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ABSTRACT

Scabies is a major and potentially growing public health problem worldwide with an unmet need for acaricidal agents with greater efficacy and improved pharmacological properties for its treatment. The objective of the present study was to assess the efficacy and describe the pharmacokinetics profile of a novel acaricide, afoxolaner (AFX), in a relevant experimental porcine model. Twelve pigs were experimentally infested and treated either with 2.5 mg/kg single dose oral AFX (n = 4), 0.2 mg/kg two-doses 8 days apart oral ivermectin (IVM, n = 4), or no treatment against scabies (n = 4). Response to treatment was assessed by reduction of mite counts in skin scrapings as well as clinical and pruritus scores over time. Plasma and skin pharmacokinetic profiles for both AFX and IVM were evaluated. AFX efficacy was 100% at days 8 and 14 post-treatment and remained unchanged until the study-end (day 45). IVM efficacy was 86% and 97% on days 8 and 14, respectively, with a few mites recovered at study-end. Clinical and pruritus scores decreased in both treated groups and remained constant in the control group. Plasma mean residence times (MRT) were 7.1±2.4 and 1.1±0.2 days for AFX and IVM, respectively. Skin MRT values were 16.2±16.9 and 2.7±0.5 days for AFX and IVM, respectively. Overall, a single oral dose of AFX was efficacious for the treatment of scabies in experimentally infested pigs and showed a remarkably long MRT in plasma and notably in the skin.

KEYWORDS *scabies, isoxazoline, afoxolaner, ivermectin, acaricide agent, porcine model*

Introduction

Scabies is an epidermal infestation caused by the mite *Sarcoptes scabiei* in humans (1). It is increasingly recognised as a large and potentially growing public health problem worldwide with a significant burden (2, 3). Prevalence is estimated to be around 100-130 million cases/year (4–7). Scabies is often perceived erroneously as causing a simple itch but over the past decade, studies emphasised its important morbidity, mostly caused by secondary bacterial infections (4). Opportunistic pathogens like *Streptococcus pyogenes* (group A streptococcus) and *Staphylococcus aureus* are commonly associated with human scabies. Especially in tropical and sub-tropical countries, these can lead to invasive bacterial infection and post-infection complications, such as post-streptococcal glomerulonephritis, acute rheumatic fever and rheumatic heart disease (8–10). The psycho-social and economic impact caused by scabies through school absenteeism or loss of work productivity due to pruritus and lack of sleep is considerable and leads to an exacerbation of poverty in affected populations (5, 11).

The currently most accepted medical intervention to treat scabies consists of multiple treatments with either one of four topical agents (5% permethrin, 10-25% benzyl benzoate, piperonyl butoxide-synergised pyrethrins, or 0.5% malathion) and/or oral ivermectin (IVM), the only systemic drug approved in some countries (12, 13). The major limitations of these anti-parasitic therapies are absence of 100% cure in the target population, poor compliance with topical application and repeated treatment schedules, limited activity against *Sarcoptes* eggs, and insufficient half-life to cover the whole 14-day life cycle of the mite (14). The risk of emergence of mite resistance is of growing concern (15, 16), especially with the increasing use of permethrin, esdepallethrin, and IVM for scabies and for other skin diseases in humans (e.g. head lice, rosacea), but also in animal parasitic diseases (17). Thus, there is an unmet

need for new acaricide molecules with greater efficacy and improved pharmacological profiles to overcome scabies and its morbidity (3).

New hopes to find an adequate treatment for human scabies are coming from the translation of molecules from the veterinary field such as moxidectin (14, 18). More recently, research has advanced that could give new perspectives for human scabies treatment. The veterinary therapeutic arsenal has been expanded with various effective ectoparasiticides (19). Afoxolaner (AFX), a member of the novel isoxazolines family, is administered orally and shows a great efficacy against fleas, ticks and mites in dogs (20, 21). AFX inhibits parasite γ-aminobutyric acid (GABA) and glutamate-gated chloride channels. Notably, AFX binds to a site distinct from the binding site of other acaricides-insecticides, among them the macrocyclic lactones (e.g. IVM) (22). AFX has advantageous pharmacokinetics and toxicity profiles with long-lasting activity (23).

We recently optimised the experimental porcine model developed by Mounsey et al. in 2010 (24) and demonstrated its usefulness for preclinical assessment of drug candidates for the treatment of scabies (18). Here, we assessed drug efficacies and pharmacokinetic profiles of a single oral dose of AFX compared with two oral doses of IVM in experimentally-infested pigs.

