Canine generalized demodicosis treated with varying doses of a 2.5% moxidectin + 10% imidacloprid spot-on and oral ivermectin: Parasiticidal effects and long-term treatment outcomes

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ABSTRACT

Advocate® (2.5% moxidectin + 10% imidacloprid) (Bayer HealthCare, Leverkusen, Germany) is a multiparasiticidal spot-on authorized for treating canine demodicosis in many countries. This blinded, randomized three-phase clinical trial compared its efficacy employing different dosing regimens with that of ivermectin. In the blinded first phase, 58 dogs suffering from generalized demodicosis were randomly assigned to one of four groups and treated with monthly, biweekly or weekly applications of Advocate®, or with oral ivermectin (IVR) at 500 µg/kg daily. Dogs were evaluated clinically and multiple skin scrapings undertaken every 4 weeks until parasitological cure was achieved (defined as two consecutive series of deep skin scrapings at monthly intervals negative for all life forms). Forty dogs completed the 16-week initial blinded phase, with 5 cases achieving parasitological cure. Five dogs were deemed treatment failures and subsequently treated with ivermectin. The treatment protocol was then changed for the remaining 35 dogs and this cross-over phase (Phase 2) was maintained for a further 8 weeks with an additional 9 dogs achieving parasitological cure. Thereafter, all remaining animals were treated with IVR until cured (Phase 3). Overall, 26 dogs achieved parasitological cure during the clinical investigation. Of these, 23 remained disease-free for at least 12 months while two were lost to follow up and one died of unrelated causes. A total of 32 (55.2%) dogs were withdrawn at various stages of the investigation including the 5 dogs that were judged treatment failures. Other reasons for withdrawal included: non-compliance, lost to follow-up, ivermectin toxicity or reasons unrelated to the investigation. No adverse effects were attributable to the use of Advocate®. Parasitical efficacy was assessed by changes in mite counts (live adult, juvenile and egg) and skin lesion extent & severity scores. Statistical significance was assessed using ANCOVA with initial mite counts or skin scores used as the covariate to account for variations in disease severity. Planned pairwise comparisons were used to identify differences between treatment groups. The efficacy of Advocate® increased with its rate of application across all measures of efficacy. Although ivermectin was shown to be more effective than...
1. Introduction

It is accepted that *Demodex canis* is a normal inhabitant of the cutaneous flora of dogs albeit in small numbers (Ravera et al., 2013) and is acquired from the dam during nursing (Greve and Gafar, 1966). The skin lesions attributable to demodicosis are a result of an abnormal proliferation of the mites often compounded by secondary bacterial infection. Demodicosis can be subdivided into localized and generalized forms as well as juvenile and adult onset, with the latter usually associated with an underlying systemic disease. Generalized demodicosis can be further subdivided into squamous and pustular forms, with the latter associated with secondary bacterial infection. It is often claimed that localized and generalized forms are distinct and separate entities, however the distinction between the two is somewhat arbitrary, and most cases of generalized demodicosis were localized at onset. The juvenile-onset generalized form is believed to be inherited with breed predilections shown especially for the American Staffordshire bull terrier, the Staffordshire bull terrier and the Chinese shar-pei with odds ratios of 35.6, 17.1 and 7.2, respectively, in a study of 2767 cases (Plant et al., 2010). Although the precise method of inheritance remains speculative, a significant association with the dog leukocyte antigen Class II alleles has recently been shown (It et al., 2010). The factors that trigger the proliferation of mites leading to generalized disease also remain speculative. It has been claimed that this results from an antigen-specific and/or generalized immunosuppression (Barta et al., 1983; Corbett et al., 1975). However, the nature of this defect and its relevance to the disease is still a subject of controversy (Lemarie and Horohov, 1996; Felix et al., 2012; Singh et al., 2010). Failure of the skin innate immunity has been implicated in the proliferation of *Demodex* mites in rosacea in man (Bevins and Liu, 2007) but its role in canine demodicosis has yet to be investigated.

