before study start. Treatments were based upon bodyweights recorded on Day 0, 30 and 60. Animals were dosed to provide the recommended dosage of 6.0–12.0 mg selamectin and 1.0–2.0 mg sarolaner per kg bodyweight. In the flea study, the positive control product was dosed following manufacturer’s label directions to deliver 10–20 mg imidacloprid and 1–2 mg moxidectin per kg bodyweight. In the tick study, the positive control product was dosed following manufacturer’s label directions to deliver 7.5–15 mg/kg fipronil. All treatments were applied topically, directly to the skin by the Dispenser.

2.3. Efficacy assessment

Parasite counts on primary cats were conducted prior to treatment on Day 0, and on Days 14, 30, 60 and 90 (±3 days of the target day). Cats were thoroughly examined (and combed using flea combs) for at least 10 min until all fleas and/or ticks were removed. The collected parasites were counted and stored in alcohol solution (at least 70%) at room temperature. The species and gender of the fleas, and the species, developmental status and gender (adults only) of the ticks were determined at a single central parasitology laboratory (Department of Parasitology and Zoology, Budapest) under a stereomicroscope using identification keys (Szabó, 1975; Hillyard, 1996; Estrada-Peña et al., 2004). Engorgement status of the ticks was determined by visual inspection of the alloscutum.

In the flea study, each primary cat was also thoroughly examined for clinical signs of flea allergy dermatitis (FAD) including but not limited to pruritus, erythema, scaling, alopecia, and dermatitis, prior to treatment on Day 0, and on Days 14, 30, 60 and 90. The Examining Veterinarian assessed the severity of the clinical signs on a four level scale as absent, mild, moderate or severe.

2.4. Safety assessment

All cats (primary and supplementary) that received at least one treatment were included in the safety assessment. All cats received a physical examination by the veterinarian prior to treatment on Day 0, and on Days 30, 60 and 90. Primary cats received an additional physical examination by the veterinarian on Day 14. All abnormal health events observed during the physical examinations by the veterinarian or observed by the owner between visits, were recorded.

2.5. Data analysis

The primary cat per household was the experimental unit. Efficacy was calculated at each post-treatment visit day (Day 14, 30, 60 and 90) as the mean percentage reduction in live parasite counts compared to the pre-treatment counts (recorded on Day 0) for each animal using the following formula:

\[
\text{% efficacy} = \frac{\text{count (Day 0)} - \text{count (post – treatment)}}{\text{count (Day 0)}} \times 100
\]

Efficacy was calculated across all flea and tick species in the flea and tick studies, respectively. Additionally, efficacy was calculated for each flea and tick species. Non-inferiority test of efficacy between selamectin/sarolaner and the positive control product
was done at each visit using a non-inferiority margin of 15% at the one-sided significance level of 0.025. If the lower limit of the 95% confidence interval of the difference in efficacy between selamectin/sarolaner and the positive control product was greater than −15% then selamectin/sarolaner was non-inferior to the positive control product at that time point. If the lower limit of the 95% confidence interval was greater than 0, percent efficacy of the combination was superior to the positive control product. The improvement in clinical signs of FAD was assessed for primary cats that were identified by the Examining Veterinarian as having FAD. The numbers and percentages of cats with each of the clinical signs of FAD were calculated by severity category.

3. Results

3.1. Animals

In the flea study, 277 primary (185 selamectin/sarolaner-treated and 92 imidacloprid/moxidectin-treated) and 170 supplementary (120 selamectin/sarolaner-treated and 50 imidacloprid/moxidectin-treated) cats were enrolled. Cats were enrolled in a total of 33 study sites in France (n = 9), Hungary (n = 7), Germany (n = 9) and Italy (n = 8). Cats in the selamectin/sarolaner-treated group had a mean age of 4.9 years (range from 3 months to 22 years) and mean weight of 3.99 kg (range from 1.0 to 9.6 kg). Cats in the imidacloprid/moxidectin-treated group had a mean age of 3.9 years (range from 3 months to 17 years) and mean weight of 4.06 kg (range from 1.0 to 8.4 kg). In total, 223 female cats (49.9% of all cats; 149 selamectin/sarolaner-treated and 74 imidacloprid/moxidectin-treated) and 224 male cats (50.1% of all cats; 156 selamectin/sarolaner-treated and 68 imidacloprid/moxidectin-treated) were enrolled. Of these 447 cats, 75.4% (n = 337) had short hair, 15.0% (n = 67) had medium hair length and 9.6% (n = 43) had long hair, with similar distribution over both treatment groups. Cats were either European shorthair (56.2%), mixed breed (24.2%), European longhair (5.6%) or any of the other 18 breeds recorded (14.0%). In the selamectin/sarolaner-treated group 10 cats (5 primary and 5 supplementary) were withdrawn for a variety of reasons, not related to treatment. In the imidacloprid/moxidectin-treated group one primary cat was withdrawn because it was hit by a car and died. Thus, 271 primary patients and 165 supplementary patients completed the flea study.

In the tick study, 200 primary (133 selamectin/sarolaner-treated and 67 fipronil-treated) and 70 supplementary (47 selamectin/sarolaner-treated and 23 fipronil-treated) cats were enrolled. Cats were enrolled on a total of 33 study sites in France (n = 8), Hungary (n = 7), Germany (n = 11) and Italy (n = 7). Cats in the selamectin/sarolaner-treated group had a mean age of 4.8 years (range from 3.5 months to 20 years) and mean weight of 4.37 kg (range from 1.3 to 8.5 kg). Cats in the fipronil group had a mean age of 4.7 years (range from 6 months to 16 years) and mean weight of 4.29 kg (range from 2.2 to 7.2 kg). In total, 104 female cats (38.5% of all cats; 67 selamectin/sarolaner-treated and 37 fipronil-treated) and 166 male cats (61.5% of all cats; 113 selamectin/sarolaner-treated and 53 fipronil-treated) were enrolled. Of these 270 cats, 78.1% (n = 211) had short hair, 13.3% (n = 36) had a medium hair length and 8.5% (n = 23) had long hair, with similar distribution over both treatment groups. Cats were either European shorthair (71.5%), mixed breed (10.4%), European midhair (5.9%) or any of the other 13 breeds recorded (12.2%). In the selamectin/sarolaner-treated group 5 cats (3 primary and 2 supplementary) and in the fipronil-treated group 2 cats (1 primary and 1 supplementary) were withdrawn for a variety of reasons not related to the treatment. Thus, 196 primary patients and 67 supplementary patients completed the tick study.

3.2. Efficacy against fleas

All fleas found on the cats were identified as C. felis. The results for the flea study are summarized in Table 1. The arithmetic means of live flea counts per cat at enrolment were 9.9 (range from 5 to 66) in the selamectin/sarolaner-treated group and 10.6 (range from 5 to 84) in the imidacloprid/moxidectin-treated group. Efficacy in the selamectin/sarolaner-treated group was 97.4%, 97.3%, 98.8% and 99.4%, and efficacy in the imidacloprid/moxidectin-treated group was 90.0%, 83.6%, 87.7% and 96.3%, on post-treatment Days 14, 30, 60 and 90, respectively. Selamectin/sarolaner was non-inferior to imidacloprid/moxidectin at all time-points.

Fourteen primary cats in the selamectin/sarolaner-treated group and two in the imidacloprid/moxidectin-treated group were identified as having FAD at enrolment. The clinical signs of flea allergy dermatitis (alopecia, dermatitis, erythema, pruritus and scaling) improved in all cats following treatment administration in both groups.

3.3. Efficacy against ticks

3.3.1. Overall efficacy against ticks

The efficacy against all tick species combined is summarized in Table 2. The arithmetic means of live tick counts at enrolment were 4.7 (range from 3 to 22) per cat in the selamectin/sarolaner-treated group and 4.6 (range from 3 to 14) in the fipronil-treated group. On post-treatment Days 14, 30, 60 and 90, efficacy was 96.7%, 92.6%, 98.8% and 99.5%, respectively in the selamectin/sarolaner-treated group, and 90.2%, 74.6%, 83.0% and 93.4%, respectively in the fipronil-treated group. The percent efficacy in selamectin/sarolaner was non-inferior to fipronil at all time-points, and superior to fipronil on Days 30 and 60.

3.3.2. Efficacy against individual tick species

At enrolment, 149 primary cats (98 in the selamectin/sarolaner-treated group and 51 in the fipronil-treated group) were infested with I. ricinus and 42 primary cats were infested with R. sanguineus (29 in the selamectin/sarolaner-treated group and 13 in the fipronil-treated group). The number of cats infested with I. ricinus and R. sanguineus, the maximum tick count at the different study days, and the efficacy is provided in Table 3. A limited number of cats (n = 16; 7 in the selamectin/sarolaner-treated group and 9 in the fipronil-treated group) was infested with D. reticulatus or with I. hexagonus (n = 14; 10 in the selamectin/sarolaner-treated group and 4 in the fipronil-treated group). Some cats harboured mixed infestations of more than one tick species. The presence of the different tick species at enrolment (Day 0) in the 4 different countries is provided in Table 4.

Treatments with selamectin/sarolaner resulted in a large decrease in the incidence of infestations for all tick species by Day 14. More specifically, efficacy of selamectin/sarolaner was 100% for I. hexagonus and D. reticulatus throughout the study. The efficacy of selamectin/sarolaner against I. ricinus was 96.1%, 89.3%, 98.7% and 99.8% on Days 14, 30, 60 and 90, respectively. The efficacy of selamectin/sarolaner against R. sanguineus was 100%, 98.0%, 99.1% and 98.6% on Days 14, 30, 60 and 90, respectively. The efficacy of fipronil against I. ricinus was low, with 84.3%, 66.5%, 75.4% and 87.7% on Days 14, 30, 60 and 90, respectively. Efficacy against R. sanguineus was 100%, 97.4%, 96.9% and 98.5% on Days 14, 30, 60 and 90, respectively. Efficacy of fipronil was 100% against D. reticulatus was >93.8% against I. hexagonus on all study days.

3.4. Safety

There were no serious treatment-related adverse events in any of the selamectin/sarolaner-treated cats. In the flea study, a
Table 1
Efficacy against fleas: the number of treated cats, arithmetic mean live flea counts, flea count range and efficacy relative to pre-treatment counts. Cats were dosed topically with selamectin/sarolaner (S/S) or with imidacloprid/moxidectin (I/M) once a month (Days 0, 30 and 60) for three months.

