Parasitol Res (2013) 112:S57–S66 DOI 10.1007/s00436-013-3281-y

Treatment of Naturally *Notoedres cati*-infested Cats with a Combination of Imidacloprid 10 %/Moxidectin 1% Spot-on (Advocate®/Advantage® Multi, Bayer)

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Abstract

Notoedric mange (feline scabies) is a rare, but highly contagious disease of cats and kittens caused by *Notoedres cati (N. cati)*, which can infest other animals and also humans. The study objective was to determine the efficacy and safety of 10 % imidacloprid/1 % moxidectin (Advocate®/Advantage® Multi spot-on for cats) against natural *N. cati* infestation in cats. Sixteen cats were randomly assigned to treatment group or negative control using pre-treatment mite counts. The treatment group received a single spot on treatment of the investigational veterinary product (IVP) according to label instructions. The control group stayed untreated. Five cats from the negative control were treated with the IVP at the end of the study and observed for 28 days to increase the treatment group. Skin scrapings and mite counts were performed 28 days post treatment (p.t.). Notoedric skin lesion assessments with clinical scoring were performed regularly. Five animals had to be removed

prematurely from the study population due to different reasons. The number of viable *N. cati* mites in all treated animals 28 days p.t. was zero compared with 2.8 ± 3.0 in the negative control, being significantly lower for treated cats (p = 0.0019, Wilcoxon test). The resulting efficacy was 100 %. Clinical cure based on skin lesion assessment was achieved 28 days p.t. in 100 % of all treated animals completing 28 study days.

The IVP was well tolerated and applied at the minimal therapeutic dose (10 mg imidacloprid/1 mg moxidectin/kg body weight) a high therapeutic efficacy in curing *N. cati* infestations and feline scabies clinical symptoms was recorded.

Fig. 1 Scanning electron micrograph of an adult *Notoedres cati* mite among hair and skin debris

Introduction

Scabies in general is an intensely pruritic, non-seasonal, contagious dermatopathy caused by rather species-specific, sarcoptiform mites (Schwartzmann 1983; Toomey 1922). However, most scabies mites are capable of causing dermatitis also in other host species. Concerning *Notoedres* spp., more than 20 species have been described, most of which are ectoparasites of tropical bats (Wall and Shearer 2000). Apart from bats, *Notoedres* mites parasitise in several other mammalian species.

Notoedric mange caused by the mite *Notoedres cati* (Astigmata: Sarcoptidae) (Fig. 1) is a cutaneous ectoparasitic disease of mammals. This disease often affects domestic cats (*Felis catus*), wild cats and more rarely it has become a major disease among wild animals in captivity and natural reserves (Valenzula et al. 2000). The condition is highly contagious and primarily occurs by direct contact between animals or by contact with infested bedding or sites recently visited by infested animals. The clinical manifestation is, as in scabies, characterised by intense pruritus, hyperkeratosis, peeling skin and lesions, especially on the face and the ears (Friberg 2006), extending to the neck, limbs and other body areas in the case of massive infestation (Fig. 2). The clinical symptoms are often

Fig. 2 Cat showing severe notoedric mange symptoms

aggravated by secondary bacterial infections, initiated by the excoriations from self-trauma (Fadok 1980), and the disease can even be lethal. Finally the mite possesses also a zoonotic potential and has been diagnosed in humans after close contact with infested animals (Chakrabarti 1986; Fujita et al. 1997).

The disease is diagnosed by direct microscopic identification of the mite, obtained by superficial skin scrapings. A differentiation of this spherical mite of approximately 200–350 µm in size from *Sarcoptes scabiei* is based especially on the criteria of medium-length, unjointed, sucker-bearing stalks on the legs, a greater number of body striations and a dorsal anus. *N. cati* mites excavate burrows up to the *stratum germinativum*, where eggs are deposited by the females. Within approximately three weeks the developmental cycle is completed (Kutzer 2000).