Results

Study design. Twelve 3-week old pigs were randomly assigned into 3 groups in January 2015 and were infested with *Sarcoptes scabiei* var. *suis* two weeks after their arrival (Fig. 1). Dexamethasone (0.2 mg/kg) was used daily during the entire study to promote initial infestation, and to increase intensity and duration of the infestation. Pigs were blinded-treated nine weeks post-infection, at day 0 (D0), in end-March 2015. The first group of pigs (n = 4) received oral AFX at the dosage of 2.5 mg/kg given once at D0. The second group (n = 4)

104 received oral IVM at the dosage of 0.2 mg/kg twice (on D0 and D8). The third group (*n* = 4)
105 was a control group and did not receive any treatment against mites. One pig (in the IVM-
106 treated group) died during the study because of a congenital malformation of the digestive
107 tract. The response to treatments was assessed by the reduction of live-mite counts in skin
108 scrapings and by the reduction of clinical and pruritus scores at different time points. The
109 endpoint was the complete absence of live mites at D14. Plasma and skin pharmacokinetic
110 profiles for both drugs were evaluated.
111
112 **Pigs assessment at baseline (D0).** At D0, pigs were 12 weeks old and their weights ranged
113 from 10.7 to 19.5 kg (mean ± SD, 15.2 ± 2.7 kg). At D0, all 12 pigs were infested with
114 scabies. No statistical difference was found between the three groups in terms of mite count in
115 skin scrapings (P = 0.944), clinical (P = 0.751) and pruritus scores (P = 0.893). Mite counts,
116 clinical and pruritus scores at baseline are shown in Figures 2A, 3 and 4, and Supplementary
117 Tables 1 and 2.
118
119 **Parasitological assessment of drug efficacy.** On D14, the endpoint, all AFX-treated pigs and
120 one out of three IVM-treated pigs were mite-free. The progression of the parasite burden in
121 the AFX- or IVM-treated and control pigs after treatments from baseline (D0) to study-end
122 (D45) is presented in Figure 2A. The partition of the parasite population between immature
123 and adult live stages detected in the scrapings is presented in Figure 2B. The percentage
124 efficacy of the treatment and the percentage reduction in the number of live mites in skin
125 scrapings over time are shown in Supplementary Table 1. On D14 the drug efficacy was
126 100% in the AFX-treated pigs compared to 95.4% (range 87.7−98.6%) in IVM-treated pigs
127 and the percentage reduction of the mite count was 100% in AFX-treated pigs compared to
128 94.7% (range 82.7−100%) in the IVM-treated pigs. From D8 post-treatment onwards to

study-end, not a single mite was detected in the scrapings of the AFX-treated pigs. In contrast, among the IVM-treated pigs, one pig was still infested with live mites at the end of the study. In all animals of the untreated control group the mite count remained constant until the end of the study. After treatment, the number of mites over time in both treated groups was statistically different from the count in the control group (P = 0.0001), and statistically different from each other (P = 0.045).

After treatment, the large majority of eggs retrieved from scrapings of all three cohorts hatched in the incubator (37°C, Relative Humidity 90%). At baseline (prior to treatment), twelve eggs from each cohort were incubated and all, except one in the control group and one in the AFX-treated group, hatched. At D2 post-treatment, hatching was observed for ten eggs out of ten, seven eggs out of eight and eight eggs out of eight, from the AFX, IVM-treated and control groups, respectively. At D4 post-treatment, hatching was observed for one egg out of one, three eggs out of three and twelve eggs out of twelve, from the AFX, IVM-treated and control groups, respectively. At D8 and D14 post-treatment, no eggs were found in scrapings from the AFX-treated and the IVM-treated animals. All the eggs from the control group hatched.

Clinical assessment of drug efficacy. The mean clinical scores over time in the three groups are presented in Figure 3 and Supplementary Table 2. Clinical lesions disappeared completely in all AFX-treated pigs whereas two out of three IVM-treated pigs still had lesions at the end of the study, albeit 93% of improvement (Table S2). After treatment, the mean clinical scores of both treated groups were statistically different from those of the control group (P = 0.0001) and statistically different from each other (P = 0.023). No clinical signs of drug intolerance were noticed during the 50-day period of observation after administration of the two drugs.

153 Side effects due to steroid long-term administration were mild (increase of the appetite and
154 hairiness).

155 Straight after treatment (D2), an increase of pruritus was observed in both treated
156 groups, followed by a decrease of the pruritus score (Fig. 4). A second peak was observed in
157 the IVM-treated group at D8, just after the second administration of IVM. After treatment, the
158 mean pruritus scores of both treated groups were statistically different from those of the
159 control group (P = 0.0001) but were not statistically different from each other (P = 0.566).
160
161 **Plasma and skin drug levels in AFX and IVM treated-groups.** Pharmacokinetics after a
162 single oral dose of AFX and double oral doses of IVM were determined in plasma, and in skin
163 (Table 1). In the plasma, the highest concentrations of both drugs were detectable within 2 h
164 after oral administration and declined in a linear manner over time (Fig. 5). There was a high
165 individual variability of AFX plasma concentration the first days post-administration,
166 certainly due to drug absorption variability. Nevertheless, AFX was detectable until the study-
167 end (D50) whereas IVM was barely detectable 5 (D5) or 6 (D14) days post first or second
168 oral administration, respectively. Less than half a day post-administration, the AFX plasma
169 maximum concentration of drug [C_{max}] was 4.4-fold higher than that of IVM. The AFX area
170 under the concentration-time curve [AUC] was ~32-fold larger than IVM. AFX exhibited a
171 long mean residence time [MRT] in plasma, with a mean MRT value of 7.1 ± 2.4 days. For
172 IVM the value was 1.1 ± 0.2 days.