Demodicosis is a frustrating disease to treat with an unpredictable course. While the majority of cases of localized disease resolve spontaneously, most cases of generalized demodicosis require aggressive miticidal and supportive therapy. Amitraz remains one of the few treatments licensed for this purpose world-wide but with widely varying reports of efficacy (Muller, 1983; Scott and Walton, 1985; Medleau and Willemse, 1995; Hugnet et al., 2001). However, due to its potential toxicity and inconsistent efficacy, there has been increasing interest in the use of the macrocyclic lactones. The most widely used is ivermectin given at 400–600 μg/kg orally once per day until parasitological cure (Muller, 2004; Muller et al., 2012). However, not only is this treatment contraindicated in collies and other herding breeds that have the ABCB1-1Δ (MDR1) mutation, but chronic toxicity is not uncommonly reported in dogs that are homozygous normal (Bissonnette et al., 2009). Other macrocyclic lactones employed with varying success are milbemycin (Holm, 2003), moxidectin (Wagner and Wendleberger, 2000) and doramectin (Murayama et al., 2010). Reported efficacy of these compounds varies, and it remains unclear as to whether they are significantly less toxic than ivermectin.

Moxidectin has more recently been introduced as a topical spot-on in association with imidacloprid, and marketed worldwide as Advocate® and as Advantage Multi® in North America by Bayer HealthCare (Leverkusen, Germany). It is licensed for the treatment of generalized demodicosis in a number of countries. Initially, it was labelled for once monthly application (Heine et al., 2005), but later studies suggested that it was more efficacious when applied more frequently (Mueller et al., 2009). Its safety at higher than labelled doses has been demonstrated in two studies (Paul et al., 2004; Fourie et al., 2009). In a previous publication, we compared the efficacy of weekly, biweekly and monthly Advocate® with ivermectin administered orally at 500 μg/kg for 16 weeks, and showed that weekly treatment was significantly more efficacious than monthly administration, and that the response to ivermectin did not differ significantly from that to weekly Advocate® (Paterson et al., 2009). We now report the results of a long-term study which includes: additional study participants, a cross-over phase and long-term follow-up.

2. Materials and methods

2.1. Dogs

Dogs included were patients presented to the St. George’s University Small Animal Clinic, St George’s, Grenada, or recruited by two welfare organizations on the island, namely the Grenada Society of Prevention of Cruelty to Animals (GSPCA) and Pothounds Against Pregnancy (PAP).

They were considered for admission to the study if suffering from generalized demodicosis—as defined by a minimum of 5 affected areas (>10 cm² each) or a single large affected body region (>100 cm²) or at least one affected paw, all confirmed by demonstration of mites upon deep skin scrapings. They were of either sex, ≥7 weeks of age and weighing >4 kg. Exclusion criteria were pregnancy, lactation or history of recent parenteral corticosteroid usage for ≥4 weeks or use of a parasiticidal agent with known demodicidal activity within the past 4 weeks.

2.2. Diagnostic workup

Potential enrollees were subjected to a full clinical examination by the Principal Investigator (PI) including
as detailed a history as was possible. Laboratory workup included a complete blood count and screening for infection with heartworm, ehrlichiosis, Lyme disease and/or anaplasmosis using either Idexx 3DX® or 4DX® SNAP® test (Idexx Laboratories, Westbrook, Maine). Clinical evidence of staphylococcal pyoderma was confirmed by cytology from either impression smears or acetate tape preparations stained with DiffQuik (Jorgensen Laboratories, Loveland, CO, USA). Bacterial culture and sensitivity testing was performed on cases with extensive lesions and/or a failure to respond to previous antibiotic therapy, by sampling intact pustules (where present) or purulent exudate (Miller et al., 2013).