Feline notoedric mange is rather uncommon to rare in most western European countries, but in regions of Italy, Switzerland, Spain, Slovenia and Croatia it is still occurring, either endemic or present in an epizootic state (Leone 2007). It has also been reported from regions of the former country Czechoslovakia (Nesvadba 1967), Poland (Gorski et al. 2004), Japan (Fukase et al. 1991), and Brazil (Alves Ferreira et al. 2010; Torres Ferreira et al. 2009) with sometimes high numbers of prevalences (20.9 % in northeastern Brazil (Torres Ferreira et al. 2009)). Even though often occurring more in focal distributions in a restricted number of countries, the zoonotic potential, the possible severity of the clinical disease in animals and the easy transmission between animals emphasises the necessity of an effective, reliable and easily applied treatment for cats.

The treatment of notoedric mange aims to eradicate the mite on the animal (Angarano and Parish 1994). There are few, if any, treatments currently licensed to treat notoedric mange in cats in Europe. There are some reports in the literature on the off-label use of treatment schemes with weekly total body lime sulfur dips (Angarano and Parish 1994), where animals often had to wear an "Elizabethan collar" until dry, or on oral respectively subcutaneous application of doramectin (Delucchi and Castro 2000; Ferrero et al. 1996) or ivermectin (Cozette 1996; Oliva and Baldi 1988; Fukase et al. 1991). More recently single treatments were done with other avermectins, e.g. selamectin (Itoh et al. 2004; Leone et al. 2003), which were reported to be successful.

The combination of 10 % imidacloprid/1 % moxidectin (Advocate®/Advantage® Multi, Bayer) is a registered feline product containing moxidectin as a very potential macrocyclic lactone. The cat product has been, among others, approved for the treatment of *Otodectes cynotis* and is expected to be as effective against *N. cati*. Moreover, the dog product Advocate® spot on for dogs containing 10 % imidacloprid/2.5 % moxidectin is approved for the treatment of *Otodectes cynotis* and *Sarcoptes scabiei* infestation (Fourie et al. 2003; Krieger et al. 2005).

The objective of this study was to determine the efficacy of a single topical application of Advocate®/Advantage® Multi at the recommended dosage (imidacloprid 10 mg/kg body weight and moxidectin 1 mg/kg body weight) in the treatment of cats naturally infested with *N. cati*.

Materials and methods

Study design

The study was conducted as a negative controlled, randomised, partially blinded, unicentre study in compliance with local regulatory requirements of Hungary, Directive 2009/09/EC, Directive 2004/28/EC amending 2001/82/EC and applying VICH Guideline on Good Clinical Practice (GCP), the guideline on statistical principles of veterinary clinical trials (EMEA/CVMP/816/00-Final) and the guideline on demonstration of efficacy of ectoparasites (The Rules Governing Medicinal Products in the European Union, Volume VII (7AE17a)). This study was authorised by the Hungarian regulatory authorities prior to implementation.

Study animals, study procedures and efficacy study

Study animals

The sixteen cats included into the study were client-owned European short-hair cats of both sexes, between 4 and 60 months of age, apparently healthy with the exception of confirmed notoedric mange infestation, based on clinical signs and positive results for viable *N. cati* mites on skin scrapings.

Study population

The study population consisted of originally eight negative control animals and eight animals treated with the investigational veterinary product (IVP) (Advocate®/Advantage® Multi – 10 % imidacloprid/1 % moxidectin). On Day 28 (study completion day) five animals of the negative control group possessing viable mite counts were treated with the IVP two days later (Day 30) and evaluated analogous to the eight previously IVP-treated cats until day 58 in order to increase the number of IVP-treated animals. From these five animals results of sample collections were evaluated within the IVP group using mite counts of Day 28 as baseline counts and mite counts of Day 58 as completion counts. An analysis of the primary and secondary efficacy criteria was performed in these five additionally IVP-treated animals in the same way as described for the original eight IVP-treated animals. Summarising, the group of animals receiving the IVP consisted of 13 cats and the negative control group consisted of eight cats. On Day –1 cats were randomly allocated to treatment or negative control group by pre-treatment mite counts determined on Day –1 in descending order. No randomisation was used for the additional five cats enrolled and treated with the IVP on Day 30.