173 Both drugs reached the skin compartment on D1 post-administration. There were good
174 correlations between plasma and skin concentrations for the two drugs (r=0.855 and r=0.804
175 for AFX and IVM respectively). Both drugs accumulated at high concentrations in the skin
176 (Fig. 6) and the C_{max} values were 9.7-fold and 1.6-fold higher than those measured in plasma
177 for AFX and IVM, respectively. Consequently, for both drugs, the skin exposure based on

178 AUC and C_{max} values was greater than the corresponding parameters in plasma, indicating
179 marked distribution into the target tissue (i.e. the skin), especially for AFX. Calculation of
180 tissue/plasma AUC ratios indicated that exposure relative to plasma was high for AFX in skin
181 (ratio: 9.2) compared to IVM (ratio: 4.8). Interestingly, AFX showed a strong persistence in
182 the skin with a mean MRT value of 16.2 ± 16.9 days, much higher than ivermectin, with a
183 MRT at 2.7 ± 0.5 days.
184

185 **Discussion**

186 Scabies mites cannot be maintained or propagated *in vitro* away from their host for
187 more than a few days (25). Therefore, the establishment of a surrogate experimental porcine
188 scabies model (24) provides real potential to conduct translational preclinical and
189 pharmacokinetic studies with a new drug candidate. Although costly, pigs represent the ideal
190 host to model human scabies. Porcine sarcoptic mange features are very similar to scabies in
191 humans (26) and pigs have unsurpassed similarities in skin anatomy, physiology and
192 immunology (27). We recently showed that this experimental porcine model was useful for
193 preclinical assessment of drug candidates (18). Our first preclinical study using this model
194 showing that moxidectin was more efficient than the regular IVM-based treatment has served
195 as baseline for a rational strategy to conduct larger high-powered efficacy studies in humans
196 (18). Even though the number of pigs involved in these pilot studies is limited, the cohort size
197 can be considered as a representative sample for proof-of-concept.

198 In this preclinical study, we demonstrated a better efficacy of a single dose
199 (2.5 mg/kg) of AFX over two doses of IVM (0.2 mg/kg) against a scabies infestation in pigs.
200 AFX achieved a complete and fast parasitological and clinical cure. This was not the case in
201 the IVM-treated group, where two pigs out of three could not be cured by the 14-day post-
202 treatment end-point. In fact, one pig out of three was still infested with live mites at the end of

the study. Laboratory or field studies looking at the efficacy of AFX (28), or other isoxazolines such as fluralaner or sarolaner (29–31) against *S. scabiei* infestation in dogs have been recently completed. In naturally infested dogs (n = 10), Beugnet et al. showed that oral AFX dosed at 2.5 mg/kg achieved also a 100% efficacy based on mite counts at D28 and D56 post-treatment. Clinical scores declined to 80% in the AFX-treated dogs *vs*. 50% in the control group (28). The general observations of the present study were strictly comparable to our first trial (18) and successfully replicated previous Australian reports (24, 32, 33) with regards to the development of disease, strengthening the use of this robust experimental model for drug development.

The population structure of the mites recovered from the skin scrapings from the pigs differed in the different treatment groups (Fig. 2B). Before the administration of treatments, we observed a homogenous partition of the immature *vs*. adult stages in all animals. This population structure remained constant in untreated pigs. Four days after the first IVM oral administration, there was a dramatic decrease of the number of adult mites, presumably killed by the drug and an increased proportion number of immature stages which corresponded to newly hatched mites from the eggs at the time of treatment. This can be explained by the absence of ovicidal activity of IVM and in accordance with the life cycle of *S. scabiei*, as previously proposed by Currie and McCarthy (34). In contrast, in AFX treated-pigs, a rapid and definitive decrease of mite count was observed. To date, there are no studies investigating the ovicidal activity of AFX. While our sampling protocol did not aim for isolating large numbers of eggs, the dataset nevertheless indicates that both drugs have limited ovicidal activity.

Drug uptake into the skin and stability under its physiological conditions are further factors that may contribute to the difference in efficacy of the two treatments. As previously reported (18), we observed a relatively short duration of the effectiveness of IVM, matching

with the presence of the drug measured in plasma and skin compartments. Hence, newly hatched mites may not have been killed, confirming the importance of the second administration of IVM. Accordingly, for maximum efficacy, the second IVM dose should be given between days 7 and 10, as soon as all eggs have hatched but before newly hatched mites have time to mate and produce a new generation of eggs. To optimize the interval of consecutive IVM treatments additional studies about egg survival and hatchability in presence of IVM are required.

The pharmacokinetics profile of AFX orally administered at a dose of 2.5 mg/kg exhibited a long elimination profile, with drug present for approximately 7 days in plasma and 16 days in skin, i.e. ~7-fold longer than that of IVM. Previous investigations in dogs with AFX orally administered at the same dosage demonstrated similar results. AFX was rapidly absorbed (around 2−4 h), with a high initial plasma peak (C_{max} 1,655 ± 332 ng/mL). The terminal plasma half-life was remarkably long at up to 15 days (15.5 ± 7.8 d) (23), consistent with the lipophilic and unionized properties of the small AFX molecule, and with its high affinity to plasma proteins shown in dogs (> 99%) (23). Our study is the first one to address AFX pharmacology parameters in the skin and further studies are needed to investigate in which layer of the skin AFX is accumulating.