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Treatment group</th>
<th>Number of cats</th>
<th>Flea Counts</th>
<th>Efficacy(^a) (%)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Arithmetic Mean</td>
<td>Range</td>
<td>Least Square Mean</td>
</tr>
<tr>
<td>0</td>
<td>S/S</td>
<td>185</td>
<td>9.9</td>
<td>5–66</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>I/M</td>
<td>92</td>
<td>10.6</td>
<td>5–84</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>S/S</td>
<td>184</td>
<td>0.3</td>
<td>0–9</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>I/M</td>
<td>90</td>
<td>1.2</td>
<td>0–17</td>
<td>90.0</td>
</tr>
<tr>
<td>30</td>
<td>S/S</td>
<td>183</td>
<td>0.3</td>
<td>0–8</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td>I/M</td>
<td>91</td>
<td>2.3</td>
<td>0–48</td>
<td>83.6</td>
</tr>
<tr>
<td>60</td>
<td>S/S</td>
<td>181</td>
<td>0.1</td>
<td>0–2</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>I/M</td>
<td>90</td>
<td>1.4</td>
<td>0–54</td>
<td>87.8</td>
</tr>
<tr>
<td>90</td>
<td>S/S</td>
<td>180</td>
<td>0.1</td>
<td>0–49</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>I/M</td>
<td>90</td>
<td>0.8</td>
<td></td>
<td>96.4</td>
</tr>
</tbody>
</table>

\(^a\) Efficacy is the arithmetic mean of percent reduction of flea counts relative to pre-treatment calculated for each cat individually.

Table 2
Efficacy against ticks: the number of treated cats, the arithmetic mean live tick counts (all tick species), tick count range and efficacy relative to pre-treatment counts. Cats were dosed with selamectin/sarolaner (S/S) fipronil topically once a month (Days 0, 30 and 60) for three months.

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Treatment group</th>
<th>Number of Cats</th>
<th>Tick Counts</th>
<th>Efficacy(^a) (%)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Arithmetic Mean</td>
<td>Range</td>
<td>Least Squares Mean</td>
</tr>
<tr>
<td>0</td>
<td>S/S</td>
<td>131</td>
<td>4.7</td>
<td>3–22</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>fipronil</td>
<td>67</td>
<td>4.6</td>
<td>3–14</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>S/S</td>
<td>128</td>
<td>0.2</td>
<td>0–3</td>
<td>96.7</td>
</tr>
<tr>
<td></td>
<td>fipronil</td>
<td>67</td>
<td>0.5</td>
<td>0–9</td>
<td>90.2</td>
</tr>
<tr>
<td>30</td>
<td>S/S</td>
<td>129</td>
<td>0.4</td>
<td>0–11</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>fipronil</td>
<td>65</td>
<td>1.3</td>
<td>0–10</td>
<td>74.6</td>
</tr>
<tr>
<td>60</td>
<td>S/S</td>
<td>128</td>
<td>0.1</td>
<td>0–2</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>fipronil</td>
<td>65</td>
<td>0.8</td>
<td>0–12</td>
<td>83.0</td>
</tr>
<tr>
<td>90</td>
<td>S/S</td>
<td>125</td>
<td>0.0</td>
<td>0–2</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td>fipronil</td>
<td>64</td>
<td>0.4</td>
<td>0–12</td>
<td>93.4</td>
</tr>
</tbody>
</table>

\(^a\) Efficacy is the arithmetic mean of the percent reduction relative to pre-treatment calculated for each cat individually.

Table 3
Numbers of cats infested with *Ixodes ricinus* and *Rhipicephalus sanguineus*, the maximum tick count for each species at the different study days, and efficacy relative to pre-treatment counts. Cats were dosed with selamectin/sarolaner or treated with fipronil topically once a month (Days 0, 30 and 60) for three months.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Study Day</th>
<th>L. ricinus</th>
<th>R. sanguineus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selamectin/Sarolaner (n = 131)</td>
<td>0</td>
<td>98 (74.8)</td>
<td>29 (22.1)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>12 (9.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>18 (17.1)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>6 (4.7)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1 (0.8)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Fipronil (n = 67)</td>
<td>0</td>
<td>51 (76.1)</td>
<td>13 (19.4)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>14 (20.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>21 (32.3)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>16 (24.6)</td>
<td>2 (3.1)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>6 (9.4)</td>
<td>1 (1.6)</td>
</tr>
</tbody>
</table>

Table 4
The number of cats infested with all ticks or with specific tick species in Hungary, Italy, France and Germany, as well as the average number of ticks and the range in tick counts.

<table>
<thead>
<tr>
<th>Number of cats infested with(^a)</th>
<th>Average number of ticks per cat</th>
<th>Range (minimum and maximum tick count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ticks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td>65</td>
<td>6.3</td>
</tr>
<tr>
<td>Italy</td>
<td>30</td>
<td>4.0</td>
</tr>
<tr>
<td>France</td>
<td>41</td>
<td>5.4</td>
</tr>
<tr>
<td>Germany</td>
<td>62</td>
<td>7.9</td>
</tr>
<tr>
<td><em>Ixodes ricinus</em></td>
<td>62</td>
<td>3–38</td>
</tr>
<tr>
<td><em>Ixodes hexagonus</em></td>
<td>7</td>
<td>3–6</td>
</tr>
<tr>
<td><em>Dermacentor reticulatus</em></td>
<td>16</td>
<td>3–17</td>
</tr>
<tr>
<td><em>Rhipicephalus sanguineus</em></td>
<td>0</td>
<td>2–43</td>
</tr>
</tbody>
</table>

\(^a\) Mixed infestation did occur.
total of nine abnormal health events were considered to be possibly related to treatment. Two selamectin/sarolaner-treated cats were reported to have a mild to moderate alopecia at the application site after one of the three treatment applications. In six other selamectin/sarolaner-treated cats, mild and transient pruritus was recorded after treatment application. In one of these six animals, itching was observed after two treatments, but in the five other cats pruritus was observed only after one treatment. In one imidacloprid/moxidectin-treated cat, hair coat discoloration was observed after one of the three treatment applications. In the tick study, 6 cats were reported with abnormal health events that were considered to be possibly linked to treatment. One cat in each treatment group displayed mild drooling immediately after treatment on Day 0. One of these cats (in the fipronil group) displayed drooling again after treatment on Day 60, but not on Day 30. In all cases, the drooling stopped within a few minutes after treatment. On Day 60, one selamectin/sarolaner-treated cat was diagnosed with a local and mild alopecia at the application site. During the immediate post-treatment observations, transient itching was observed in two selamectin/sarolaner-treated animals and in one fipronil-treated animal. These abnormal health events occurred at a similar low frequency in both treatment groups and are events (transient drooling and scratching at the application site) that are not unexpected following a topical application in cats.

4. Discussion

The new spot-on formulation of selamectin plus sarolaner for cats (Stronghold® Plus) administered topically at monthly intervals was safe and highly effective against natural flea and tick infestations in cats. The efficacy of selamectin/sarolaner was non-inferior to imidacloprid/moxidectin (Advocate® for Cats, Bayer) and fipronil (Frontline® Spot-on, Merial), for efficacy against fleas and ticks, respectively.

High efficacy against fleas was expected, given the known pulicidal efficacy of sarolaner (Simparica®; McTier et al., 2016) and selamectin (Stronghold®; Benchaoui et al., 2000; McTier et al., 2000; Ritzhaupt et al., 2000; Paarlberg et al., 2013). Furthermore, the efficacy of new spot-on formulation of selamectin plus sarolaner against fleas was also confirmed in laboratory studies (Becksi et al., 2017). Treatment with selamectin/sarolaner reduced the severity of clinical signs associated with FAD in cats, as previously described for selamectin treatment (Dickin et al., 2003). The low efficacy of imidacloprid/moxidectin against fleas observed in this field study, with efficacy above 95% efficacy only achieved after three monthly treatments, was more surprising given the 4 week persistent efficacy claim and the absence of imidacloprid resistance put forward in Kopp et al. (2013) and Dryden et al. (2011). The lower efficacy may possibly reflect increased tolerance to imidacloprid in the field following continued use, as decreasing efficacy of imidacloprid against the KS1 strain towards the end of the one month treatment interval was indeed reported before in experimental studies (Dryden et al., 2005).

The new spot-on formulation of selamectin/sarolaner for cats was also found to be highly efficacious against all tick species, with overall tick efficacy >92.6% at all time-points. High and persistent efficacy against I. ricinus, H. hexagonus, R. sanguineus and D. reticulatus was previously confirmed in laboratory studies (Geurden et al., 2017). Selamectin/sarolaner was non-inferior to the control product fipronil at all time-points, and was superior on Days 30 and 60. The overall efficacy of fipronil against all tick species was below 90% on Day 30 and Day 60, and the efficacy against I. ricinus specifically was below 90% at all time-points. As reported before (Becksi et al., 2017; Geurden et al., 2017), the new spot-on formulation of selamectin plus sarolaner for cats provides 5 weeks of persistent efficacy against fleas, I. ricinus and I. hexagonus and 4 weeks against R. sanguineus and D. reticulatus, which was confirmed in the field in the current studies.

Under field conditions, cats are continuously exposed to re-infestations by fleas and ticks from the environment. Therefore, ectoparasiticide should provide persistent efficacy until the end of the treatment period to protect the animals from re-infestation. This is not only needed to decrease the immediate clinical impact of these ectoparasites on infected cats, but is also important in order to help reduce the spread of vector-borne pathogens either because of the direct clinical relevance to the cat population and to break the epidemiological cycle of these pathogens. The new spot-on formulation of selamectin plus sarolaner for cats provides the high and persistent efficacy against both ticks and fleas needed to achieve this.

5. Conclusions

The new spot-on formulation of selamectin plus sarolaner for cats administered topically at monthly intervals at the commercial dose range of 6.0–12.0 mg selamectin and 1.0–2.0 mg sarolaner per kg bodyweight, was safe and highly effective against natural infestations of fleas and ticks on cats. Specifically under conditions when cats were continuously exposed to natural re-infestations with ticks and fleas, new spot-on formulation provided consistent, high efficacy through each monthly dosing interval.

Conflict of interest

The studies reported here were funded by Zoetis. RF was in independent investor and conducted the species identification of all fleas and ticks. All other authors were current employees of Zoetis. All authors assisted with the design and conduct of the studies, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

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References


Efficacy and speed of kill of a new spot-on formulation of selamectin plus sarolaner against flea infestations in cats

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A B S T R A C T

The efficacy of a new spot-on formulation of selamectin plus sarolaner against induced flea infestations in cats was confirmed in three placebo-controlled, blinded studies. Purpose-bred adult cats (n = 8/group) were blocked by pre-treatment flea counts and randomly allocated to treatment with either a placebo or with the spot-on formulation at the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight. Treatments were applied topically once on Day 0. All cats were infested with approximately 100 unfed, adult Ctenocephalides felis prior to treatment and at weekly intervals for 5 weeks. In Studies 1 and 2 comb counts were conducted to determine the numbers of viable fleas 24 h after treatment and subsequent weekly infestations. In Study 3, flea counts were conducted at 6, 12, 24 and 48 h after treatment and 3, 6, 12 and 24 h after subsequent weekly infestations to evaluate the speed of kill against fleas. Cats in the placebo-treated groups maintained flea infestations throughout all studies. In Study 1, no live fleas were found on any of the treated cats, resulting in 100% efficacy for 5 weeks after a single treatment (P < 0.0001). In Study 2, selamectin/sarolaner reduced flea counts by 92.4% immediately after treatment and by 97.7%–100% after re-infestations for five weeks (P ≤ 0.0001). In the speed of kill study, selamectin/sarolaner started killing fleas within 12 h after treatment administration and within 6 h following re-infestation for at least 28 days. Efficacy was 98.1% by 24 h after treatment and 100% within 24 h after re-infestations for 5 weeks. Success was observed of a new spot-on formulation of selamectin plus sarolaner at the minimum dose rapidly and consistently kills fleas on cats for at least 5 weeks.