Accommodation

Cats were acclimatised to the study site for at least four weeks prior to treatment. During this time, cats were kept in two separate heated and air conditioned rooms.

In one of these rooms individual cages for eight cats of the IVP treatment group were placed. After randomisation and treatment (Day 0) and from Day 30 onwards (treatment day for the five additional cats), the cats were kept in individual cages for three days to avoid cross contamination. From Day 4 respectively Day 34 onwards, IVP treated cats were housed together in one room until the end of the study. Untreated control cats were also group-housed in one room, strictly separated from the IVP-treated cats. Cats were provided with commercial dry and canned food and water *ad libitum*.

Study procedures

Clinical observations

Physical examination including body weighing was performed at baseline (Day –1) prior to study inclusion, on Day 29 for the five additional cats and whenever daily general health observations demanded a further physical examination based on clinical signs. A final physical examination was performed at study completion or at premature study removal of an animal. Assessment of notoedric skin lesions and observation of the application site was performed on Days –1, 0, 1, 7, 14, 21 and 28, as well as on Days 30, 31, 37, 44, 51 and 58 for the five additional cats or any other day of study completion, if earlier removal from the study was required. For all enrolled cats general health observations were performed on a daily basis during the whole study duration by observing appetite, demeanour, urination and defaecation.

Skin lesion assessment

For the assessment of notoedric skin lesions, severity and extent of the lesions on each cat were assessed before and after treatment on the days given above along with the following scores.

Score for severity of notoedric lesions:

0 = no skin lesions, no alopecia, no scratching

1 = mild skin lesions, mild alopecia, occasionally scratching

2 = moderate skin lesion, moderate alopecia, intensive scratching, scratching wounds,

3 = severe skin lesion, severe alopecia, thick/crusty and scabby appearance of the skin, intensive scratching, scratching wounds

Score for extension of notoedric lesions:
0 = no skin lesions
1 = <50 % of the body skin surface affected
2 = ≥50 % of the body skin surface affected

Both scores were added up and expressed as a *Notoedres*-induced skin lesions score (NISLS) with sum values between 0 and 5. A final clinical assessment of *Notoedres*-induced skin lesions was performed based on NISLS on Day 28 (Day 58 for the five additional cats) or any other earlier day of removal.

The following outcomes were possible:
– clinical cure → NISLS reduced to zero
– clinical improvement → NISLS <50 % of NISLS on Day 0 (Day 30)
– clinical failure → NISLS ≥50 % of NISLS on Day 0 (Day 30)

Skin scrapings

Skin scrapings (about 1 cm² each) from three different sites suspected of being infested and subsequent mite counts were performed on Days –1 and 28, and on Days 28 and 58 (for the five further cats), respectively, or at premature study removal. For laboratory examination these three samples from one cat were evaluated as one sample per study day.
For the skin scrapings, hair of the cats was removed over the sampling areas, and skin scrapings were made with a 2-cm blade so that capillary oozing occurred. Immediately afterwards the removed material (i.e. hair and skin scrapings) was sowed in a Petri plate. The plate was kept at 30 °C for 15 minutes, before counting of the viable mites started. After counting of all viable mites and prior to the counting of dead mites respectively mite eggs, complete material was sowed in 10 % potassium hydroxide (KOH). In general, mites (viable and dead) were counted ensuring that the complete material collected was observed.
The quantitative examination was carried out with a stereoscope at 40x enlargement. The entire procedure was completed on site (Day 0 and Day 28) or in a designed laboratory (Day 58) by blinded laboratory personnel.

Safety criteria

Safety of the IVP was assessed by documenting all adverse events during the whole study duration.