Data in pigs can provide interesting insight for projection and comparison with human pharmacokinetics (35). We found here that AFX and IVM both potentially have no ovicidal activity. In contrast to IVM it seems that due to the long plasma and the skin persistence of AFX at an effective dose, newly hatched mites are killed and the parasitic life cycle is completely interrupted. Indeed, AFX could be given as a single dose, thereby conferring a major advantage of assuring better treatment adherence, a determinant factor for drug efficacy in resource-poor communities where scabies is endemic.

AFX is considered as a safe drug. So far, no adverse clinical signs have been observed in previous studies in dogs, even after six times oral administration with up to 5 times the maximum exposure dose (22, 36). Mammalian chloride channels of rat brain cells showed no significant response to isoxazolines in binding assays (37), indicating that the binding site (NCA−II) of these channels to AFX is either not present or of low sensitivity (38).

In summary, AFX demonstrated high efficacy in treating scabies in this preclinical study in pigs and combined with an interesting pharmacokinetic profile, it guarantees a long-lasting activity assuring a convenient dosing as a unique oral administration.

Materials and Methods

Experimentally infested pigs. All procedures were approved by our Institutional Animal Care and Use Committee, *Comité d'éthique pour l'expérimentation animale*, Anses/EnvA/Université Paris-Est Créteil, France (approval no: 02515.03). The animals were handled in accordance with guidelines established by the French and European regulations for care and use of animals for scientific purposes (Articles R.214-87 to 214-137 du Code Rural et de la Pêche Maritime, Décret 2013-118, and European Directive 2010/63/UE). The ARRIVE guidelines were used to design and report the study (39). Procedures were in accordance with the method described by Mounsey et al. (24) and optimized by Bernigaud et al. (18).

Twelve three-week old *Sus scrofa domesticus* « Large white » breed, female from the same pig farm (Gambais, France) were housed at the Centre de Recherche Biomédicale in the Ecole nationale vétérinaire d'Alfort, France (http://www.vet-alfort.fr/web/fr/103-centre-de-recherche-biomedicale.php). The mean weight (± SD) at the arrival was 7.15 kg (± 0.64). Pigs were healthy and initially free of sarcoptic mange and they had never received any antiparasitic treatments. At their arrival, drawing lots randomly allocated pigs into three

277 groups (n = 4). To reduce stress and to acclimate, pigs were housed two weeks before starting
278 the study in small groups of the same gender. Pigs where placed in similar experimental
279 climate-controlled units by group (temperature of 21°C ± 2°, humidity 50% ± 10%, surface of
280 12 m^2). Environmental enrichment included wood shavings on concrete floors that were
281 cleaned once daily. Feed was given once a day and tap water was continuously provided. A
282 12/12h light/dark cycle was maintained (on at 7am and off at 7pm). A physical examination
283 of each animal by a veterinarian was performed twice a week before treatment and daily after
284 treatment for general health conditions, ascertained management according to animal welfare
285 standards. Care was taken to reduce stress or pain of the pigs. Invasive procedures such as
286 blood samples and skin biopsies were kept to a minimum and performed under a short-term
287 mild sedation, using a mixture of 0.2 mL/kg chlorhydrate of ketamine (Ketamine 1000®,
288 Virbac, Carros, France) and 0.02 mL/kg of xylazine (Rompun® 2%, Bayer Healthcare, Loos,
289 France) given by a single intra-muscular injection. The synthetic glucocorticoid immune-
290 suppressant dexamethasone (Fagron SAS, Thiais, France) was used to promote initial
291 infestation, increase intensity and duration of infestation. A daily oral dosage of 0.2 mg/kg
292 dexamethasone was administered. Dexamethasone treatment was initiated one week prior to
293 infestation and continued during the entire study period. The infestation was accomplished by
294 directly introducing mite-infected skin crusts deep into the ear canals of the pigs. Crusts were
295 obtained from a previous cohort of pigs initially infected with crusts of naturally infected pigs
296 coming from a farm in Brittany (Dominique Dreau, Saint-Allouestre, France) (18). Crusts
297 were collected in the morning and inoculated to pigs on the same day. Crusts were dissected
298 into small pieces (approximately 0.5 cm^2) containing between 600 and 800 mites. During the
299 procedure, the pigs were put under mild sedation for 15 min to prevent the dislodgement of
300 the crusts by agitation and to ensure successful infestation.
301

Drugs. AFX (Nexgard®, Merial/Boehringer, Inc., Lyon, France) was a 68-mg soft, beef-flavoured chew (for dogs weighing between 10.1 and 25kg). IVM was the human formulation (Stromectol®, MSD France, Courbevoie, France) provided as 3-mg tablets. Pigs were weighed on day 0 to calculate the dose of treatment required. The pigs were fed with their normal ration of food immediately after drug administration. Pigs were hand pilled to ensure accurate and complete dosing. Researchers involved in performing assessments and observations did not administer the treatments to pigs. No other acaricide or endectocide treatment was used throughout the study.