2.3. Pre-enrolment treatments

Participants were treated for endoparasites as appropriate with pyrantel pamoate (Columbia Laboratories, Lexington, Kentucky) or praziquantel/oxanthel pamoate/pyrantel pamoate (Paratak Plus®, Bomac, Auckland, New Zealand). No other parasitidical products were permitted with the exception of a pyrethrin spray for flea control where required (Adams Flea and Tick Spray™, Farnham Pet Products, Phoenix, Arizona). Dogs testing positive for Dirofilaria immitis received a minimum of one dose of ivermectin (Heartgard®, Merial, Duluth, Georgia) as a microfilaricide in addition to acaricide therapy with melarsamine hydrochloride (Immiticide®, Merial). Those with clinical or serological evidence of tick-borne disease (Ehrlichia canis or Anaplasma platis) were treated with a 3-week course of doxycycline at 5–10 mg/kg twice daily (multiple manufacturers). Where antibiotic therapy was required for pyoderma, a minimum of 3 weeks of oral cepalexin (multiple manufacturers; 22–30 mg/kg twice daily) was given or an alternative antibiotic if indicated by results of sensitivity testing. Antibiotic therapy was continued or repeated during the trial as necessitated by clinical judgement. Supplemental topical antibacterial therapy was also employed using SulfOxydex®, Oxydex® or Malaseb® shampoo (DVM Pharmaceuticals distributed by Teva Animal Health, St. Joseph, MO). Shampoo therapy was not permitted 48 h either side of the day on which topical treatment was administered.

2.4. Demodicidal products employed

Advocate® (spot-on)(Bayer HealthCare) which contains 10% imidacloprid and 2.5% moxidectin (ADV) applied at the recommended dose: dogs 4–10 kg, 1.0 mL; dogs 10–25 kg, 2.5 mL; dogs 25–40 kg, 4.0 mL.

Ivomec® (Merial) containing 10 mg/mL of ivermectin (IVR) administered orally at 500 μg/kg once daily.

2.5. Therapeutic protocol and blinding

2.5.1. Phase 1 (blinded)

When admitted to the study, dogs were assigned to one of four treatment groups by the trial technician, according to a previously determined, computer-generated randomization schedule namely:

1. ADV1, Advocate® once monthly according to the manufacturer’s instructions.
2. ADV2, Advocate® every 2 weeks (biweekly).
3. ADV4, Advocate® weekly.
4. IVR, ivermectin at 500 μg/kg orally once daily.

Medications were dispensed according to the assigned protocol by the technician in sufficient quantities for one month, and were administered at the clinic on the first occasion. The owner or responsible person was instructed regarding the maintenance of the blinding which was accepted as part of the treatment consent form. The blinding was maintained for 16 weeks or until parasitological cure, whichever came first. Parasitological cure was defined as two consecutive series of skin scrapings undertaken at monthly intervals that were negative for all life forms, both dead and alive. It was deemed ethically unacceptable to maintain an animal on a regimen that might prove ineffective, and so any dog that failed to show decreasing mite counts or whose clinical signs worsened (unless due solely to pyoderma) when assessed at 8, 12 or 16 weeks, was judged to be a treatment failure. These dogs were removed from the study and subsequently treated with IVR. Any case whose owner was non-compliant (NC) (defined as failing to adhere to the therapeutic protocol or failing to present for re-evaluation as scheduled) was also similarly removed from the trial.

2.5.2. Phase 2 (cross-over)

Again, in order to avoid maintaining a patient for a long period on a protocol that could prove to be relatively ineffective, the regimen was changed for those who failed to achieve parasitological cure within 16 weeks. From this point on the study was unblinded and the treatment protocol changed as follows:

- ADV1 (Advocate® once monthly) → ADV2 (Advocate® biweekly)
- ADV2 (Advocate® biweekly) → ADV4 (Advocate® weekly)
- ADV4 (Advocate® weekly) → IVR (ivermectin daily)
- IVR (ivermectin daily) → ADV4 (Advocate® weekly)

This regimen was maintained for a further 8 weeks, with monthly assessments, until parasitological cure. If the first negative skin scraping was achieved during the 8th week of this phase, the dog was maintained on the same regimen for a further 4 weeks until the second negative scraping resulted.

2.5.3. Phase 3 (final cure)

Any animal failing to achieve parasitological cure (or one negative scraping) by the end of the 8 weeks of Phase 2, continued into Phase 3 and was treated with IVR until parasitological cure occurred.

2.5.4. Long-term follow up

Following parasitological cure, whenever it occurred, the animals continued to be monitored by the PI for a minimum of 12 months via telephone contact and/or clinical examination. Owners were asked to examine the pet for development of alopecia, crustating or any dermatological
problems that resembled their presenting signs. Where any skin problems were reported, the patient was evaluated at the clinic and multiple skin scrapings undertaken. All animals were examined by the PI at the end of the 12-month follow-up period.