Treatment application

Cats of the treatment group received Advocate®/ Advantage® Multi spot-on for cats according to body weight using the minimum therapeutic dose of 0.1 ml/kg body weight on Day 0 (original eight cats) or Day 30 (additional five cats).
In order to keep partial blinding, separate personnel for clinical and laboratory examinations were used. No deblinding was done for the first study period from Day 0 to Day 28. On Day 28, blinding was broken to continue with the five control animals possessing positive mite counts for further 28 days (Day 30 to Day 58).

Statistical analysis

Data were statistically examined using SAS® statistical analysis software of SAS Institute Inc., Cary, NC, USA (version 9.2). Summary statistics including arithmetic and geometric means, minimum, maximum and median were provided for all counts, percentages or continuous parameters of interest. Primary efficacy objective was to compare the moxidectin-treated group with the negative group with respect to parasitological cure (therapeutic efficacy), i.e. complete elimination of viable mites (of all three life stages) 28 days post treatment (p.t.). Therefore, five cats which were removed prematurely from the study were excluded from the analysis. Secondary efficacy objective was to compare the reduction of severity and/or extension of skin lesions caused by notoedric mite infestation (clinical cure).

The criterion for efficacy was the difference between the arithmetic means of the treatment group and the untreated group 28 days p.t.

Percent efficacy was calculated using arithmetic means according to the formula:

$$\%\ \text{efficacy} = \frac{100 \times (\text{mean viable count [negative control]} - \text{mean viable count [IVP-treated group]})}{\text{mean viable count [negative control]}}$$

The following statistical methods were used for each evaluation criterion:

Primary efficacy criterion (number of viable mites (larvae, nymphs or adults) in skin scrapings) at Day 28 or Day 58, with one-sided test of superiority as statistical approach, was calculated with Wilcoxon-Mann-Whitney test for the treated group vs. the untreated group.

The secondary efficacy criterion (improvement of skin lesions) was calculated separately for each post-baseline period in percent animals with improvement, worsening and no change. Treatment group was compared using the Mantel-Haenszel chi-square statistic.

For the safety criteria (adverse events), treatment group comparison using Fisher's exact test calculating the number and percentage of animals with at least one adverse event was calculated. The level of significance was set at p = 0.05, all tests performed were two-sided.

Results

Sixteen cats, which fulfilled the inclusion criteria, were enrolled in the study. Eight cats were treated with the IVP on Day 0, and five additional cats from the negative control group with viable mite counts on Day 28 were treated with the IVP on Day 30, resulting in 13 IVP-treated cats in total, while eight cats were grouped in the negative control group on Day 0. Five out of these 16 cats (two in the IVP-treated group and three in the negative control) terminated the study earlier than Day 28 due to severe notoedric mange (four animals) or other reasons (one animal) (see also Safety assessment). Analyses of the efficacy criteria were calculated for the Per Protocol (PP) population, consisting of all animals which completed the 28 study days after treatment, i.e. six cats treated on Day 0 plus five cats treated on Day 30 and five negative controls.

Efficacy parameters

Primary efficacy criterion – viable mite counts

The primary efficacy criterion in this study was the viable mite count post treatment. The individual mite counts of the study animals are listed in Table 1.

The number of viable mites was reduced by 100% in all animals treated with the IVP which completed 28 study days. For detailed viable mite count data and percent efficacies in the different study groups see Table 2.

Secondary efficacy criterion – improvement of skin lesions

In the PP population, all animals were cured in the IVP group (100.0%) 28 days p.t. compared to one animal (20.0%) in the negative control group (p = 0.009; Mantel-Haenszel test). Within the same population, first clinical improvement (NISLS < 50% of NISLS on Day 0/Day 30) was observed on Day 7 within the IVP group (one animal), and first clinical cure (NISLS reduced to zero) was observed on Day 14 (one animal in the IVP group). For detailed data see Table 3.

Safety assessment

Safety was assessed based on all study animals receiving at least one dose of the IVP and all animals from the negative control group. Adverse events which were documented for animals of the control group which received the IVP on Day 30 were evaluated within the IVP-treated group. Therefore, safety criteria were evaluated for 13 animals treated with the IVP compared to eight animals of the negative control group.