Parasitological and clinical assessment - data scoring. The first experimental phase was the progression of the scabies-infestation for 9 weeks after infection. At week 9 post-infection (day 0), treatments were administered. The second experimental phase was the assessment of drug efficacy and the pharmacokinetics study from day 0 to day 50 after treatment. Figure 1 illustrates the study design. The primary outcome was based on the reduction in the number of live mites counted in the skin scrapings after treatment. The end-point was the complete absence of live mites at day 14 post−treatment. Mites were collected and counted in skin scrapings, taken on day 0 (just before treatment) and subsequently on days 2, 4, 8, 10, 14, 21, 28, 35, and 45 post-treatment to estimate the percentage efficacy of treatment and the percentage of mite count reduction. Skin scrapings were obtained from each pig, around 0.2 g of crusts were scraped using a scalpel blade from the ears until blood seeped from the abrasion. Samples were examined in a Petri dish within 2 h after collection. Under a light heat source, mites were encouraged to crawl out of the crusts. The mites were examined under a stereomicroscope (Nikon©, SMZ645). Only live mites were counted and the number of life stages (adult or immature stages) was noted. Immature stages included larvae and nymphs.

A clinical score (Fig. 3) was used based on the skin surface affected by scabies lesions (scale

from 0 to 6: 0, 0%; 1, <10%; 2, 10–29%; 3, 30–49%; 4, 50– 69%; 5, 70–89%; 6, 90–100%), the intensity of the erythema of the skin (from 0 to 4: 0, no erythema; 1, mild; 2, moderate; 3, severe; 4, extremely severe), and the intensity of the encrustment (from 0 to 4: 0, no crust; 1, grey to white, thin and irregular 1–2 mm crust; 2, 2–5 mm crust; 3, grey-brown >5 mm crust; and 4, >5 mm, hard crust). The score was calculated for five anatomic sites (ears, legs, tail, back, and head) and added up. Clinical examination and scoring of animals were carried out weekly after infestation and on day 0 (just before treatment) and subsequently on days 2, 4, 8, 10, 14, 21, 28, 35, and 45 post-treatment. All animals were individually examined. Photographs were taken from each pig.

Pigs were observed weekly for 15 min to record pruritus. Movements in response to pruritus, such as flapping of the ears, rubbing on a surface, scratching ears with a posterior leg were recorded. Scoring of pruritus was carried out after infestation and on day 0 (just before treatment) and subsequently on days 2, 4, 8, 10, 14, 21, 28, 35, and 45 post-treatment. All animals were individually examined.

To estimate the hatchability of the eggs, eggs were collected from the skin scrapings taken at day 0 (just before treatment) and subsequently on days 2, 4, 8, and 14 post-treatments. Each time, 10 eggs were collected from each group in a sterile plastic Petri dish. The eggs were placed in an incubator at 37°C and 90% relative humidity and observed in 24 h intervals.

Afoxolaner and ivermectin pharmacokinetics analysis. Blood samples were collected by jugular vein puncture on heparinized tubes (BD Vacutainer®, BD-Plymouth, UK) on day 0 (just before treatment) and subsequently on hours 2, 4, 6, 24, and days 2, 4, 5, 8 (4 h after the second administration of drug), 10, 14, 21, 28, 35, 45, and 50 post-treatments. Plasma samples were prepared by centrifugation of blood samples at 2,000 *g* for 10 min. Skin biopsies were made by using a standard 5-mm-diameter punch biopsy tool (KAI Europe®, GmbH,

Germany) to extract a piece of epidermis and dermis from the neck region of the pigs on day 0 (just before treatment) and subsequently on days 1, 2, 4, 5, 8 (4 h after the second administration of drug), 10, 14, 21, 28, 35, 45, and 50 post-treatment. Plasma and tissue samples were stored at -20°C until drug analysis. IVM concentrations were measured in plasma and skin by high performance liquid chromatography (HPLC) with fluorescence detection using a procedure previously described and validated (18, 40). The procedure was performed in the Toxalim laboratory, INRA, Toulouse, France. AFX concentrations were measured in plasma and skin by liquid chromatography-mass spectrometry (LC-MS) in the Merial/Boehringer laboratories in Missouri (plasma analyses) and New Jersey (skin analyses), USA. The extracted analyses were chromatographed by reverse-phase HPLC and quantified by a triple quadrupole mass spectrometer system using the electrospray interface (41, 42). For IVM concentrations, the linearity was similar in the plasma and in the skin ($r = 0.99$ over a 0.1–100 ng/mL concentration range) and the limit of quantitation (LOQ) were 0.05 ng/mL in the plasma, and 0.1 ng/g in the skin (18). For AFX concentrations, the lower LOQ was 1 ng/mL in plasma and in skin. The pharmacokinetics parameters were determined using a non-compartmental analysis (Kinetica computer program version 4.2, InnaPhase®, Philadelphia, PA). The area under the concentration–time curve (AUC) and the mean residence time (MRT) were calculated from the time of administration to the time of the last measurable concentration (t_{last}), using the arithmetic trapezoidal rule. The peak plasma concentration (C_{max}) and time of peak plasma concentration (T_{max}) were read from the plotted concentration versus time for each pig.