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1. Introduction

Flea infestations are very common in cats, although they may only be recognized as a nuisance by pet owners particularly when they are accompanied by pruritus or when infestations become particularly heavy. Severe flea infestations may cause anaemia, particularly in young cats (Dryden, 1989). Cats with access to the outdoors are particularly at risk of flea infestations because of their more frequent contact with feral animals that are reservoirs for fleas (Rust, 2005). While flea infestations are known to peak during the warmer summer months, adult fleas also survive and reproduce in the colder months in home environments, where immature stages may survive for several months. Year-round flea control that provides efficacy against the immature stages of fleas should therefore be considered for pets in most geographic areas (Rust and Dryden, 1997). In addition, fleas are the intermediate hosts for the tapeworm, Diphylidium caninum, and can transmit a number of pathogens, including zoonotic pathogens such as Rickettsia felis (Pérez-Osorio et al., 2008) and Bartonella henselae (Chomel et al., 1996; Breitschwerdt and Kordick, 2000). Given the high prevalence and the pathogenic potential and zoonotic relevance of fleas (Beugnet and Franc, 2012), effective flea control is a concern for pet owners and veterinarians.

Most parasitcides have adulticidal activity (e.g. nitfenpyram, spinosad, fipronil) or adulticidal and larvicidal activity, but are not effective against flea eggs (e.g. imidacloprid, indoxacarb). Selamectin is the only molecule with efficacy against the adult and larval stages of fleas and against flea eggs (McTier et al., 2000b; Dryden et al., 2007). Selamectin is also unique with its broad spectrum of activity, not only providing protection against flea infestations, but also against ear mites, lice, heartworm, and gastrointestinal nematode infections in cats (Fisher et al., 2007).

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Sarolaner is a novel isoxazoline with potent activity against a wide range of ectoparasites (McTier et al., 2016), and broadens the efficacy spectrum to include ticks.

A series of laboratory studies were conducted to confirm the efficacy of the new spot-on formulation of selamectin plus sarolaner, administered at the minimal dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight in the treatment and control of cats for one month.

2. Materials and methods

Three placebo-controlled, blinded and randomized studies were conducted. The studies were conducted in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention, control of flea and tick infestation on dogs and cats (Marchiondo et al., 2013) and complied with Good Clinical Practice and VICH guideline GL9 (EMEA, 2000). Study protocols were reviewed and approved by the local and/or Zoetis Institutional Animal Care and Use Committee.

2.1. Animals

The studies used adult purpose-bred cats of both sexes, ranging in age from 9 months to 8 years. All cats were in good health at enrolment as confirmed by a physical examination by a veterinarian, and had undergone a wash-out period sufficient to ensure that no residual efficacy remained from any previously administered compounds. Cats were housed individually in enclosures with no physical contact between them that conformed to accepted animal welfare guidelines. Cats were acclimatized to the facilities for at least a week prior to treatment. Cats received an appropriate maintenance ration of a commercial feed for the duration of the study. Water was available ad libitum.

2.2. Experimental design

All cats were observed for general health at least twice daily throughout the studies.

To determine host suitability, cats were infested with approximately 100 fleas prior to treatment. The fleas were removed and counted 24 h after infestation. Cats with the highest live flea counts were selected for inclusion. In Studies 1 and 2, one placebo-treated group and one selamectin/sarolaner-treated group were enrolled (n = 8/group). In Study 3 (speed of kill), eight groups of cats were utilized (n = 8/group); four groups received placebo and four groups received selamectin/sarolaner. In each study, the cats were blocked by flea count and then randomly assigned to either placebo treatment or treatment with selamectin/sarolaner in a randomised complete block design.

2.3. Treatment administration

Treatments were administered topically directly to the skin in a single spot at the base of the neck in front of the shoulder blades on Day 0. All treatments were administered at a dose volume of 0.1 mL per kg bodyweight providing the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight in the treated groups. The placebo formulation was identical to the selamectin plus sarolaner combination product, but only contained the excipients. Clinical observations were conducted pre-treatment and at 1, 3, 6 and 24 h after treatment.

2.4. Flea infestations and flea counts

Each cat was infested with approximately 100 unfed viable adult Ctenocephalides felis on Days −1, 6, 13, 20, 27 and 34 in Studies 1 and 2 and on Days −1, 7, 14, 21, 28, and 35 in Study 3. Infestations were performed by applying the fleas directly to the fur while the cats were restrained for a few minutes until the fleas dispersed into the hair coat. Fleas for all studies were obtained from local laboratory colonies that had all been genetically enriched with fleas within 1–2 years of the study. Study 1 and 3 used the same flea strain that originated from the USA, Study 2 used a separate flea strain that originated from Europe.

Flea counts were conducted by systematically combing the hair coat of each cat with a fine-toothed flea comb for at least 10 min and removing all fleas. Any animal on which fleas were found in the last 5 min was combed for an additional 5 min. All live fleas, including those incapable of maintaining upright orientation and/or coordinated movement at the time of their removal from the cats were counted. Protective clothing was changed between animals. In Studies 1 and 2, flea counts were conducted 24 h after treatment and after weekly re-infestations. In the third study, flea counts were conducted 6, 12, 24 and 48 h after treatment and 3, 6, 12 and 24 h after weekly re-infestations in separate pairs of placebo-treated and selamectin/sarolaner-treated groups. All personnel conducting parasite or other observations were blinded to treatment allocation.

2.5. Data analysis

Flea counts of individual cats were ln(count + 1) transformed prior to analysis in order to stabilize the variance and normalize the data. Transformed counts were analyzed using a general linear mixed model for repeated measures (SAS 9.3, Cary NC). The model included the fixed effect of treatment, day of study, and the interaction between treatment and day of study. The random effects included block, the interaction between block and treatment, and error. Hypothesis testing was two-sided at the significance level of 0.05. Percent efficacy relative to the control group was calculated using the Abbott formula: [(C − T)/C] × 100, where C = arithmetic or geometric mean flea count for the control group and T = arithmetic or geometric mean flea count for the treated group.

3. Results

3.1. Efficacy

The results of the efficacy assessments (Studies 1 and 2) are summarized in Table 1. In Study 1, placebo–treated cats maintained flea infestations throughout the study with arithmetic mean counts between 89.4 and 94.6 fleas. No live fleas were recovered from any selamectin/sarolaner–treated cat at any post–treatment count. Efficacy was thus 100% through Day 35 after a single treatment. Live flea counts were significantly lower for the selamectin/sarolaner–treated animals compared to placebo at all time-points (P < 0.0001).

In Study 2, cats in the placebo–treated group maintained flea infestations throughout the study with arithmetic mean counts between 65.8 and 95.4 fleas. In the selamectin/sarolaner–treated group, efficacy based on arithmetic mean flea counts was 92.4%, 99.9%, 100.0%, 99.9%, 97.7% and 97.7% on Days 1, 7, 14, 21, 28 and 35, respectively. Live flea counts were significantly lower for the selamectin/sarolaner–treated cats compared with placebo at all time-points (P < 0.0001).

In the speed of kill study (Study 3), all placebo–treated groups maintained flea infestations throughout the study, with arithmetic mean counts between 72.0 and 90.9 fleas (Table 2). A significant reduction in flea counts was observed within 12 h after treat-
3.2. Health observations

No adverse events related to treatment with selamectin/sarolaner occurred in any study. In Study 2, two placebo-treated and three selamectin/sarolaner-treated cats had abnormal health events. One cat in the placebo-treated group and two in the selamectin/sarolaner-treated group showed upper respiratory and ocular signs and received antibiotic therapy. One placebo-treated cat had mild diarrhea on Day 6 and received treatment with metronidazole. One selamectin/sarolaner-treated cat had mild scratch wounds on Day 3. Two placebo-treated cats were removed from Study 3 due to abnormal health. One cat was removed on Day 3 because of oral ulcers which required hospitalization for treatment. The other cat developed flea allergy dermatitis by Day 22 that required medical intervention.

4. Discussion

The results of the present studies confirm the high efficacy of a new spot-on formulation of selamectin plus sarolaner at the minimum label dose against fleas for at least five weeks, and show that the product starts killing fleas within 12 h after treatment and within 6 h after subsequent re-infestations for a month.

The main objective of flea control in cats is to kill the fleas rapidly to provide quick relief of the clinical signs associated with fleas, such as pruritus and flea-allergy dermatitis (FAD). Further, fleas need to be killed within 24 h, before they can lay eggs, contaminate the environment and initiate the flea life-cycle (Rust and Dryden, 1997). A fast pulicidal effect is also necessary to reduce the risk of disease transmission and to break the epidemiological cycle of these flea-borne diseases. The new spot-on formulation of selamectin plus sarolaner started killing fleas within 12 h after treatment administration and within 6 h after re-infestation. It is known that the fast-killing effect of selamectin is combined with a significant reduction in flea blood-feeding (88.9%–97.4%) for at least
28 days after a single treatment, leading to fewer FAD symptoms (McCoy et al., 2008). In contrast, fipronil treatment had a significant effect on blood feeding of fleas for only 7 days and imidacloprid for only 14 days after a single treatment in the same study.

Flea treatments are not only expected to have a rapid onset of effect, but also to provide long-lasting, persistent efficacy to protect the animals from re-infestation from the environment. In line with these expectations, the current studies demonstrated that selamectin/sarolaner provided high efficacy for the treatment and persistent control of flea infestations in cats for at least 5 weeks after a single administration. The results are in line with the high efficacy demonstrated for the topical formulation containing selamectin alone against fleas (McTier et al., 2000a; Dryden et al., 2004; Schenker et al., 2003; Franc and Yao, 2007). Selamectin is also known to have a long-lasting ovicidal and larvalcidal effect (McTier et al., 2000b), and almost complete inhibition of egg production was also confirmed for selamectin/sarolaner (Vatta et al., 2017). The rapid and persistent efficacy of selamectin/sarolaner in combination with its potent effects on flea reproduction breaks the flea life cycle fast and persistently, reducing the infestation pressure in the immediate home environment of cats, thus providing complete protection against fleas. The impact of the high and fast efficacy of selamectin/sarolaner was also confirmed under field conditions where the treatment was also successful in the reduction of the clinical signs of FAD in client-owned cats (Geurden et al., 2017).