2.6. Data recorded

The majority of the assessments were undertaken by the PI, but where she was unavailable one of two other veterinarians who were specifically trained for the project performed the examinations. The following series of data was recorded at each monthly re-evaluation:

2.6.1. Mite counts

At the initial presentation, mite counts were undertaken from three affected areas. Absolute numbers of each life stage (adults, juvenile (larvae/nymphs), eggs) were counted. Live and dead adults were recorded separately based upon the presence or absence of movement of mouthparts or legs (Miller et al., 2013) when examined within 2–3 h of sampling, and the live/dead ratio was noted. Mite counts on some animals were extremely high, so when counts of any life stage exceeded 50, the results were reported as >50 and assumed to be 50 for purposes of statistical analysis. The same areas were sampled at each subsequent evaluation. Where new lesions developed they were also sampled at the discretion of the PI in order to further assess clinical progress, but these mite counts were not included in the dataset.

2.6.2. Skin lesion extent and severity score

This was based upon the principles of the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) (Olivry et al., 2007) but adapted for the lesions of generalized demodicosis. The extent of dermatological lesions including erythema, scales/ crusts, comedones/papules/pustules and alopecia was documented by assigning each lesion type a score of between 0 (normal) and 6 (extremely severe) for each of 36 body areas. The maximum total possible score was thus 864.

2.7. Statistical methods

Statistical analyses were performed with Minitab 16® (Minitab Inc, State College, Pennsylvania) with P-values less than 0.5 considered significant. For Phase 1 (blinded), the per cent change in mean counts for each life stage as well as skin lesion extent and severity score was calculated and the reduction examined for significance by analysis of covariance (ANCOVA) using the initial score as the covariate to account for variations in the disease severity at the outset. In the event that significant differences were found, planned pairwise comparisons were conducted. The adult mite live/dead ratio was also evaluated for prognostic significance. For Phase 2 (cross-over), mean scores at the end of this phase were compared with those at the end of Phase 1 (blinded) and evaluated using one-sided paired t-tests. The long-term follow-up data was utilized to evaluate treatment efficacy based on the number of dogs remaining in remission for a minimum of 12 months. Both the response to treatment and the time to parasitological cure were calculated based upon the initial group assignment, but are obviously reflective of a combination of treatments applied due to the cross-over design of this investigation.

2.8. Institutional approval

The clinical investigation protocol was approved by the St. George’s University Institutional Animal Care and Use Committee.

3. Results

3.1. Demographics of the study participants

A total of 58 dogs were enrolled between December 2005 and October 2008 (Table 1). The majority of dogs (n = 38) included were the local mixed breed known as “Pothound”. The “Pompek” was the next most represented breed, however this more accurately reflects the local small breed dog as opposed to a true first generation cross between Pomeranian and Pekingese. There were 31 males (9/31 neutered) and 27 females (12/27spayed). Intact females were by chance evenly distributed across the groups. Ages were not always precisely known but ranged from 3 months to 12 years. Three cases were classified as adult onset, and the remainder as juvenile onset. Some cases of juvenile onset were up to 4 years of age when enrolled due to either previous treatment failures or chronic untreated disease. Sixteen dogs (28%) had received prior treatment with ivermectin, amitraz or other unknown medication.

3.2. Clinicopathological findings

Thirty-eight cases (66%) had pustular demodicosis, and the remaining 20 (34%) had the squamous form. Antibiotics employed were generally cephalosporins or less commonly, fluoroquinolones. One case developed a multi-drug resistant *Staphylococcus pseudintermedius* infection which responded slowly to a 16-week course of chloramphenicol. Predisposing causes for two of the three adult-onset cases were severe ehrlichiosis and chronic corticosteroid usage. No contributing cause was identified in the third case. Ten juvenile-onset cases with ehrlichiosis and one with *D. immitis* infection were treated as described above before beginning the clinical trial.
Table 2
Summary of results from the 16-week Phase 1 of the trial (blinded). Efficacy of the assigned treatment protocol is reported as a per cent decrease in mite count or improvement in skin lesion extent and severity score.