Statistical analysis. The non-parametric Kruskal-Wallis test was used in order to compare the groups at baseline. The primary outcome was based on the reduction in the number of live mites counted in skin scrapings following treatment. The percentage of efficacy was

calculated according to the following formula: Efficacy (%) = [(C – T)/C] x 100 where C was the arithmetic mean number of live mites for the control group and T was the arithmetic mean number of live mites for the treated group for each time point. The percentage reduction of the mite count was calculated according to the formula: Reduction (%)= [(Mpre – Mpost) / Mpre] x 100 where Mpre was the arithmetic mean number of live mites at baseline (day 0), and Mpost the arithmetic mean number of live mites post-treatment (days 2, 4, 8, 10, 14, 21, 28, 35, and 45). The decrease over time in mite count and in clinical and pruritus scores within each group of pigs was tested for significance ($P < 0.05$) by repeated measures in a mixed model with a robust variance estimate using STATA version12® software. We use a negative binomial regression model to assess the relationship between parasites (variable to explain), treatments and time (40, 41). Pharmacokinetics parameters obtained in the different groups were compared by a non-parametric Mann-Whitney test at a significance level of $P < 0.05$.

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Competing Interests.

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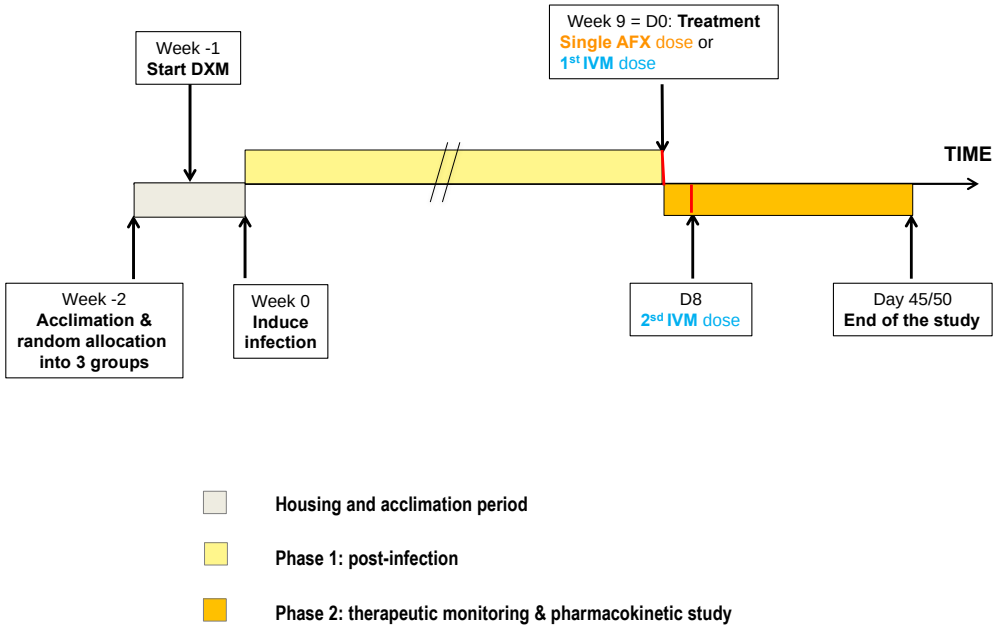
TABLE 1 Pharmacological parameters for AFX and IVM in plasma and in skin after oral administration to scabies-infested pigs[a]

	C_{max} (ng/mL)	T_{max} (d)	$AUC_{t\text{-}last}$ (d . ng/mL)	$MRT_{C\text{-}last}$ (d)
PLASMA				
AFX	196 ± 160.2	0.4 ± 0.4	1,217.5 ± 751.5	7.1 ± 2.4
IVM	44.2 ± 11.6*	0.2 ± 0.0	38.7 ± 17.9	1.1 ± 0.2*
SKIN				
AFX	1,909.2 ± 962.4	1.25 ± 0.5	11,159.3 ± 6,158.4	16.2 ± 16.9
IVM	72.0 ± 40.2*	1.0 ± 0.0	186.2 ± 104.8	2.7 ± 0.5*

[a]Values are presented as means ± SD of AFX and IVM in the plasma and in the skin of pigs following oral intake. C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration; $AUC_{t\text{-}last}$, area under the plasma curve concentration-time curve from time zero after first-administration to the last sampling time point with a measurable concentration; $t_{1/2}$, terminal elimination half-life; $MRT_{C\text{-}last}$, mean residence time; –, could not be determined; d, day; * $P < 0.001$ vs. AFX (compared with a non-parametric Mann-Whitney test).

659 **FIG 1** Study design showing the three experimental phases[a]



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662 [a]DXM, dexamethasone; D, day; AFX, afoxolaner; IVM, ivermectin