No local reactions at the application site were reported. In total, six adverse events were reported

Table 1 Viable mite count data of the study animals

	Animal no.	Day –1	Day 28		Day 58
IVP-treated group	1	2	0		
	2	1	0		
	3	1	excluded [a]		
	4	44	0		
	5	1	0		
	6	2	0		
	7	1	excluded [b]		
	8	2	0		
AM ± SD		8.7 ± 17.3	0.0 ± 0.0		
Negative control	9	2	2	Additionally IVP-treated on Day 30	0
	10	1	1		0
	11	1	1		0
	12	7	excluded [c]		
	13	13	8		0
	14	1	excluded [d]		
	15	3	2		0
	16	4	excluded [e]		
AM ± SD		4.0 ± 5.1	2.8 ± 3.0	AM ± SD	0.0 ± 0.0
				AM ± SD (IVP group extended by 5 additional cats)	0.0 ± 0.0

AM = arithmetic mean; **SD** = standard deviation; **IVP** = investigational veterinary product
[a] excluded Day 4 (death); [b] excluded Day 25 (death); [c] excluded Day 0 (death); [d] excluded Day 7 (removal); [e] excluded Day 2 (removal)
In the analysis the five cats which were removed prematurely from the study are not included.

Table 2 Overview on mean viable mite counts (arithmetic mean) and percent efficacies in the different study populations 28 days post treatment

Evaluation population	Mean viable mite count (IVP-treated group)	Mean viable mite count (negative control)	Percent efficacy
Initial population (6 IVP, 5 neg. controls)	0.0	2.8	100.0 %
Per Protocol (PP) population (inclusive 5 additional cats; 11 IVP, 5 neg. controls)	0.0	2.8	100.0 %

Table 3 Improvement in skin lesions based on NISLS

	IVP-treated group (n = 11)	Negative control (n = 5)
1 day p.t.		
Clinical failure	11 (100.0 %)	5 (100.0 %)
Total	11 (100.0 %)	5 (100.0 %)
7 days p.t.		
Clinical improvement	1 (9.1 %)	0 (0.0 %)
Clinical failure	10 (90.9 %)	5 (100.0 %)
Total	11 (100.0 %)	5 (100.0 %)
14 days p.t.		
Clinical cure	1 (9.1 %)	0 (0.0 %)
Clinical improvement	1 (9.1 %)	0 (0.0 %)
Clinical failure	9 (81.8 %)	5 (100.0 %)
Total	11 (100.0 %)	5 (100.0 %)
21 days p.t.		
Clinical cure	7 (63.6 %)	0 (0.0 %)
Clinical failure	4 (36.4 %)	5 (100.0 %)
Total	11 (100.0 %)	5 (100.0 %)
28 days p.t.		
Clinical cure	11 (100.0 %)	1 (20.0 %)
Clinical failure	0 (0.0 %)	4 (80.0 %)
Total	11 (100.0 %)	5 (100.0 %)

NISLS: *Notoedres*-induced skin lesions score; **p.t.**: post treatment

for six animals – three among three animals (23.1 %) in the IVP group and three among three animals (37.5 %) in the negative control group. This difference was not statistically significant ($p > 0.6$; Fisher's exact test). Three animals died during the study, stating a serious adverse event – two from the IVP-treated group and one from the negative control group. In detail, one cat of the IVP-treated group died on Day 4 due to very severe *Notoedres* infestation (clinical symptoms of hypothermia, apathy and anorexia). A second cat of the IVP-treated group was found dead on Day 25. For both cats, results of the pathology examination excluded relation with the IVP treatment. The third adverse event within the IVP group was observed in one cat 24 days p.t. presenting with hypothermia, apathy and anorexia. This cat recovered completely. None of these adverse events were suspected to be related to the study medication. Within the negative control group one cat died on Day 0 due to a very severe *Notoedres* infestation with clinical symptoms of hypothermia, diarrhoea and dyspnoe. Two further cats showed adverse events in form of alopecia, apathy and pyodermatitis, respectively hypothermia in a very severe state, so that these animals were removed from the study for animal welfare reasons on Day 2 and Day 7, respectively.