<table>
<thead>
<tr>
<th>Range</th>
<th>Mean total mite counts</th>
<th>Mean total live adults</th>
<th>Mean total juvenile mites</th>
<th>Mean total egg counts</th>
<th>Mean total skin lesion extent and severity score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADV1 (%)</td>
<td>ADV2 (%)</td>
<td>ADV4 (%)</td>
<td>IVR (%)</td>
<td></td>
</tr>
<tr>
<td>203–330</td>
<td>45</td>
<td>72</td>
<td>82</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>67–123</td>
<td>28</td>
<td>56</td>
<td>83</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>34–58</td>
<td>39</td>
<td>93</td>
<td>90</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>37–77</td>
<td>24</td>
<td>88</td>
<td>77</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>155–193</td>
<td>–20</td>
<td>12</td>
<td>57</td>
<td>74</td>
<td></td>
</tr>
</tbody>
</table>

ADV1 = Advocate® applied monthly; ADV2 = Advocate® applied biweekly; ADV4 = Advocate® applied weekly; IVR = oral ivermectin.

3.3. Withdrawals and treatment failures

Thirty-two cases were withdrawn at various points during the study. Five dogs were deemed treatment failures—two dogs each from the ADV1 and ADV2 treatment groups, and one from the Advocate® weekly (ADV4) treatment group. The remaining withdrawals were: 2 dogs that developed ivermectin toxicity, 11 cases of non-compliance, 6 deaths from causes unrelated to the study and 8 dogs were lost to follow-up.

3.4. Phase 1 (blinded)

Forty dogs completed the first phase, with 5 cases achieving parasitological cure—one from the ADV1 group, and two each from the ADV2 and IVR groups. Most of the dogs enrolled were very severely affected, with the majority of total mite counts from the 3 scrapings exceeding 100, with some greater than 500. These numbers represent underestimates, as each life stage count was capped once a count of 50 for that particular life stage was reached.

3.4.1. Parasiticidal effects

The mean counts of all life forms decreased during the 16-week period of evaluation across all treatment groups (Table 2). Pre-post ANCOVA analysis revealed significant differences between groups ($P < 0.001$) for all measures of efficacy. The significant differences for each life form were as follows:

(i) Mean total counts: while there was no significant difference in efficacy between monthly (ADV1) and biweekly (ADV2) application, weekly application (ADV4) had a significantly greater effect than did biweekly application ($P = 0.03$) and oral ivermectin (IVR) was significantly more efficacious than was ADV weekly application ($P = 0.003$) (Fig. 1a, Table 2). Since the total mite count includes dead adults, arguably the measurement of live adults, juvenile forms and eggs may be more reliable indicators.

(ii) Mean total live adult mites: similar to the total mite counts, IVR treatment led to a significantly greater reduction in live adults than did ADV4 ($P = 0.014$), and ADV4 was significantly more effective than was ADV2 ($P = 0.048$) (Table 2). There were no significant differences observed between monthly and bi-weekly application.

(iii) Mean total juvenile mites: for this measure of efficacy, ADV2 was more effective than was ADV1 ($P < 0.01$) and IVR was more effective than was ADV4 ($P < 0.001$). Biweekly application (ADV2) proved more effective than weekly treatment (ADV4) ($P < 0.001$) (Table 2).

(iv) Mean total egg counts: as was the case for the juvenile counts, the effect of ADV2 on mean total egg counts was in fact significantly greater than that of ADV4 for this measure ($P = 0.023$) and IVR was more effective than was ADV4 ($P < 0.001$) (Table 2).

(v) Live/dead mite ratio: the ratio of live to dead adult mites proved to be prognostically non-informative in this study.

Fig. 1. (a) Per cent reduction in mean total mite counts at end of the 16-week Phase 1 (blinded). ADV4 was more effective than ADV2 ($P = 0.03$) and IVR was more effective than ADV4 ($P = 0.003$). (b) Per cent reduction in mean skin lesion extent and severity scores at end of the 16-week Phase 1 (blinded). ADV4 was more effective than ADV2 ($P < 0.001$) and IVR was more effective than ADV4 ($P < 0.001$).
(vi) Exponential decay models: the relative effectiveness of the three dosages of Advocate® on total mite counts is depicted in Fig. 2 with IVR included as a reference. Monotonically decreasing exponential trends over time were observed across the treatment groups, which illustrate the apparent dose-response relationship.