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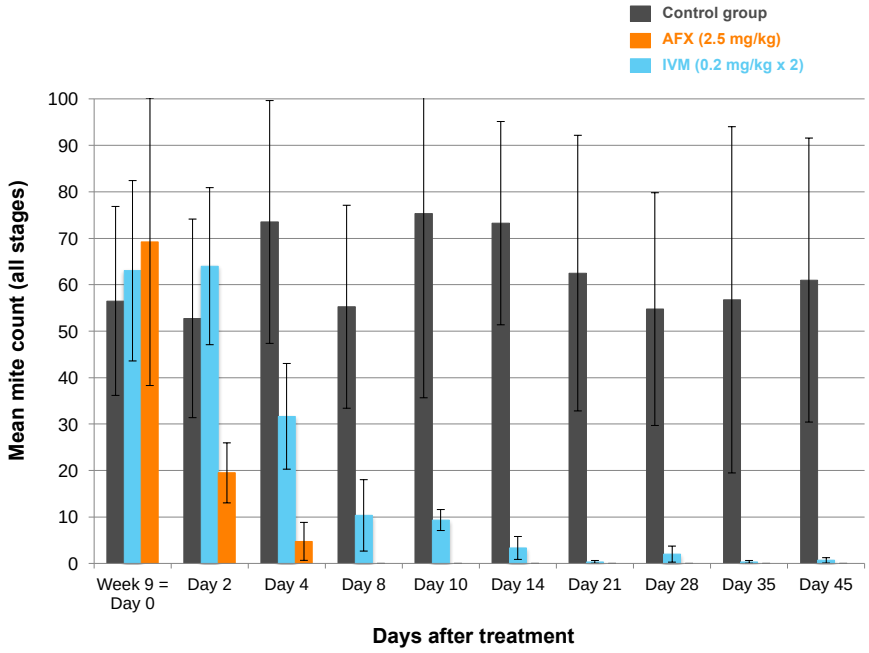
FIG 2 A. Mite count (mean ± SD) for the AFX- or IVM-treated and control pigs over time after treatments from baseline (D0) to study-end.

B. Partition of the different life-stages (adult or immature) recovered from the skin scrapings collected from the AFX- or IVM- or control pigs over time after treatments from baseline (D0) to study-end[a].

A.



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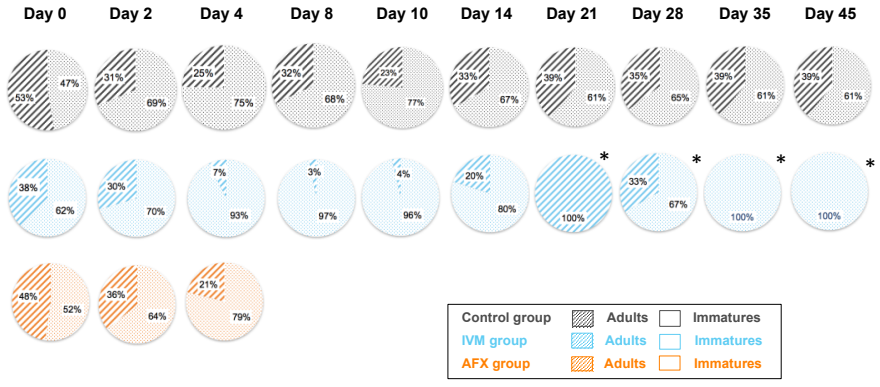

[a] *, less than 10 mites were recovered in total from the skin scrapings; AFX, afoxolaner; IVM, ivermectin.

685 **FIG 3** Clinical scores (mean ± SD) for the AFX- or IVM-treated and control pigs over time from

686 scabies-infesction to study-end[a]

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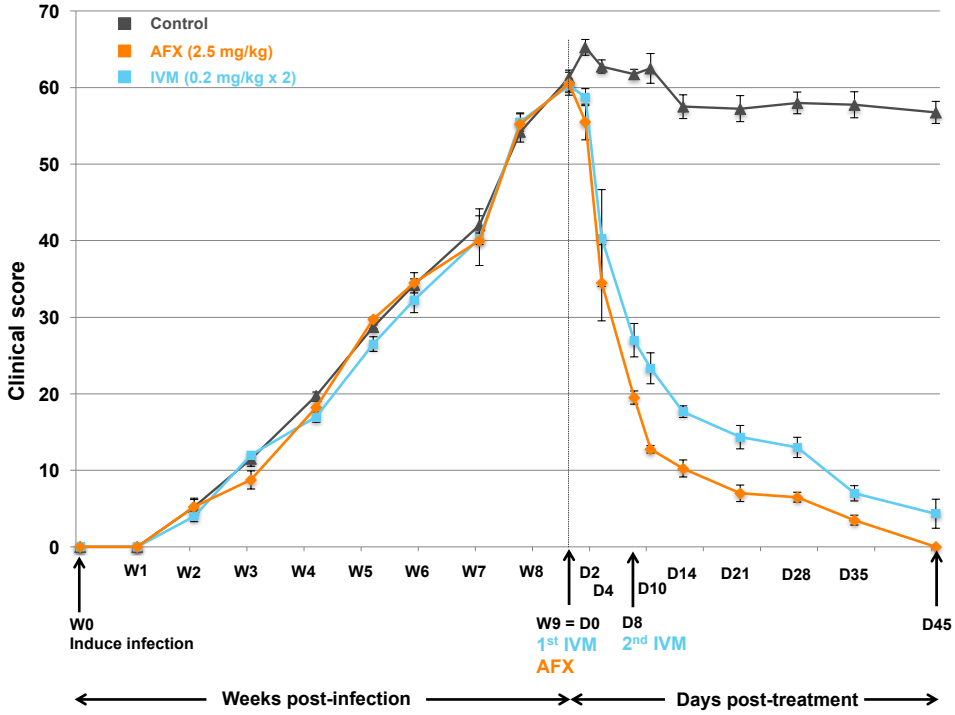

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690 [a]The manifestation of scabies infestation in the skin areas of five anatomic sites (ears, legs, tail, back,

691 and head) was monitored weekly. Clinical scores are based on the skin surface affected by scabies

692 lesions (scored 0–6: 0, 0%; 1, <10%; 2, 10–29%; 3, 30–49%; 4, 50– 69%; 5, 70–89%; 6, 90–100%),

693 intensity of skin erythema (scored 0–4: 0, no erythema; 1, mild; 2, moderate; 3, severe; 4, extremely

694 severe), and the encrustment intensity (scored 2× 0–4: 0, no crust; 1, grey to white, thin and irregular

695 1–2 mm crust; 2, 2–5 mm crust; 3, grey-brown >5 mm crust; and 4, >5 mm, hard crust). The score was

696 calculated for the 5 different anatomic sites and added. W, week; D, day; AFX, afoxolaner; IVM,

697 ivermectin.

