Effectiveness of a single application of 0·25% fipronil solution for the treatment of hirstiellosis in captive green iguanas (*Iguana iguana*): an open-label study

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SUMMARY

Hirstiella spp. are common ectoparasites of captive green iguanas (*Iguana iguana*). Suggested treatments are empirical and some of them are of low efficacy and potentially toxic. The objective of this open-label study was to investigate the short-term efficacy and safety of a single application of 0·25% fipronil solution for the treatment of hirstiellosis. The skin of 50 green iguanas was thoroughly examined with the aid of bright light and magnifying lenses. A total of 21 iguanas were found to be infested, harbouring 1–24 mites (median: 5). All 35 mites collected from 17 iguanas were identified as *Hirstiella* sp. Both infested and non-infested lizards, sharing the same enclosure, were carefully wiped with 0·25% fipronil solution. The safety and the efficacy of the treatment were evaluated after 2 days in 47/50 (94%) and 7 days in 29/50 (58%) iguanas. Compared with pre-treatment levels, the parasitic load did not changed significantly on the second day but was significantly lower on day 7 ($P = 0·006$). No adverse reactions were noticed. Based on these results a single whole-body application of 0·25% fipronil solution can be considered a safe and effective treatment for the reduction of parasitic burden in captive green iguanas infested by *Hirstiella* sp. mites.

Key words: fipronil, green iguana, *Hirstiella*.

INTRODUCTION

During the last decades reptiles, such as lizards, snakes and chelonians, have became popular pets (Stahl, 2003; Hoppmann and Barron, 2007; Gazyağcı *et al*. 2011; White *et al*. 2011). Virtually all imported reptiles can be infested by a large number of ectoparasites, some of them having zoonotic significance. However, in most countries there is no screening for these parasites before importation (Schultz, 1975; Arnold, 1986; Goldberg and Bursey, 1991*b*; Harvey-Clark, 1995; Marano *et al*. 2007; Pasmans *et al*. 2008; Delfino *et al*. 2011; Hellebuyck *et al*. 2012).

Ectoparasites may infest green iguanas (*Iguana iguana*), a common pet in our area, and cause skin lesions that can become secondarily infected, anaemia, and transmission of pathogens such as haemogregarins and *Leishmania* spp. (Mader *et al*. 1986; Harvey-Clark, 1995; Bannert *et al*. 2000; Walter and Shaw, 2002; White *et al*. 2011; Hellebuyck *et al*. 2012). Suggested ectoparasiticidal treatments are empirical and some of them of low to moderate efficacy or potentially toxic (White *et al*. 2011; Hellebuyck *et al*. 2012).

Fipronil solution 0·25% is commercially available in a spray form (Frontline® spray, Merial) licensed for the treatment and prevention of flea and tick infestations in dogs and cats. However, it is commonly used as an extra-label medication for the treatment and prevention of various mite infestations in dogs, cats and horses, due to its wide acaricidal activity (Koutinas *et al*. 2001; Curtis, 2004; Rendle *et al*. 2007). Although it has been proposed as an effective treatment against mites and ticks in lizards, when applied topically every 1–2 weeks (Gazyağcı *et al*. 2011; Hellebuyck *et al*. 2012), there are few objective data to substantiate the safety and the efficacy of this treatment modality.

The aim of this open-label study was to investigate the short-term efficacy and safety of a single application of 0·25% fipronil solution for the treatment of mites, subsequently identified as *Hirstiella* sp., in captive green iguanas.

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MATERIALS AND METHODS

Animals

A total of 50 green iguanas (*I. iguana*), kept in pet shops located in Athens and Thessaloniki, Greece, were examined. They were considered healthy by pet shop owners and were kept for commercial purposes. Some of these green iguanas had been imported from Belgium (11/50 – 22%) and from Germany (5/50 – 10%), but the majority (34/50 – 68%) had been obtained from Greek breeders. They had been kept in the pet shops for 2–8 weeks (median: 2·5 weeks) in collections of 2–10 (median: 9) animals, sharing the same enclosure. They were fed on dry iguana formula and fresh vegetables. Wood shavings were used as substrate, all enclosures were clean and regularly disinfected and no ectoparasiticidal treatments had ever been applied. The age of these 50 iguanas ranged from 3·5 to 9 months (median: 7·5 months) and their body weight from 30–206 g (median: 78 g). Informed owners' consent was obtained before their inclusion into the study.