3.4.2. Clinical response
Mean skin lesion extent and severity scores improved in all treatment groups except ADV1 which demonstrated a 20% worsening of clinical disease during the 16-week period of evaluation (Fig. 1b, Table 2). Pre-post ANCOVA again revealed significant differences between groups (P<0.001). Weekly application (ADV4) was significantly more effective than biweekly application (ADV2) (P<0.001), but there was no difference between the latter and ADV1 (P = 0.595). IVR was significantly more effective than ADV4 (P<0.001).

3.5. Phase 2 (cross-over)
Thirty-five dogs proceeded from Phase 1 (blinded) to Phase 2 (cross-over), which was no longer blinded and 28 dogs completed this phase. Nine dogs achieved parasitological cure during this phase: five in the group changing from IVR to AVD4, two in the group changing from AVD4 to IVR, two in the group changing from AVD2 to AVD4 and none of those changing from ADV1 to ADV2. It should be noted that in four instances, the dogs received their first negative scraping at the end of the two months and were maintained on the same protocol until their second negative scraping and hence parasitological cure resulted.

The statistical significance of the measured changes in mite counts and skin scores that occurred during the cross-over phase are reported in Table 3. No significant improvements resulted from changing from ADV1 to ADV2. Those cases that changed from ADV2 to ADV4 showed significant reductions in total mites and in juvenile mites (P=0.02 and 0.037, respectively). Those dogs proceeding from ADV4 to IVR showed significant improvements in all parameters, and in the case of dogs changing from IVR to ADV4, significant improvements were noted in total mite counts and lesions scores (P=0.039 and 0.040, respectively).

3.6. Phase 3 (final cure)

Nineteen dogs that failed to achieve parasitological cure during the first two phases proceeded to Phase 3 (final cure) and were treated with oral ivermectin. Seven of these were withdrawn with the remaining 12 dogs achieving parasitological cure.

3.7. Long-term follow up

The 26 dogs that achieved parasitological cure during the various phases of the study were monitored for a minimum of a further 12 months. Two were lost to follow up, one died of unrelated causes, but the remaining 23 were still disease-free 12 months later. Two cases relapsed after the end of the follow up period—one 15 months after achieving parasitological cure, and the other after 31 months.

3.8. Time to parasitological cure

Each participant’s time to parasitological cure and the study phase in which this occurred, are summarized in Table 4. Five cases cured within Phase 1 (blinded), 9 during (or shortly after) Phase 2 (cross-over) and 12 during Phase 3 (final cure). The longest time to cure was 54 weeks and a number of dogs required treatment in excess of 30 weeks. The mean times to parasitological cure per treatment group ranged from 23.4 to 38.6 weeks. Based upon the data generated, a generalized linear model was developed using exponential response and Poisson counts. The resultant model can be used to predict the estimated number of weeks to parasitological cure based upon treatment protocol and the patient’s initial mite count (Fig. 3). It should be emphasized that each treatment group actually represents a combination of treatments, but of importance is the fact

Table 3
Statistical significance of improvements in mite counts and skin scores during the 8-week Phase 2 of the trial (cross-over) using one-sided paired t-test. (Gray denotes significance at P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Adult mites</th>
<th>Juvenile mites</th>
<th>Mite eggs</th>
<th>Total mite count</th>
<th>Skin lesion score</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADV1 → ADV2</td>
<td>0.789</td>
<td>0.287</td>
<td>0.320</td>
<td>0.542</td>
<td>0.142</td>
</tr>
<tr>
<td>ADV2 → ADV4</td>
<td>0.051</td>
<td>0.037</td>
<td>0.110</td>
<td>0.020</td>
<td>0.056</td>
</tr>
<tr>
<td>ADV4 → IVR</td>
<td>0.010</td>
<td>0.045</td>
<td>0.026</td>
<td>0.007</td>
<td>0.013</td>
</tr>
<tr>
<td>IVR → ADV4</td>
<td>0.500</td>
<td>–</td>
<td>0.039</td>
<td>0.040</td>
<td></td>
</tr>
</tbody>
</table>

ADV1 = Advocate® applied monthly; ADV2 = Advocate® applied biweekly; ADV4 = Advocate® applied weekly; IVR = ivermectin.
that irrespective of treatment protocol, the weeks to cure increases proportional to the initial mite count.