5. Conclusions

A single topical administration of a new spot-on formulation of selamectin plus sarolaner at the recommended minimum dose provided rapid, highly effective treatment of existing flea infestations and persistent control of fleas on cats for a month. A single treatment started to kill an existing flea infestation within 12 h and subsequent re-infestations within 6 h.

Conflict of interest

The studies reported here were funded by Zoetis. CB, JAC, AFV, VLK, DL and DR are current employees of Zoetis or were employed by Zoetis at the time of the studies. All authors assisted with the design and conduct of the studies, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

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References


Research Paper

Efficacy of a new spot-on formulation of selamectin plus sarolaner for cats against adult *Ctenocephalides felis*, flea egg production and adult flea emergence

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**A B S T R A C T**

A new spot-on formulation of selamectin plus sarolaner was evaluated against fleas for adulticidal efficacy, and for the effect on egg production and hatching when applied to flea-infested cats. Ten male and ten female adult domestic shorthair cats were randomly assigned to one of two treatment groups based on pre-treatment flea counts. Cats received topical treatment on Day 0 in a single spot to the dorsal scapular area with either a placebo formulation or with the combination formulation at the minimal dose of 6.0 mg selamectin plus 1.0 mg sarolaner per kg bodyweight. On Days 1–5, 12, 19, 26 and 33, cats were infested with approximately 100 (±5) unfed *Ctenocephalides felis* fleas. At 24 h after treatment or 48 h after subsequent flea infestation, cats were housed for a 20-h period in a cage to allow collection of flea eggs. At the end of this period, flea eggs were collected from the cages and cats were combed to remove and count live fleas. Emerged viable larvae and emerged adult fleas were counted 3 days and 35 days, respectively, after egg collection. The new spot-on formulation of selamectin plus sarolaner provided 100% efficacy against adult fleas up to Day 36 following a single application. Fleas on placebo-treated cats produced large numbers of eggs throughout the study, with individual counts ranging from 110 to 1256 eggs. Following treatment, four flea eggs were collected from a single selamectin/sarolaner-treated cat on Day 29, but there were no eggs collected from any other selamectin/sarolaner-treated animal during the study. No larvae or adult fleas developed from these four eggs. From the eggs collected from the placebo-treated cats, the mean percentage of live larvae and adults that emerged ranged from 67.3% to 84.2% and from 50.7% to 81.8%, respectively. A single topical treatment with a new spot-on formulation of selamectin plus sarolaner at the minimum label dose thus controlled fleas on cats and was 100% effective in preventing flea reproduction for over one month after treatment.

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1. Introduction

The cat flea, *Ctenocephalides felis felis*, is distributed globally and is a major ectoparasite of cats and dogs (Rust, 2016; Rust and Dryden, 1997). The adult flea lives on the host and the female lays eggs which fall from the animal and develop into larvae in the environment (Dryden, 1989). Here the larvae feed on dried blood in the feces of the adult flea as well as other organic material in the environment (Rust, 2005; Rust and Dryden, 1997). The late third larval instar spins a cocoon and pupates. After the pupal-imaginal molt, the adult fleas remain in a pre-emerged, quiescent stage in the cocoon for varying periods of time, but are stimulated by pressure and heat to emerge rapidly (Silverman and Rust, 1985). This greatly facilitates contact of the emerging adult with a host. On the host, the adult fleas mate and within 24–36 h after the first blood meal, females start laying up to 50 eggs per day (Dryden, 1989). Traditional management of flea infestations have relied upon the elimination of the existing adult fleas on the animal, the elimination of fleas acquired from the environment and the prevention of subsequent re-infestation (Siak and Burrows, 2013).

Insect growth regulators, including lufenuron and pyriproxifen, that disrupt the development of eggs and larvae have been used with success in reducing adult flea emergence and consequent host infestation (Jacobs et al., 1996; Maynard et al., 2001), but cats are
at risk of re-infestation from reservoir hosts, especially those with access to the outdoors (Fisher et al., 1996). Combining an adulticide with an insect growth regulator, on the other hand, has been shown to effectively manage flea infestations by killing adult fleas re-infesting the host while providing control of the juvenile stages in the environment (Dryden, 2009; Dryden and Broce, 2002). The use of a highly effective adulticide with rapid onset of activity will kill any fleas re-infesting the host, but it also has the potential to kill the fleas before they lay eggs, effectively preventing the contamination of the environment in the first instance (Jacobs et al., 2001). For this strategy to be effective, the adulticide must be able to stop the fleas from feeding on the host’s blood within 24 h in order to stop egg production (Dryden et al., 2007).

Selamectin (Revolution® or Stronghold®, Zoetis) is a topically applied macrocyclic lactone that controls many ecto- and endoparasites on cats including fleas, ear mites, heartworm and gastro-intestinal nematodes (Boy et al., 2000; McTier et al., 2000a,c; Six et al., 2000a,b). Despite this broad spectrum of efficacy, selamectin has little reported effectiveness against ticks on cats at the labeled rates (Fisher and Shanks, 2008; Bishop et al., 2000). Ticks are increasingly being recognized as serious ectoparasites of cats, possibly due to increasing incidence of infestation as well as a greater awareness of their role in the transmission of a number of serious pathogens, including some that are known to be zoonotic (Beugnet and Marié, 2009; Claerebout et al., 2013; Lappin et al., 2015; Otranto et al., 2017). Sarolaner is a novel isoxazoline (McTier et al., 2016) that has been shown to have excellent efficacy against fleas and ticks on dogs for at least 35 days following oral administration (Six et al., 2016a,b) and similar efficacy has been demonstrated following topical administration of selamectin plus sarolaner to cats (Geurden et al., 2017; Becskai et al., 2017). A new spot-on formulation of selamectin plus sarolaner has been developed to provide broad spectrum protection against the common internal and external parasites of cats with sarolaner providing tick efficacy and complementing the insecticidal effects of selamectin. Here we report a study evaluating the effect of a new spot-on formulation of selamectin plus sarolaner for cats on fleas and flea reproduction.

2. Materials and methods

The study followed recommendations for evaluating the efficacy of parasiticide for the treatment, prevention and control of flea and tick infestations on cats and dogs as discussed in the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Marchiondo et al., 2013) and complied with Good Clinical Practices, VICH guideline GL9 (EMEA, 2000). The experimental design broadly followed that used by Six et al. (2016c) to evaluate the effect of treatment with sarolaner on flea egg production and development in dogs. The study protocol was approved by the local Institutional Animal Care and Use Committee and was conducted within accepted animal welfare guidelines. Cats were housed individually in enclosures that ensured no direct contact between cats. All personnel responsible for animal husbandry, conducting observations, or performing flea infestations or counts were masked to treatment allocation.

2.1. Animals and parasites

Ten male and 10 female adult (12–35 months of age; 2.1–7.0 kg), purpose-bred, domestic shorthair cats were used in this study. The 20 cats used in this study were selected from a pool of 30 cats that had undergone an adequate wash-out period from any previous exposure to parasiticide and were in good health at study initiation. On Day –9, all cats were infested with 100 (±5) unfed, adult cat fleas. On Day –6, the cats were combed to count and remove fleas and the 20 cats with the highest live flea counts were enrolled in the study. The flea (C. felis) colony was initially established from a laboratory strain from North Carolina and was enriched with field-collected fleas obtained from untreated animals locally in Arkansas, with the last infusion of wild fleas to the colony about 18 months prior to conduct of the study.

2.2. Experimental design and methods

Cats were acclimated to the study conditions for at least seven days prior to first infestation. Each cat was examined by a veterinarian to determine suitability prior to inclusion in the trial and then observed for general health at least once daily throughout the study. For infestations, cats were held and 100 (±5) cat fleas (~1:1 sex ratio) were applied to the cats and allowed to disperse into the fur. For all post-treatment infestations, fleas were infested at a site distal to the treatment application site. Personnel conducting flea counts were trained in protocol procedures and the standard practices in use at the test facility. Protective gloves and clothing were changed between cats to reduce cross-contamination. Each cat was thoroughly combed for at least 10 min with commercial fine-toothed combs to count and remove fleas. Care was taken to ensure that all areas of the cat were combed, including the head, dorsum, lateral aspects and ventrum. All live fleas were counted, including those incapable of maintaining upright orientation and/or co-ordinated movement.

At 48 (±2) hours after each flea infestation, each cat was housed for approximately a 20-h period in a cage designed to allow collection of flea eggs. At the end of the period, the fur of the cat was ruffled vigorously by hand to dislodge any eggs retained in the fur and the flea eggs were collected from the cage floor pan and all eggs were counted. Cats were then combed to remove and count the adult fleas. Up to 200 eggs were randomly selected for each cat, half were transferred to a container and maintained in an incubator for 3 days under appropriate conditions for egg hatching and then emerged viable larvae were counted. The other half were transferred to a container with an appropriate growth medium and maintained in an incubator under appropriate conditions for flea development and emerged adult fleas were counted after 35 days.

The day on which the cats were administered the treatment was defined as Day 0 for the study. Prior to treatment the cats were ranked by descending Day −6 flea count and randomly allocated to treatment with placebo or selamectin/sarolaner, to a total of 10 cats per treatment group. On Day −1 (approximately 24 h prior to treatment), all cats were infested with fleas. On Day 0, control cats were dosed with placebo vehicle at 0.1 mL/kg body weight. The selamectin/sarolaner-treated cats received the combination product at a dose volume of 0.1 mL/kg body weight to provide a dose of 6.0 mg selamectin plus 1.0 mg sarolaner per kg body weight. Both treatments were applied topically to the skin at the base of the neck directly in front of the shoulder blades using an appropriately sized disposable syringe. Cats were assessed for overall health and administration sites were evaluated prior to treatment and at approximately 1, 3, 6, and 24 h after dosing. Administration sites were further evaluated on Days 3 and 5, and again on Day 36.

On Day 1, cats were placed in the egg collection cages. On Day 2, eggs were collected and the cats combed to count and remove fleas. Subsequent infestations were performed on Days 5, 12, 19, 26 and 33.

2.3. Data analysis

The experimental unit for analysis was the individual animal. Using SAS version 9.4 (SAS Institute, Cary, NC, USA), separate statistical analyses were performed for flea counts, egg counts (except Day 1), percent egg hatch and percent adult emergence for each
Table 1  

<table>
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<tr>
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Means in each column with different superscript letters are significantly different; P < 0.001.