Clinical examination, counting, collection and identification of mites

The entire skin surface of the iguanas was carefully examined under bright light, with the aid of magnifying lenses for the presence of mites. The skin folds around the head and in axillary and inguinal areas were carefully unfolded for better visualization of the mites (Hoppmann and Barron, 2007). When two or more mites were present, at least one was collected, after gentle superficial scraping with a blunted scalpel blade, transferred into a separate bottle with 90% alcohol solution and identified to the genus level. However, if more than one mite could not be found in any of the iguanas sharing the same enclosure, one of the parasitized animals was randomly selected for mite sampling.

Treatment and evaluation of safety and efficacy

All 50 iguanas, both infested and non-infested, were treated with a 0·25% fipronil solution (Frontline® spray, Merial) that was wiped over their skin with a piece of cotton, taking care to avoid eye and nostril contact; also, cotton tip applicators were used to apply the solution on the skin folds. Attention was paid not to remove the mites from the surface of the skin during fipronil application. Treatment was performed in an open space after gentle manual restraint in order to keep stress of the animals to a minimum. All treated animals were kept out of their enclosures for at least 30 min and until they were dry, to avoid alcohol inhalation. The quantity of the solution used for each iguana was measured by weighing the bottle before and after the treatment. No environmental treatment as well as any other kind of treatment on the animals was permitted for the duration of the study.

Re-examinations, scheduled for the 2nd and 7th day post-treatment, included investigation for possible adverse reactions, physical examination and counting of mites. No mites were collected at re-examinations.

Statistical analysis

The number of mites that were left before treatment (after sample collection for species identification) was compared with the number of mites found on the same animals after 2 and after 7 days using the related-samples Wilcoxon Signed rank test. The level of significance was set at 0·05 and the analysis was performed in SPSS 20.0 for Windows.

RESULTS

Mites were observed on 21/50 (42%) iguanas and at least one animal was found to be infested in each enclosure. Eleven (22%) lizards presented with skin lesions consisting of cutaneous hyperpigmentation and very small crusts at the site of mite attachment. The number of mites in the 21 infested iguanas ranged from 1 to 24 (median: 5) per animal. A total of 35 mites were collected from 17 animals (1–4 mites per animal; median: 2 mites per animal) and they were all identified as *Hirstiella* sp. (Fig. 1).

After mite collection, at least one parasite had been left on 17 iguanas (range: 1–21 mites per animal; median: 3) (Fig. 2). Quantity of the 0·25% fipronil solution that was applied to the 50 iguanas ranged from 1·5 to 12·5 g/iguana (median: 4 g), corresponding to 0·04–0·13 g/g body weight (median: 0·09 g/g body weight) or approximately to 0·1–0·325 (median: 0·225) mg of the active substance/g body weight.

Forty seven iguanas were available for the first re-examination, 2 days after treatment, including 15/17 animals that had been left with parasites before treatment and 32/33 that had no obvious parasites before treatment. In the former, parasites were found in 10/15 (66·7%) with their numbers ranging from 1 to 84/iguana (median: 2 parasites), whereas in the latter only 2/32 (6·25%) harboured 1 and 8 parasites, respectively (Fig. 2). The parasitic load did not differ from that before the treatment in the whole group ($P = 0{\cdot}176$), in the iguanas that had been left with parasites before treatment ($P = 0{\cdot}087$) and in those with no parasites before treatment ($P = 0{\cdot}180$).

Twenty-nine iguanas were available for the second re-examination, 7 days after treatment, including 11/17 animals that had been left with parasites before treatment and 18/33 that had no visible parasites before treatment. In the former, parasites were found in 2/11 (18·2%) animals that harboured 1 and 2 mites,

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Fig. 1. Adult *Hirstiella* sp. collected from a green iguana.