Discussion

N. cati, the feline sarcoptic mite, can infest cats of all ages. In severe cases the originally infested areas of head, ears and neck may change to a generalised infestation and might be lethal, if not treated. This could also be observed in the underlying study, where the general condition of severely infested cats deteriorated so much that removal from the study was necessary or even death was observed. This possible severe disease outcome and the zoonotic potential after intensive contact, causing papulovesicular lesions accompanied by intense pruritus in humans (Chakrabarti 1986), emphasise the necessity of an effective treatment in cats. Previous treatment schemes using ivermectin (e.g. Cozette 1996; Oliva and Baldi 1988; Fukase et al. 1991), have been replaced by the off-label use of other avermectins (e.g. selamectin). However, in many European countries, no product is licensed, and especially concerning the use of ivermectin, sudden death has been reported in kittens (Merck Veterinary Manual 1998).

The product used in the study is a registered product for cats. Its efficacy against *N. cati* infestation was proven based on the superiority of the IVP in treatment of cats naturally infested with *N. cati* mites compared to an untreated control group. In order to increase the sample size and for the reason of animal welfare, five of eight untreated control cats which still had viable mite counts on Day 28 were additionally treated with the IVP on Day 30. Regarding the primary efficacy criterion (i.e. viable mite counts p.t.), all IVP-treated cats had negative viable mite counts 28 days after treatment resulting in 100 % efficacy against *N. cati* infestation.

Concerning the safety evaluation, it can be concluded that the IVP treatment was well tolerated in all cats and no drug-related adverse events occurred.

However, the data also showed that the treatment with 10 % imidacloprid/1 % moxidectin might not prevent a serious lethal outcome of a very severe notoedric mite infestation; it seems to take more than four days to eradicate the mites (positive mite count of one IVP-treated cat upon death on Day 4). Secondary lesions may even last longer, which emphasises the need to start treatment as early as possible.

Concerning the efficacy of a 10 % imidacloprid/1 % moxidectin treatment, convincing results were achieved in mite count data 28 days after treatment as well as in clinical cure, showing clinical cure in 100 % of all animals already 28 days after treatment, compared to 80 % clinical failure in the negative control group at the same time, thus offering an effective, safe alternative of notoedric mange therapy.

Conclusion

Superiority of the IVP group compared to the untreated control group was shown. Considering only animals which were observed for the planned duration of 28 days (PP population), zero mite counts in the IVP group and 2.8 (± 3.0) (arithmetic means ± standard deviation) in the untreated control group (Mann-Whitney statistic = 1; p = 0.0019, Wilcoxon test, one-sided) were recorded, resulting in a reduction of viable mites by 100 %.

Clinical scoring documenting clinical cure was first observed on Day 14 (one animal). Twenty-eight days after treatment, all IVP-treated cats (100 %) were clinically cured.

Ethical standards

The study was conducted as a negative controlled, randomised, partially blinded, unicentre study in compliance with local regulatory requirements of Hungary and in accordance with Good Clinical Practice (GCP) standards based on VICH GL9, EMEA/CVMP/816/00-Final, Directive 2009/09/EC, Directive 2004/28/EC amending 2001/82/EC and The Rules Governing Medicinal Products in the European Union, Volume VII (7AE17a). It was authorised by the Hungarian regulatory authorities prior to implementation.

Conflict of interest

The study was funded by Bayer Animal Health GmbH, Germany. Gabriele Petry is an employee of Bayer Animal Health. Klaus Hellmann and Dejan Cvejic are employees of KLIFOVET AG, which was contracted to manage and monitor the study by Bayer Animal Health. Balazs Capari is an employee of Kapriol Bt, which was contracted to carry out the in-life phase of the study. Friederike Krämer is an employee of the Free University Berlin and was supporting in the documentation of the study data.

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