\* Percent efficacy relative to the placebo-treated control.

examination day. Data were analyzed using a general mixed linear model that included the fixed effect of treatment and the random effects of block and error. Testing was at the two-sided significance level α = 0.05. Least squares means (LSMeans, used as arithmetic means) were computed and compared for each group on each day. Percent efficacies, relative to the non-treated control group and based on LSMeans, were calculated as follows:

\[
\%\text{Efficacy} = \left( \frac{\text{LSMean Control} - \text{LSMean Treated}}{\text{LSMean Control}} \right) \times 100
\]

3. Results

3.1. Efficacy

All cats included in the study demonstrated that they were adequate hosts for fleas, with the Day – 6 counts ranging from 66 to 97 fleas. The mean flea counts in the placebo-treated cats ranged from 67.3 to 84.3 (Table 1), demonstrating that these control cats had adequate flea infestations throughout the study. No live fleas were recovered from any selamectin/sarolaner-treated cat, thus percent reduction in flea count compared to placebo was 100% on all days. Mean flea counts were significantly lower than the placebo-treated group on all days (P < 0.001).

Flea eggs were recovered in large numbers from the majority of placebo-treated cats throughout the study (Table 2). On Day 2, flea eggs were only counted until the 200 required for incubation had been collected, and on all placebo-treated cats at least 200 eggs were produced. For subsequent study days, the mean egg counts ranged from 368.8 to 625.2 eggs per cat and over 200 eggs were collected from each cat (range = 205 to 1256) except for a single animal on Day 36 with only 110 flea eggs. Four eggs were collected from a single selamectin/sarolaner-treated cat on Day 29; no eggs were otherwise collected from any selamectin/sarolaner-treated animal throughout the study. Thus, a single topical application of the new spot-on formulation of selamectin plus sarolaner was 99.9% effective in reducing egg production at Day 29 and 100% effective on all other days for at least one month after treatment. The mean percentages of live larvae that hatched from the eggs collected from the placebo-treated cats ranged from 67.3% to 82.4% while the mean percentage of adults that emerged ranged from 50.7% to 81.8% (Table 2). There were no eggs to incubate with the exception of the four eggs collected from the one selamectin/sarolaner-treated cat on Day 29, and no live larvae or adults were obtained. Both the percent egg hatching and adult emergence for treated cats was significantly lower than that for placebo-treated animals on all days (P < 0.001).

3.2. Health and cosmetic observations

There were no abnormal health observations noted in any cat throughout the study. Both placebo and the new spot-on formulation of selamectin plus sarolaner applied easily and no run off or reaction to treatment was observed. Minor cosmetic observations of greasiness and hair spiking were noted in both groups for a few hours after treatment and had resolved completely within 24 h.

4. Discussion

In the current study, a single topical treatment with the new spot-on formulation of selamectin plus sarolaner at the minimum label dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg body-weight was evaluated in cats. Adult flea counts were performed 48 h after treatment and 72 h after re-infestation, and 100% control of adult fleas was achieved for up to 36 days after treatment. Previous studies also confirmed that the spot-on formulation of selamectin plus sarolaner has high pulicidal efficacy within 24 h, and starts killing fleas within 12 h after treatment administration and 6 h following weekly re-infestations for 28 days (Becskei et al., 2017). As previously demonstrated, efficacy within 24 h is required to stop flea feeding and subsequent egg production (Dryden et al., 2007). The high and rapid efficacy of selamectin/sarolaner thus disrupts the flea life cycle by killing adult fleas before they can lay eggs.

The 100% reduction in adult flea counts and the 99.9% reduction in egg production after treatment was expected as both selamectin and sarolaner as individual treatments have rapid onset of activity and effectively reduce flea reproduction and development (McTier et al., 2000b; Six et al., 2016c). Despite the difficulties in comparing results across different studies, it appears that the combination product has equivalent if not better efficacy than selamectin alone. In four dose confirmation studies in cats using selamectin alone, flea efficacy at the 72-h counts in cats that were not bathed was mostly 100% on Day 7 and Day 14. Efficacy dropped below 100% on Day 21 and Day 30, but efficacy was never less than 98.0% (McTier et al., 2000a). In another study, efficacy for selamectin alone on Days 3, 10, 17, 24 and 31 was 99.3%, 100.0%, 100.0%, 99.7% and 97.3% based on these 72-h flea counts (Dryden et al., 2007). In the present study, efficacy was 100% at the 48-h count on Day 2 and 100% at each 72-h count thereafter up to 36 days after a single treatment. In the study by Dryden et al. (2007) and a study by McTier et al. (2000b) which both used a similar design to the present study, a small number of flea eggs were collected from the selamectin-treated cats after each infestation. For example, in Dryden et al. (2007), percent reduction in egg production between 48 and 72 h after treatment or re-infestation ranged from 95.35% to 100% for 5 weeks after treatment, with eggs collected on all occasions but Day 10 and Day 16. In the present study, efficacy against egg production was 100% for 5 weeks post-treatment with the exception of Day 29, when the reduction was 99.9%.

Although the direct ovicidal and larvicidal effect of the new spot-on formulation of selamectin plus sarolaner could not be evaluated in this study as virtually no eggs were produced after treatment, the high reduction in adult flea counts and egg production for a month has a similar outcome in terms of environmental contamination. Effective flea control not only aims to kill the adult fleas, but also aims to disrupt the flea life cycle and as such to decrease the environmental infestation pressure (Dryden, 2009; Dryden and Broce, 2002; Jacobs et al., 2001). For cats living in a flea-infested environment, monthly treatment with the spot-on formulation of selamectin plus sarolaner will rapidly reduce the existing flea burden on the animals and cause the deposition of eggs in the environment to stop, for the month-long treatment period. The product will also kill adult fleas that emerge from the infested environment and infest the host. Because these fleas are killed before they can successfully reproduce, they do not contribute to the re-infestation of the environment. Furthermore, the dander from cats treated with topical selamectin has been found to inhibit the hatching of flea eggs and kill exposed flea larvae (McTier et al., 2000b).
A single topical application of the new spot-on formulation of selamectin plus sarolaner at the minimum label dose was 100% effective in preventing flea reproduction for at least one month after treatment.

Conflict of interest

The study was funded by Zoetis. AV, SH, VK and TG are current employees of Zoetis. JAC and DR are former employees of Zoetis who played an integral part in the study planning, execution and write-up. WRE was an independent investigator contracted for this study. All authors assisted with the design and conduct of the study, interpretation of the data and preparation of the manuscript. There were no conflicts of interest that could have influenced the conduct and reporting of this study.

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References


Research paper

Efficacy of a new spot-on formulation of selamectin plus sarolaner in the treatment of *Otodectes cynotis* in cats

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**A B S T R A C T**

The efficacy of a new spot-on formulation of selamectin/sarolaner was evaluated against induced *Otodectes cynotis* infestations in cats in two randomized, blinded studies. Fourteen and 16 cats were randomly assigned to treatment groups in Studies 1 and 2, respectively. On Day 0, animals were either treated with placebo or with the spot-on formulation at the minimal dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight. Treatments were administered topically at the base of the neck. Presence of live mites was evaluated 14 days after treatment administration by otoscopic examination and total live mite counts (adults plus immature) were conducted on Day 30 by ear lavage. Efficacy was calculated based on the reduction of mean total live mite counts on Day 30 in the selamectin/sarolaner-treated group versus the placebo-treated group.

There were no treatment-related adverse reactions during the studies, apart from one cat in each treatment group with alopecia at the administration site. In both studies combined, live mites were present on Day 14, in 14 out of 15 cats in the placebo-treated groups and in 2 out of 15 cats in the selamectin/sarolaner-treated groups. On Day 30, the arithmetic mean live mite counts were 576.9 and 875.8 in the placebo-treated groups and 5.8 and 4.7 in the selamectin/sarolaner-treated groups, in Studies 1 and 2, respectively. The live mite counts were significantly (*P* ≤ 0.0021) lower in the selamectin/sarolaner-treated groups compared to the placebo-treated groups with efficacies of 99.2% and 99.3%, in Studies 1 and 2 respectively.

A single administration of a new spot-on formulation of selamectin/sarolaner at the minimum dose was safe and highly efficacious in the treatment of ear mite infestations in cats.

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1. Introduction

Otodacariosis or otodectic mange is diagnosed in up to 66% of *otitis externa* cases in cats (Nardoni et al., 2014; Perego et al., 2014) and it has recently been reported that *Otodectes cynotis* is the most prevalent ectoparasite of cats in Europe (Beugnet et al., 2014). Most often infestations in cats are only recognized by owners when intense pruritus accompanies the infestation. Upon ear examination, erythema and a dark brown, coffee-ground-like, ceruminous otic exudate is a characteristic finding in the ear canal of infected animals (Sotiraki et al., 2001; Curtis, 2004; Muller and Kirk, 2013). The otitis triggered by the mites may severely impact the well-being of the animal, and in some cases may progress to cause rupture of the tympanic membrane leading to disease of the middle ear or central nervous system. While *O. cynotis* most commonly inhabits the ear canals of the host, ectopic infestations of the head, neck, tail head and trunk can occur and zoonotic infections have also been reported (Curtis, 2004; Muller and Kirk, 2013). While diagnosis is relatively straightforward in most cases by visualization of mites on otoscopic examination or microscopically in aural swabs, treatment may be challenging.

Licensed treatments for ear mite infestations in cats include otic suspensions containing acaricides such as permethrin, thiabendazole and monosulfiram, but also products that do not contain an active ingredient with recognized acaricidal activity e.g. Surolan ear drops, Canauoral ear drops (Curtis, 2004 Yang and Huang, 2016). When combined with anti-inflammatory, antibacterial and antifungal agents, otic formulations help to rapidly reduce inflam-
mation and pruritus, and treat secondary infections locally. These topical formulations however have a limited residual action and require reapplication up to twice daily for several weeks to ensure that all immature stages are also exposed to the drug. Due to the contagious and non-host-specific nature of ear mite infestations, all animals in the household should be treated to avoid re-infestations. The intra-aural route of treatment may therefore be time-consuming in multi-pet households and can be difficult to execute in cats, particularly when the ears are painful. In severe *otitis externa* and with occluded ear canals, aural treatment may be less effective. As *O. cynotis* may infest body areas outside the ear canals and may survive in the environment for up to 12 days, thus causing re-infestations after aural treatments are concluded, systemic treatment that also reaches the ectopic sites and has residual activity is advocated (Müller and Kirk 2013 Yang and Huang, 2016). For the treatment of cats, topical spot-on formulations containing systematically active moxidectin or selamectin provide an attractive alternative to aural treatments, because of their ease of administration just once a month (Six et al., 2000; Fourie et al., 2003). A recent systematic review of the literature suggests recommending the use of selamectin or moxidectin spot-ons for the treatment of ear mite infestations in cats (Yang and Huang, 2016). Systemically active ectoparasitides of the isoxazoline family also seem to have mitidical activity as well, at least in dogs. To date, selanolaner is the only member of this class of molecules that is licensed for the treatment of sarcoptic mange mites in dogs (Beckesi et al., 2016). Its activity against Demodex and Otodectes mites in dogs following oral administration has also been demonstrated (Six et al., 2016).