### 3.9. Adverse reactions

Side effects that have been reported with the use of Advocate® include transient pruritus, erythema and vomiting (Fourie et al., 2009), but in this trial no adverse reactions of any type were seen with any of the three dosage schedules. Two dogs developed ivermectin toxicity during the trial and were subsequently withdrawn from the study due to the toxicity. Two further dogs that had already been withdrawn from the study [one for non-compliance, the other due to treatment failure] continued on ivermectin at the protocol dosage but developed toxicity associated with long-term treatment. All cases had moderate to severe neurotoxicosis with bilateral mydriasis, decreased to absent pupillary light response, ataxia and generalized muscle weakness. Two dogs had severe-to-complete visual impairment with concurrent absence of the menace response, as well as generalized muscle tremors and vomiting. The most severe case progressed to coma shortly after admission to the clinic which lasted for 12 h. All cases recovered fully within 5–7 days with mydriasis being the last clinical sign to resolve. One case had been treated with ivermectin for 6 weeks, two others followed more chronic administration (~8 months) and the fourth case is suspected to have resulted from accidental double-dosing on the same day. At the time of the intoxications, none of the dogs were receiving any drugs that inhibit P-glycoprotein which would have increased the animal’s risk for CNS toxicity. Two of the dogs were lost to follow up and the
remaining two resumed treatment on either ivermectin tapering up to 500 µg/kg orally per day or the ADV4 schedule and continued to parasitological cure.

4. Discussion

Fifty-eight dogs, including a variety of pure breeds, some cross-breeds and the local mixed breed—Grenadian pothound, were enrolled into the study. The Pothound is the most prevalent breed type on the island, and although no precise epidemiological studies have been undertaken, it is commonly affected with demodicosis. In Grenada, there is a large stray population and many animals remain sexually intact. It is a matter of speculation as to whether the subjectively high incidence of demodicosis in the dog population can be accounted for by the gene pool and facilitated by random and generally uncontrolled breeding, or whether it results from the presence of numerous factors that are often viewed as predisposing, e.g. poor nutritional status, frequent endo- and ecto-parasitism (Plant et al., 2010) and endemic infectious diseases, such as ehrlichiosis and heartworm disease.

Unfortunately, 55% (32/58) of study participants were withdrawn from the study during the course of the trial. The most common reason was owner non-compliance despite the fact that all services and miticidal products were provided without charge. Among the dogs withdrawn were five dogs that were deemed treatment failures—the treatment failure criterion was introduced to safeguard the health and welfare of dogs whose poor response to the assigned treatment could constitute a welfare issue. Nevertheless, these dogs continued to be treated with ivermectin although their data was not included in the statistical analysis of the current investigation.

All 26 dogs remaining in the study went on to parasitological cure and the 23 that could be followed remained in remission for at least one year—representing a ~100% cure rate. Two dogs were lost to follow-up during this time and another died one month before completing the 12-month follow-up. Many of the animals in this study were severely affected since most had never received prior treatment or were poorly managed for months to years resulting in chronic disease and high mite counts with secondary bacterial infection. Consequently, individual mite life stage counts often exceeded 100 for any given sample site, and so for practical purposes counting ceased at 50. While this may have led to an underestimation of mite counts and ultimately the therapeutic efficacy in the early stages of the investigation, it would have had no effect on the robustness of the later analyses. The cure rates in this investigation are higher than all studies in the literature where similarly defined cure rates were <90%. However, direct comparison of clinical trials for the treatment of canine generalized demodicosis is difficult as there is a lack of defined standards of therapeutic efficacy. Some studies report rate of cure based on the number of animals achieving parasitological cure without long-term follow-up or even based on single negative skin scrapings, while others have reported efficacy based on the decrease in mite counts. This investigation followed the currently recommended standard of efficacy based on the number of animals remaining in remission for at least one year following parasitological cure.