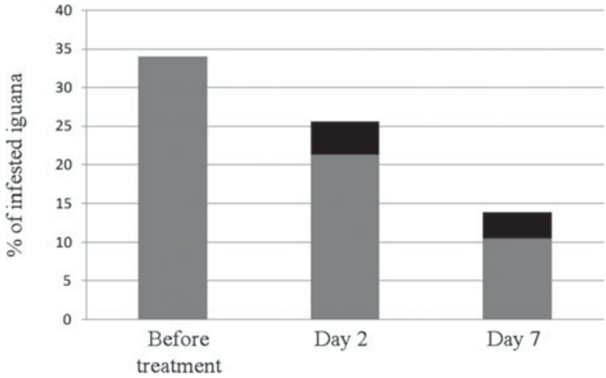

Fig. 2. Percentage of infested iguana before treatment and 2 and 7 days after a single application of 0·25% fipronil solution. Grey bars represent those animals that had been left with at least one mite after mite collection for species identification and black bars represent those iguanas without visible mites before treatment.

respectively, whereas in the latter only 1 mite was seen in 1/18 (5·6%) iguana (Fig. 2). The parasitic burden was lower than before treatment in the whole group ($P = 0{\cdot}006$) and in the iguanas that had been left with parasites before treatment ($P = 0{\cdot}005$) and did not differ significantly for those with no parasites before treatment ($P = 0{\cdot}317$).

No adverse reactions due to the use of fipronil were noticed.

DISCUSSION

Hirstiella sp. (Acari, Pterygosomatidae) is a common ectoparasite of wild and captured lizards (Hoppmann and Barron, 2007). Many species have been recognized, including *H. bakeri, H. boneti, H. diolii, H. jimenezi, H. pelaezi, H. pyriformis, H. stamii* and *H. trombidiiformis*, but only *H. diolli* and *H. stamii* have been isolated from captured or wild-caught green iguanas (Newell and Ryckman, 1964; Mader *et al.* 1986; Walter and Shaw, 2002; Paredes-León and Morales-Malacara, 2009; Corn *et al.* 2011). These zoonotic mites, which appear as brown, orange or red spots depending on the degree of blood engorgement, may cause dermatitis (dark-coloured plaques, erythema, swelling, ulcers), dysecdysis, anaemia and debilitation and they can transmit pathogens (Mader *et al.* 1986; Harvey-Clark, 1995; Walter and Shaw, 2002; Mitchell and Colombini, 2003; Stahl, 2003; Hoppmann and Barron, 2007). In the present study 23/50 (46%) of the iguanas that were examined at least once and 15/29 (51·7%) that were available for all three examinations were found to be infested (data not shown). Considering that at least one infested animal was found in each enclosure and the difficulty in diagnosing mild infestations (Hoppmann and Barron, 2007) it is possible that these figures represent an underestimation of the true rate of infestation. The presence of skin lesions was not of diagnostic help because they were present in less than half of the infested iguanas.

Various antiparasitic remedies have been used in lizards, including immersion in tepid water, whole body application of olive oil, organophosphates, carbamates, pyrethrin or pyrethroid sprays and shampoos and ivermectin injections or sprays (Mader *et al.* 1986; Harvey-Clark, 1995; Mader, 1995; Mitchell and Colombini, 2003; Hoppmann and Barron, 2007; Hellebuyck *et al.* 2012). However, none of these treatment protocols has been thoroughly evaluated in terms of safety and efficacy and most of them appear to be either potentially toxic, at least in some lizard species, or of low efficacy (Mader, 1995; Széll *et al.* 2001).

Fipronil is a phenylpyrazole compound that exerts its ectoparasiticidal activity through the antagonism of gamma-aminobutyric acid-gated chloride channels thus leading to parasite hyperexcitability and death (Gant *et al.* 1998). It has been anecdotally recommended for the treatment of mite infestations in lizards (Hellebuyck *et al.* 2012) and there is a single report of its safety and effectiveness in a parasitized green iguana (Gazyağcı *et al.* 2011). In our study, a single whole-body wipe with 0·25% fipronil solution resulted in a significant decrease of mite load after a 7-day period but not 2 days post-treatment. This delay may be explained by the rather slow onset of action of this compound (McCoy *et al.* 2008). Another explanation could be the movement of moribund mites, that had not been detected before treatment because they were hiding beneath the scales of the iguanas (Delfino *et al.* 2011), towards the skin surface. This may account for the high number of

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mites (up to 84 mites) found in some iguanas on day 2 post-treatment and for the appearance of mites on two iguanas that had been considered parasite-free before treatment. Unfortunately, no mites were collected at this time point to examine their viability.