In order to provide an easy to use, broad spectrum endo- and ectoparasite treatment option for cats, sarolaner has been added to selamectin in a new spot-on formulation to broaden the activity to include ticks, against which selamectin has weak efficacy. In the current studies, the efficacy and safety of the new combination product when applied at the minimum dose of 6.0 mg/kg selamectin and 1.0 mg/kg sarolaner, was evaluated against induced aural infestations of *O. cynotis*.

### 2. Materials and methods

Two placebo-controlled, randomized, blinded studies were conducted in two separate laboratories and in compliance with Good Clinical Practice (EMEA, 2000). Study protocols were reviewed and approved by the Institutional Animal Care and Use Committees of Zoetis and the study sites. Masking of both studies was assured through the separation of functions. All personnel conducting observations or animal care, or performing mite counts were masked to treatment allocation.

#### 2.1. Animals

In total 30, purpose-bred, domestic short hair cats (15 males and 15 female), with induced infestations of *O. cynotis* and aged between 4 months and 8 years were included in the studies. Mite infestations were induced by the inter-aural transfer of mites from naturally infested donor animals. Cats were included in the studies if they harbored at least 5 live mites in at least one ear.

All cats were to be in good health at enrolment as established by a physical examination by a veterinarian. In both studies, cats were individually housed in enclosures that allowed for auditory and visual but no physical contact between cats and conformed to accepted animal welfare guidelines. The investigator confirmed that the cats had undergone an adequate washout period to ensure that no residual ectoparasiticide efficacy remained from any previously administered compounds. Cats were fed an appropriate maintenance ration of a commercial feed for the duration of the study. Water was available *ad libitum*. Cats were acclimated to the study conditions for at least 7 days prior to treatment and were observed for general health at least twice daily. To avoid cross-infestation or contamination of cats when handling them, study personnel changed protective clothing between cats.

#### 2.2. Treatment administration

Cats were blocked based upon whether they had bi- or unilateral infestations at study start and upon body weight and then were randomly assigned to placebo-treated or selamectin/sarolaner-treated groups. In total, 15 cats (7 in Study 1 and 8 in Study 2) per group received a single treatment with a placebo solution or with selamectin/sarolaner on Day 0. All treatments were applied topically in one single spot directly to the skin at the base of the neck at the dose volume of 0.1 mL/kg providing the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight in the treated groups and only the excipients in the placebo-treated groups. Cats were assessed for overall health prior to treatment and at 1, 3, 6 and 24 h after dosing. Evaluations of the application site were performed at approximately 1, 3, 6 and 24 h after treatment and again at 3, 5 and 30 days after treatment.

#### 2.3. Efficacy assessments

Cats were examined otoscopically for the presence of live mites on Day 14 and by total ear mite counts on Day 30. For total mite counts, cats were sedated and each ear was flushed and the ear canal contents were processed separately. The ear canals were filled with 5% docusate sodium (Docusoral® Typharm Ltd, Norwich) and massaged to loosen the contents. The docusate sodium solution was then removed from the ears and poured through a 38 mm sieve.

The ears were then flushed with a warm saline solution which was poured through the same sieve. The ears were examined otoscopically and if needed the flushing process was repeated until the ear canals were assessed as clean. The contents of the sieves were transferred to a Petri dish for examination under a stereo microscope. All live mites (adults, larvae and nymphs) were counted and the counts for the two ears were summed for each animal’s total ear mite count.

#### 2.4. Data analysis

All calculations were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). The individual cat was the experimental unit. Efficacy (% reduction) was calculated using the Abbott formula: \( \frac{[(C – T)]}{C} \times 100 \), where \( C \) = arithmetic mean mite count for the control group and \( T \) = arithmetic mean mite count for the treated group.

For the statistical comparison, mite counts were transformed by the \( \ln (\text{count} + 1) \) transformation prior to analysis in order to stabilize the variance and normalize the data. Total live *Otodectes* mite counts at Day 30 were analyzed using a general linear mixed model including the fixed effect of treatment and random effects of block and error. Differences were assessed at the two-sided significance level \( \alpha = 0.05 \).

### 3. Results

#### 3.1. Efficacy

In the two studies combined, the otoscopic examinations on Day 14 diagnosed live mites in 14 of the 15 placebo cats, and in 2 of the 15 selamectin/sarolaner-treated cats. At study completion on Day 30, live mites were recovered from all 15 placebo-treated cats with mite counts per cat ranging from 1 to 3923. In Study...
Table 1
Arithmetic (geometric) mean Otodectes cynotis counts, mite count range, and percent efficacy relative to placebo for cats 30 days after a single topical treatment with selamectin/sarolaner.

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n = 7)</td>
<td>Selamectin/Sarolaner (n = 7)</td>
</tr>
<tr>
<td>Mean mite counts</td>
<td>576.9 (301.6)</td>
<td>4.7 (1.3)</td>
</tr>
<tr>
<td>Mite count range</td>
<td>9–1176</td>
<td>0–28</td>
</tr>
<tr>
<td>Percent efficacy</td>
<td>99.2 (99.6)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.0003</td>
<td></td>
</tr>
</tbody>
</table>

1. following repeated and extensive ear lavage, low numbers of ear mites were visualized in the ear canals of six of the seven placebo-treated cats. At most an additional 21 mites were noted in any single placebo-treated cat, but these mite counts were not added to the total counts. No ear mites were visualized in situ in any of the selamectin/sarolaner-treated cats following ear lavage. The arithmetic mean mite counts in the placebo-treated groups were 576.9 and 875.8 and for the selamectin/sarolaner-treated groups the counts were 4.7 and 5.8 in Studies 1 and 2, respectively. The range, arithmetic and geometric mean mite counts are provided in detail in Table 1. The mean mite counts for the selamectin/sarolaner-treated groups were significantly lower than those for the placebo-treated groups in both studies (P = 0.0003 and P = 0.0021). A single topical treatment with the new spot-on formulation of selamectin/sarolaner at the recommended minimum dose resulted in 99.2% and 99.3% efficacy in the two studies (Table 1).

3.2. Health observations

There was one adverse event observed in each group that was likely related to treatment administration in both studies combined. In one placebo-treated cat, small pustules were observed at the administration site pre-treatment. The cat developed alopecia and erythema starting 24 h after treatment in the same area. In a selamectin/sarolaner-treated cat, alopecia was observed at the administration site starting 3 days after treatment. None of these conditions required medical treatment and both were ongoing at the end of the study. A number of cats in both treatment groups had transient cosmetic observations at the application site, including greasiness, matting, spiking of the hair and white deposits, all of which are typical of a spot-on application. Four additional abnormal health observations were reported during the studies, none of which were related to treatment administration. One cat in the selamectin/sarolaner-treated group had open pyometra on Day 0 and received antibiotic treatment for the duration of the study. Another selamectin/sarolaner-treated cat was observed with salivation pre-treatment, during treatment administration, and at 3 and 24 h after treatment. Two cats, one in the placebo-treated group and one in the selamectin/sarolaner-treated group vomited after sedation for the ear lavage on Day 30.

4. Discussion

The two studies reported here demonstrate the efficacy of the new spot-on formulation of selamectin/sarolaner against O. cynotis in cats. A single topical administration at the minimum recommended dose resulted in ≥99.2% reduction in mite counts compared to placebo.

The level of infection in the current studies was very high, with mean ear mite counts of 576.9 and 875.8 in the placebo-treated groups. In studies that enumerated mites but used naturally infected cats, mean mite counts for placebo groups were 8.3, 41.8 (Shanks et al., 2000) and 27.5 (Roy et al., 2011). The high infestation level in the cats in the current studies underlines the appropriateness of using induced infestations in controlled feline studies, and lends further support to the high efficacy of the new spot-on formulation of selamectin/sarolaner under high infestation pressure.

This level of efficacy is similar to what is reported for topically administered products currently licensed for the treatment of ear mite infestations (Shanks et al., 2000; Six et al., 2000; Davis et al., 2003; Davis et al., 2007). Nevertheless there were several differences in the methods used in the current studies compared to previous reports. The current studies used cats with induced O. cynotis infestations, while previous controlled laboratory studies enrolled cats with natural infestations (Shanks et al., 2000; Fourie et al., 2003; Roy et al., 2011). Due to the induced nature of infestations, the clinical signs of otocariosis were not evaluated in the current studies. While clinical signs are relevant for the owners and veterinarians to guide the choice of therapy and monitor treatment outcome, clinical signs do not directly infer the severity of ear mite infestations (Curtis, 2004). It has been observed that cats with severe otitis externa often harbor only few mites, and conversely, cats can be asymptomatic carriers of ear mites (Sotiraki et al., 2001; Akucewich et al., 2002). Therefore the assessment of efficacy in the current study was based purely upon acaricidal effect. Further, the complete ear lavage method used in the current study enabled the enumeration of mites present in the ear canals. This is a higher level of scrutiny than conducting otoscopic evaluations only to detect mites (Davis et al., 2007), microscopic examination of aural swab samples (Scherk-Nixon et al., 1997; Nunn-Brooks et al., 2011) or the combination of both (Engelen and Anthonissen, 2000; Fourie et al., 2003; Blot et al., 2003; Farkas et al., 2007). A similar ear lavage technique was reported in one only set of studies (Shanks et al., 2000) while mechanical removal of all the auricular secretion was performed in other studies (Roy et al., 2011, 2012).

5. Conclusions

A single topical administration of a new spot-on formulation of selamectin/sarolaner at the minimum recommended dose was safe and highly effective in the treatment of O. cynotis infestation in cats.