Although all dogs achieving parasitological cure remained in remission for this period, two cases subsequently relapsed. One dog relapsed 15 months later, after his owner was absent for an extended period of time and was suspected to have been malnourished during this period, while the other dog who was suffering from concurrent allergic skin disease relapsed 31 months following parasitological cure. To the author’s knowledge, glucocorticoids were not used to manage the allergic disease. The fact that these relapses occurred more than one year after parasitological cure serves to reinforce the fact that dogs who have had generalized demodicosis can relapse at any time and owners should be warned of possible reoccurrence, especially following episodes of psychological or physiologic stress. Recently, Colombo et al. (2012) reported results from a pilot study which suggested that once monthly Advocate® may be effective in the prevention of relapse of disease in cases of recurrent generalized demodicosis. Therefore, routine use of this product should be considered following miticidal treatment as it may aid in the prevention of subsequent relapse.

Among the five dogs achieving parasitological cure during the initial 16-week blinded phase, two received biweekly application of Advocate®. Coincidentally, these were the least affected of all study participants, and might have progressed to self-cure even without intervention. These two cases appear to be outliers relative to the rest of the dogs in this treatment group and consequently, enhanced the apparent efficacy of biweekly application of Advocate® in this investigation. Although both dogs met the inclusion criteria, it may be important to define minimum mite counts as a further inclusion criterion in future clinical trials. This contention is reinforced by the finding that the time to cure was shown to increase as a function of the initial mite count across all treatment groups (Fig. 3).
Pharmacokinetic studies have shown that the moxidectin in Advocate® reaches a steady-state serum concentration (∼36 μg/L) after four to five monthly applications and that this is significantly higher than the maximum serum concentration following a single dose (15.1 μg/L) (Anon, 2009). It is thus likely that weekly application of Advocate® enables the serum concentration to reach these elevated steady-state levels more rapidly and that these higher concentrations exert more significant miticidal effects. In addition, the fact that the Demodex mite has a relatively short life cycle of 18–24 days (Soulsby, 1982) suggests that treatment should be administered at least twice monthly for effective disruption of the mite life cycle.

Although weekly application of Advocate® proved efficacious even in the face of severe disease, the response to daily ivermectin was superior. This is in contrast to our previous report which was based on smaller numbers and showed no significant difference between the two treatment protocols (Paterson et al., 2009). However, several cases of ivermectin toxicity subsequent to chronic use were encountered during this trial, whereas no adverse reactions followed the application of Advocate® at any of the dosage schedules employed. The safety of Advocate® has been further established in two published studies. Fourie et al. (2009) reported that 8 dogs with undetermined ABCB1-Δ status had no significant adverse reactions when treated weekly with 5x the recommended dose for 17 weeks. In an earlier blinded study, 9 collies that had previously shown signs of toxicity to the administration of 120 μg/kg of ivermectin were given 3 applications of Advocate® at 5x the recommended dose at monthly intervals and showed no signs of toxicity (Paul et al., 2004). Although the ABCB1-Δ status was not determined, the fact that these animals came from a genetic pool of collies with well-documented sensitivity to ivermectin suggests that they were likely homozygous mutants.

5. Conclusions

This was one of the most detailed long-term studies on generalized demodicosis to be undertaken. Of those animals whose owners were fully compliant and could be followed, 100% achieved parasitological cure. Many animals required >30 weeks of treatment, with a maximum of 54 weeks. Irrespective of the treatment protocol, the time to cure increased as a function of the initial mite count. The efficacy of Advocate® increased with the frequency of application at the labelled dose. During the course of the investigation, the European registration was changed to permit weekly application in the face of severe disease, whilst maintaining monthly application in cases of mild-to-moderate disease. However, it is the opinion of the authors that weekly application is the preferred approach in all cases of canine generalized demodicosis. In this and other studies, it was shown to be without side-effects and with a wide margin of safety—even in ivermectin-sensitive collies. While ivermectin was more effective, toxicity was encountered. Perhaps controversially, the latter drug has been recommended as a treatment for severe generalized demodicosis under the 2011 clinical practice guidelines (Mueller et al., 2012), but it is not licensed for the treatment of this condition in any country of the world. Further studies could be warranted to investigate the use of Advocate® at higher doses and/or a greater frequency of application, to ascertain whether an equivalent response to that achieved with ivermectin might result without the associated toxicity, thus adhering to the cascade principle operative in many countries.

Conflict of interest statement

Bayer Animal Health did not play a role in the study design, data collection, data analysis and preparation of this manuscript.

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