The reduced parasitic density on the 7th day post-treatment cannot be attributed to natural death or spontaneous detachment of the mites; even when wild-caught lizards were kept in isolation, another Pterygosomatid mite, *Geckobiella texana*, remained attached for approximately 28 days (Goldberg and Bursey, 1991*a*) and in infested collections, like those that have been used in the present study, *Hirstiella* sp. is expected to be able to continue its life cycle on a permanent basis. This is further supported by the long time period that the infested iguanas had been in their current enclosures at the beginning of the trial (up to 9 months) without addition of new animals into their groups. Unsanitary cage conditions, poor husbandry and crowding predispose lizards to heavy infestations (Mader, 1995). However, none of the above was considered problematic at the initial visit and all these factors remained fairly constant during the study period. For this reason the reduced parasitic density cannot be explained by improved management of these animals. Therefore, the reduced number of parasites 7 days post-treatment must be attributed to the therapeutic intervention, even though a placebo group that would have been necessary to definitively prove this claim was not included in the trial.

Environmental treatment including cleansing of the enclosure, change of the substrate, removal of porous substances, and application of parasiticides such as formalin, dichlorvos, pyrethrin, pyrethroids, fipronil or injectable ivermectin diluted in water has been suggested as having a significant role in the control of ectoparasites in captive lizards (Mader *et al*. 1986; Mader, 1995; Mitchell and Colombini, 2003; Hoppmann and Barron, 2007; Pasmans *et al*. 2008; Gazyağcı *et al*. 2011). Although exposure to some of the above ectoparasiticides may be dangerous for the health of the animals (Harvey-Clark, 1995; Adeyemi and Adedeji, 2006), simple cleaning measures are expected to be innocuous and have the potential to increase the efficacy of the treatment. However, such measures were not implemented in the current open-label study because they would interfere with the interpretation of the results. In addition, the duration of the effect of fipronil was not investigated. Anecdotal information has suggested applying fipronil every 1–2 weeks (Hellebuyck *et al*. 2012), a much shorter interval than that usually utilized in dogs and cats. Although frequent treatment would be reasonable since iguanas lack sebaceous glands that act as the reservoir of fipronil in dogs and cats, additional studies are clearly needed to determine the optimal treatment intervals. Furthermore, it remains unknown if fipronil treatment is also effective against other mite species and ticks that may infest captive green iguanas.

As there are no published toxicological studies for fipronil in green iguanas and its use in this species is extra-label, its application should be performed with caution and after owners' informed consent (Hellebuyck *et al*. 2012). Fipronil has been found to be toxic for the fringe-toed lizard, *Acanthodactylus dumerili*, when it was ingested at a dose of 30 μg/g body weight (Peveling and Demba, 2003). In our study a much higher dose was applied (median: 0·09 g of the 0·25% solution/g body weight corresponding to approximately 225 μg of fipronil/g body weight) without observing side-effects. This may be explained by the different route of exposure (oral *vs* epicutaneous), the lack of self-grooming in iguanas and to the overestimation of the actual quantity of fipronil that was applied, since the method we used to calculate the dose does not account for the quantity that was absorbed by the cotton pad and for the quantity that evaporated during wiping. Caution is advised against the use of more concentrated fipronil solutions (i.e. the spot-on formulations) and the commercially available combination products also containing S-methoprene and amitraz.

Based on these results of the efficacy and safety of 0·25% fipronil solution, this treatment may be considered not only for those pet green iguanas with obvious parasites and/or skin lesions, but also for all green iguanas before their importation into non-native countries, in order to avoid the introduction of naïve parasites which may potentially infest wildlife animals (Oliver *et al*. 1993; Ippen and Zwart, 1996; Bram and George, 2000; Fèvre *et al*. 2006; Corn *et al*. 2011).

In summary, a single application of 0·25% fipronil solution, over the body, using cotton pads, resulted in significant reduction of *Hirstiella* sp. numbers in captive green iguanas after a 7-day period without drug-associated side-effects. Due to the extra-label use of fipronil in this animal species, informed owner's consent should be always obtained before use.

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