Conflict of interest

The studies reported here were funded by Zoetis. CR is an independent investigator contracted for one of these studies. All the other authors are or were employees of Zoetis at the time the study was conducted. All authors assisted with the design and conduct of the studies, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

References


Research paper

Efficacy of a new spot-on formulation of selamectin plus sarolaner against *Ancylostoma tubaeforme* and *Toxocara cati* in cats

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**A B S T R A C T**

The efficacy of a new spot-on formulation of selamectin plus sarolaner for cats was evaluated against induced infections with *Ancylostoma tubaeforme* (hookworm) and *Toxocara cati* (roundworm). Five laboratory studies were conducted using adult, purpose-bred cats. Four of the studies were designed to evaluate efficacy of the combination against *A. tubaeforme*, the dose-limiting gastrointestinal nematode species for selamectin. In two of these studies non-interference between selamectin and sarolaner was also evaluated. The fifth study evaluated efficacy of the combination against mixed infections of *A. tubaeforme* and *T. cati*. The hookworm isolates in three studies were of US origin, as was the roundworm isolate. In the two remaining studies, cats were inoculated with a hookworm isolate of European origin. Cats were inoculated with 150 (±50) to 200 (±50) infective hookworm larvae 30–42 days prior to treatment and with 400 infective roundworm eggs 60 days prior to treatment. Cats were ranked by pre-treatment faecal egg counts and randomly allocated to different treatment groups. In all studies, cats were treated at the minimum label dose to provide 6.0 mg selamectin per kg bodyweight. All animals were euthanized 7–10 days after treatment for worm counts. Efficacy was calculated based on the reduction of the geometric mean worm counts in the treated groups versus the placebo-treated control groups.

The efficacy against adult hookworms was 99.2%, 94.3% and 100% in three of these studies, and was lower in the remaining two studies. The efficacy against *T. cati* was 100%. Furthermore, non-interference between sarolaner and selamectin was demonstrated. Thus, a single topical application of the new spot-on formulation of selamectin plus sarolaner at the minimum label dose is effective in the treatment of adult hookworm and roundworm infections in cats.

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1. Introduction

Infections with the gastrointestinal nematodes *Ancylostoma tubaeforme* and *Toxocara cati* are common in cats, although the reported prevalence does vary depending on region or epidemiological background of the animal (Anderson et al., 2003; Barutzki and Schaper, 2011; Beugnet et al., 2014; Capári et al., 2013; Knaus et al., 2014; Ngui et al., 2014; Rebbein et al., 2014; Wright et al., 2016; Zanzani et al., 2014). Both hookworm and roundworm infections may directly impact the health of cats and are known to cause retarded growth, failure to thrive and diarrhoea. Although the clinical impact of these nematodes on cats is not always apparent, the presence of these parasites may be unacceptable to cat owners from an aesthetic and hygienic point of view. In addition, cats represent potential reservoirs for the zoonotic *A. tubaeforme* hookworms and *T. cati* roundworms. Regular treatment of gastrointestinal nematodes is therefore recommended (ESCCAP, 2010; CAPC, 2016), and especially for *T. cati* monthly treatments are required to prevent patent infections and to decrease the risk of zoonotic transmission. It has recently been demonstrated in the US that cats more commonly shed *Toxocara* eggs than dogs (Lucio-Forster et al., 2016). Moreover, mixed infections of gastrointestinal parasites and ectoparasites are frequently reported in cats (Beugnet et al., 2014; Knaus et al., 2014; Little et al., 2015), warranting the use of broad spectrum antiparasitic treatment.

The present studies were conducted to confirm the efficacy of a new spot-on formulation of selamectin plus sarolaner against *A. tubaeforme* and *T. cati*. Selamectin is known to be effective against the adult stages of both nematode species and to significantly reduce egg excretion (McTier et al., 2000; Six et al., 2000; Altreuther et al., 2005). Besides its efficacy against these gastrointestinal nematodes, selamectin is known to prevent infections
with heartworm and to treat a range of ectoparasites, including fleas and ear mites. In the new combination, sarolaner is added to expand the efficacy range to ticks. As it has been demonstrated that *A. tubaeforme* is the dose-limiting nematode for selamectin in cats (McTier et al., 2000), four of the present studies evaluated efficacy against induced hookworm infections, and the fifth study evaluated efficacy against a mixed hookworm and roundworm infection.

2. Materials and methods

Four laboratory studies (Studies 1–4) were conducted using the final formulation of selamectin/sarolaner to confirm the efficacy against *A. tubaeforme*. Study 5 was conducted using a close to final formulation to evaluate efficacy against mixed infections with *A. tubaeforme* and *T. cati*. All studies were conducted in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al., 1994) and with VICH guideline GL20 (2001). All studies were approved by the Zoetis Ethical Review Committee and by the site-specific Ethical Review Committee.

2.1. Animals

All animals had undergone a wash-out period sufficient to ensure that no residual anthelmintic efficacy remained from any previously administered compounds, and were in good health at enrollment. Fourteen (14) to 32 purpose-bred cats of both sexes were enrolled depending on the study. Cats ranged in age from 8 to 24 weeks at inoculation. Female cats were neither pregnant nor lactating. Each cat was individually identified by a unique and permanent identifier. Cats were housed in groups of two to three cats before treatment, and in individual indoor pens after treatment such that no physical contact was possible between them. Cats were fed an appropriate maintenance ration of a commercial feline diet for the duration of the study. Water was available *ad libitum.*

2.2. Study design

In all studies, general health observations were performed at least once a day from the start of the acclimation period until the end of the study. Cats were acclimatized for at least 7 days prior to inoculation. Cats were weighed prior to treatment on Day 0. In Studies 1–4, animals were dosed with the commercial formulation at the minimum label dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight (Table 1). Study 5 was conducted using a similar formulation at 6.0 mg/kg selamectin but twice the minimum label dose of 2.0 mg/kg sarolaner. All treatments were applied topically at a single spot just cranial to the scapulae at a dose volume of 0.1 mL/kg. Each cat was observed after dosing for potential adverse events associated with treatment, and again for general health and any reaction to treatment approximately 1, 3, 6, and 24 h after treatment.

Studies 1 and 2 were designed to test the efficacy of selamectin/sarolaner and to confirm the non-interference between sarolaner and selamectin in the combination. A total of four groups each containing eight animals were enrolled. A placebo was applied to the animals in the control group, one treatment group received selamectin/sarolaner, and the remaining two treatment groups received either selamectin or sarolaner alone. In both studies, cats were infected with a US hookworm isolate (Cheri-Hill Kennel isolate). In Study 1, 200 (±50) infective *A. tubaeforme* larvae were given to each cat 33 days prior to treatment. If a cat vomited after infection, a similar dose of infective larvae was provided again. Seventeen of the 32 cats (4 placebo-treated cats; 5 selamectin/sarolaner-treated cats; 3 sarolaner-treated cats and 5 selamectin-treated cats) in Study 1 were reported to have vomited after initial inoculation, and were re-inoculated with a full infection dose of 200 (±50) infective larvae. In Study 2, 150 (±50) infective larvae were given to each cat 31 days prior to treatment. Studies 3 and 4 were designed as dose confirmation studies, comparing the efficacy of selamectin/sarolaner at minimum label dose with a placebo. Two groups of either seven (Study 4) or eight (Study 3) cats were allocated to the treatment or placebo group. In both studies, cats were infected with 150 (±50) infective *A. tubaeforme* larvae of an EU isolate (Albanian isolate). The susceptibility of the EU isolate to selamectin was confirmed in a faecal egg count reduction test with multiple faecal samplings after treatment. In Study 3, infection was performed 30 days prior to treatment, whereas in Study 4 infection was performed 42 days prior to treatment. Study 5 was designed as a dose confirmation study, with two groups of 10 cats each. Cats were infected with 400 infective *T. cati* (Cheri-Hill Kennel isolate) eggs 60 days prior to treatment and with 150 (±50) infective *A. tubaeforme* larvae (Cheri-Hill Kennel isolate) 30 days prior to treatment. Both the hookworm and roundworm isolates originated from the US.

2.3. Faecal egg counts and randomisation

In Studies 1, 2, 3 and 5, faecal egg counts were performed once prior to treatment, between Day –4 and Day –3. The cats were blocked based on the pre-treatment *A. tubaeforme* faecal egg counts, and randomly allocated to treatment groups using a randomized complete block design. Cats were placed in their allocated individual pens prior to treatment. Before necropsy, faecal egg counts were performed again. In Study 4, additional pre-treatment faecal egg counts were performed, as described in Table 4. All faecal egg counts were performed using a quantitative flotation method, with a minimum sensitivity of 5 eggs per gram of faeces.

2.4. Necropsy

Food was withheld from the cats 12–24 h prior to necropsy. All cats were humanely euthanized by intravenous injection of an approved euthanasia solution containing pentobarbital at the label indicated dosages. After euthanasia, the entire gastrointestinal tract (from distal esophagus to rectum) was removed. The gastrointestinal tract was split longitudinally and the mucosal surface scraped to remove attached hookworms, and roundworms where applicable. The contents and scrapings from the gastrointestinal tract were washed over a sieve with a maximum aperture size of 250 μm (No. 60 Standard Testing Sieve or suitable alternative). The remaining small intestines were either washed under pressure (Studies 1, 2 and 5) or soaked (Studies 3 and 4) as follows: the intestines were placed in approximately 2L containers with pre-warmed 0.9% saline, in an incubator/room at approximately 32 °C to 38 °C, to stimulate the release and sedimentation of adherent parasites. After approximately 2 h, the small intestines were stripped again under tap water through the sieve. The total saline solution was sieved, through the same sieve, to recover parasites from the intestine and sediment. After washing or soaking, the contents of the sieve were rinsed into a container, preserved in alcohol or formalin and examined under a stereo microscope for nematodes. Adult worms were identified and counted.

2.5. Data analysis

The experimental unit was the animal. Prior to statistical analysis, adult worm counts were natural logarithm transformed (log(x+1)). The general linear mixed model was used to analyze the logarithm-transformed adult worm counts. The model included
the fixed effect of treatment and the random effects of block and error for all studies except Study 3. The random effects for Study 3 were the housing unit, block within housing unit and error. A two-tailed 5% level of significance was used to determine whether the treatment effect was significant.

Least squares means and standard errors were calculated and 95% confidence intervals constructed for each treatment. Geometric means (back-transformed least squares means) and corresponding back-transformed 95% confidence intervals were reported, along with minimum and maximum values for the raw data.

Percent effectiveness for selamectin/sarolaner, sarolaner or selamectin with respect to the placebo were calculated using geometric means (back-transformed least squares means) based on the formula \( [(C – T)/C] \times 100 \), where \( C = \) mean of adult worm counts for the placebo control group and \( T = \) mean of adult worm counts for the treated group.

3. Results

3.1. Efficacy based on necropsy worm counts

The worm counts and anthelmintic efficacy in the five studies as well as the statistical comparisons between placebo-treated control group and the treated groups are provided in Table 2. In all studies an adequate infection with hookworms was obtained in the placebo-treated animals, although the mean number of adult worms recovered from controls varied between studies. The worm counts after infection with the US isolate (Studies 1 and 2) were in general higher compared to the EU isolate (Studies 3 and 4). The efficacy of selamectin/sarolaner against adult hookworms was above the 90% regulatory threshold for efficacy in Studies 2, 4 and 5, with a reduction in geometric mean adult worm counts of 99.2%, 94.3% and 100%, respectively compared to the placebo-treated group. In Study 1, the efficacy in the selamectin/sarolaner-treated groups and in the selamectin-treated groups was 84.1% and 90.4%, respectively. In Study 3, the efficacy of selamectin/sarolaner was 42.1%. Efficacy against T. cati in Study 5 was 100%.

The non-interference of sarolaner with the efficacy of selamectin against adult A. tubaeformae was demonstrated in Studies 1 and 2, as the worm counts in the cats treated with sarolaner were not significantly different from the placebo-treated cats, whereas the worm counts in the selamectin-treated and in the selamectin/sarolaner-treated groups were significantly \((P \leq 0.002)\) reduced compared to the placebo-treated cats, confirming the efficacy of selamectin at the minimum label dose against adult hookworms both as a stand-alone product and in the new topical formulation.

3.2. Faecal egg counts

The faecal egg counts in the placebo-treated animals in Studies 1–4 are provided in Table 3. In all studies animals were excreting eggs prior to treatment, and the mean number of eggs increased in the period between the pre-treatment (Day –4 or –3) and the necropsy (Day 7 or 10) faecal egg counts. A substantially higher increase (135%) in faecal egg counts was however observed in Study 3 compared to the other three pivotal efficacy studies (Table 3) suggesting that in Study 3, at least part of the infective larvae were still immature at the time of treatment and developed into adult worms in between treatment and necropsy.

The faecal egg counts in the animals of both groups prior to treatment in Study 4 are provided in Table 4. The number of positive cats increased between Day 27 and 34. The mean faecal egg counts increased between Day 27 and 37 after inoculation and plateaued afterwards (Day 38–40).

3.3. Health observations and application site reactions

No adverse events related to treatment with selamectin/sarolaner were observed. A number of cats in all treatment groups had transient cosmetic observations at the application site, including greasiness, matting, and spiking of the hair, all of which are typical of a spot-on application. These application site reactions were generally observed between 1 and 24 h after application but not afterwards. In one cat treated with selamectin/sarolaner, white deposits were recorded on Day 3 but not Day 5 after treatment.

4. Discussion

The results of the current studies demonstrate that the new spot-on formulation of selamectin plus sarolaner at the minimum label dose providing 6.0 mg/kg selamectin is effective in the treatment of adult A. tubaeformae infections in cats. Moreover, it was confirmed that sarolaner does not interfere with the efficacy of selamectin against hookworm infections. The efficacy of the new spot-on formulation of selamectin plus sarolaner against adult T. cati was (100%) which agrees with the previously published efficacy for selamectin alone (McTier et al., 2000).

The 42.1% reduction in mean worm counts in Study 3 was unexpected given the well-established efficacy of selamectin against adult A. tubaeformae (McTier et al., 2000; Six et al., 2000), the high efficacy of selamectin/sarolaner observed in the other studies, and the non-interference between sarolaner and selamectin. Potential causes related to the design and execution of Study 3, including underdosing of animals, undetermined conditions at the study site, resistance of the European A. tubaeformae isolate to selamectin and a mistake in dosing of animals, were all ruled out based on the study records and follow-up analyses. Therefore, the appropriateness of the study design and especially the timing of treatment after inoculation were evaluated. According to the relevant VICH (GL20) and WAAVP (Jacobs et al., 1994) guidelines, cats should be treated >21 days after infection with third stage larvae in order to evaluate efficacy against adult A. tubaeformae worms. The proposed timing for treatment is further supported by the pre-patent period (18–28 days) described for A. tubaeformae (Bowman et al., 2008). The 30-day interval between inoculation and treatment in Study 3 therefore seemed appropriate. However in Study 4, the increase
in the number of positive cats between 27 and 34 days after inoculation, as well as the gradual increase in the pre-treatment faecal egg counts between 27 and 38 days after inoculation and the plateauing of faecal egg counts between 38 and 40 days after inoculation, suggest that the EU hookworm isolate used in Studies 3 and 4 requires more than 30 days to develop into a fully mature hookworm population. In addition, the faecal egg counts of the placebo-treated animals in Study 3 indicate a substantially higher increase in faecal egg counts between the pre-treatment and necropsy faecal egg count compared to the other three efficacy studies, suggesting that in Study 3 at least part of the infective larvae were still immature at the time of treatment and developed into adult worms in between treatment and necropsy. The above data suggest that the timing of treatment in Study 3 was not appropriate to evaluate efficacy against the adult stages of this particular *A. tubaeforme* isolate. This was confirmed by the high efficacy (94.3%) in Study 4 against the same *A. tubaeforme* isolate as used in Study 3 but after an additional 12 days of pre-treatment larval development. These observations are not contradicting the current understanding of the *A. tubaeforme* life cycle. The pre-patent period described is to be between 18–28 days (Bowman et al., 2008), but this is based on a fairly limited number of experiments (Okoshi and Murata, 1967a,b; referenced in Bowman et al., 2008). Differences from these experiments regarding development time were previously noted (von Samson-Himmelstjerna et al., 2003). Considering that the pre-patent period only describes the time required to the first measurable faecal egg excretion, the pre-patent period should not be considered as a measure of maturation for the entire worm population in the infected animal. Furthermore, the range in pre-patent period as currently described (18–28 days) illustrates that different maturation times between hookworm isolates are known. Differences concerning the progress and outcome of larval development within individual hosts and dependent on infection dose have indeed been observed (Onwuliri et al., 1981; von Samson-Himmelstjerna et al., 2003).

The efficacy of the new spot-on formulation of selamectin plus sarolaner in Studies 1, 2, 4 and 5 was 84.9%, 99.2%, 94.3% and 100%, respectively. The lower efficacy (84.9%) in Study 1 may have been due to the protocol requirement to re-infect in case of emesis after inoculation. This was included into the study protocol to ensure adequate infection and was done for 17 of the 32 cats in the study, evenly distributed over the different treatment groups, but potentially introduced a bias in the efficacy evaluation. Therefore, for all

### Table 2
The number of animals in each treatment group, the geometric mean worm counts, and the efficacy against induced *Ancylostoma tubaeforme* and *Toxocara cati* infections in cats.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>N of animals</th>
<th>Geometric mean worm counts with 95% Confidence limits</th>
<th>Efficacy</th>
<th>Control vs. treated (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>Placebo</td>
<td>8</td>
<td>105.0 (72.5–151.9)</td>
<td>84.1%</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>(A. tubaeforme US isolate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>8</td>
<td>16.7 (8.0–33.7)</td>
<td>90.3%</td>
<td>0.0020</td>
</tr>
<tr>
<td></td>
<td>Sarolaner</td>
<td>8</td>
<td>94.7 (72.2–124.2)</td>
<td>9.80%</td>
<td>0.6053</td>
</tr>
<tr>
<td></td>
<td>Selamectin</td>
<td>8</td>
<td>10.1 (2.5–34.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 2</td>
<td>Placebo</td>
<td>8</td>
<td>79.7 (46.3–136.8)</td>
<td>99.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(A. tubaeforme US isolate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>8</td>
<td>0.6 (0.0–1.8)</td>
<td>99.9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sarolaner</td>
<td>8</td>
<td>92.6 (53.8–158.8)</td>
<td>0.00%</td>
<td>0.6799</td>
</tr>
<tr>
<td></td>
<td>Selamectin</td>
<td>8</td>
<td>1.1 (0.3–2.7)</td>
<td>98.6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Study 3</td>
<td>Placebo</td>
<td>8</td>
<td>14.6 (3.7–51.2)</td>
<td>42.1%</td>
<td>0.0614</td>
</tr>
<tr>
<td></td>
<td>(A. tubaeforme EU isolate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>8</td>
<td>8.5 (1.8–30.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 4</td>
<td>Placebo</td>
<td>7</td>
<td>5.1 (3.4–7.6)</td>
<td>94.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(A. tubaeforme EU isolate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>7</td>
<td>0.3 (0.0–0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 5</td>
<td>Placebo</td>
<td>10</td>
<td>27.7 (17.4–31.2)</td>
<td>100%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>(A. tubaeforme US isolate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>10</td>
<td>0.0 (0.0–3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 5</td>
<td>Placebo</td>
<td>10</td>
<td>7.1 (5.5–18.2)</td>
<td>100%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>(T. cati US isolate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selamectin/Sarolaner</td>
<td>10</td>
<td>0.0 (0.0–0.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3
The arithmetic mean pre-treatment and necropsy faecal egg counts (*Ancylostoma tubaeforme*) in the placebo-treated animals.

<table>
<thead>
<tr>
<th>Study</th>
<th>Hookworm isolate used</th>
<th>Pre-treatment faecal egg count</th>
<th>Necropsy faecal egg count</th>
<th>Increase</th>
<th>Day of pre-treatment faecal egg count</th>
<th>Day of necropsy faecal egg count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>US isolate</td>
<td>1707</td>
<td>2079</td>
<td>22%</td>
<td>Day 30</td>
<td>Day 40</td>
</tr>
<tr>
<td>Study 2</td>
<td>US isolate</td>
<td>1281</td>
<td>1858</td>
<td>45%</td>
<td>Day 27</td>
<td>Day 41</td>
</tr>
<tr>
<td>Study 3</td>
<td>EU isolate</td>
<td>617</td>
<td>1450</td>
<td>135%</td>
<td>Day 26</td>
<td>Day 40</td>
</tr>
<tr>
<td>Study 4</td>
<td>EU isolate</td>
<td>600</td>
<td>700</td>
<td>17%</td>
<td>Day 38</td>
<td>Day 51</td>
</tr>
</tbody>
</table>

* The same US isolate was used in Studies 1 and 2, and the same EU isolate was used in Studies 3 and 4.

* Days after inoculation: pre-treatment faecal egg counts were performed 3 or 4 days prior to treatment; necropsy faecal egg counts were performed 7–10 days after treatment.

### Table 4
The number of positive cats, the range and arithmetic mean pre-treatment faecal egg counts in Study 4.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Days after inoculation</td>
<td>27</td>
<td>34</td>
<td>37</td>
<td>38</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>Number of positive cats</td>
<td>7</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Arithmetic mean FEC</td>
<td>68</td>
<td>361</td>
<td>493</td>
<td>664</td>
<td>682</td>
<td>486</td>
</tr>
<tr>
<td>Range in FEC</td>
<td>0–250</td>
<td>100–1100</td>
<td>100–1350</td>
<td>100–1850</td>
<td>100–2250</td>
<td>100–1050</td>
</tr>
</tbody>
</table>

* 14 cats were enrolled in this study.

* FEC: faecal egg count in eggs per gram of faeces (epg) for the 14 animals included in the study.
solution against naturally acquired nematode and cestode infections in domestic cats. Parasitol. Res. 107 (Suppl. 1), S52–S56.