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699 **FIG 4** Pruritus scores (mean ± SD) for the AFX- or IVM-treated and control pigs over time from

700 scabies-infestation induction to study-end[a]

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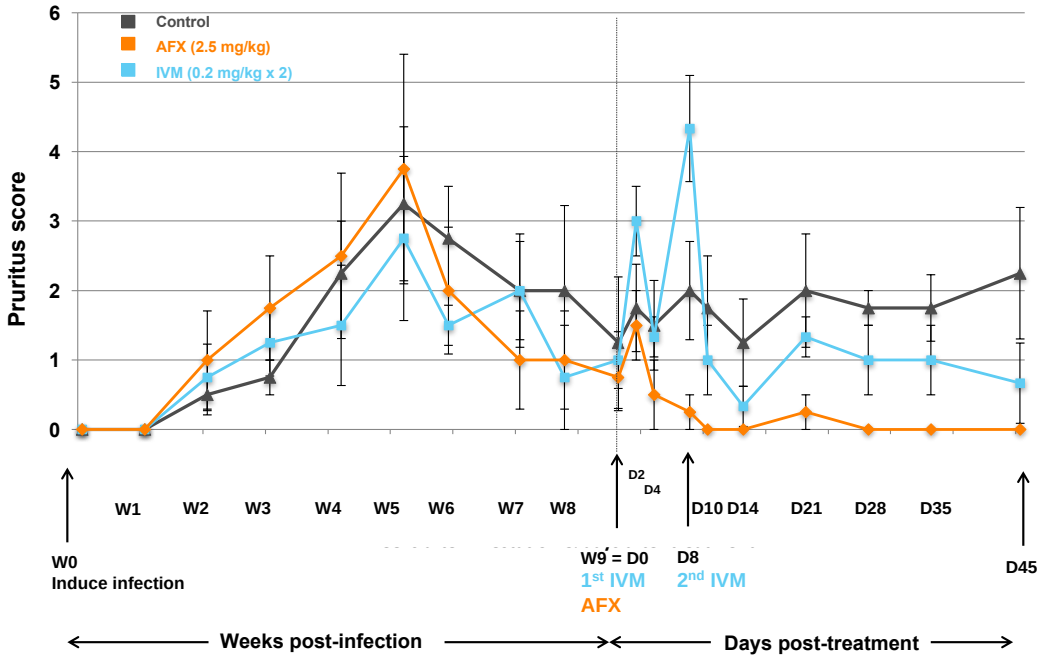

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704 [a]D, day; W, week; AFX, afoxolaner; IVM, ivermectin.

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712 **FIG 5** Mean concentration-time profiles (mean ± SD, ng/ml) in plasma following oral administration

713 of AFX or IVM in scabies-infested pigs[a]

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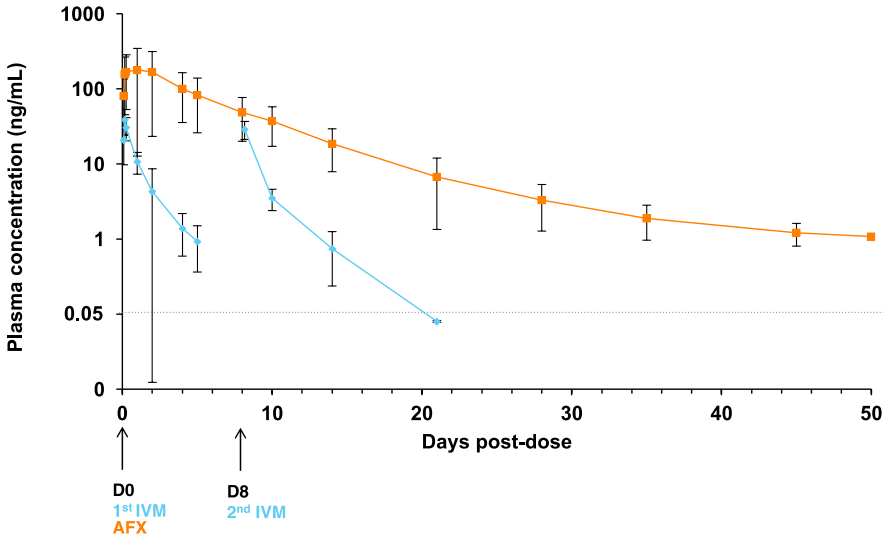

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718 [a]Concentrations measured on hour 2, 4, 6 and 24 of D0 for AFX and IVM and on D2, 4, 5, 8 (4 h after the

719 second IVM dose), 10, 14, 21, 28, 35, 45, and 50 post-treatment are depicted.

720 D, day; AFX, afoxolaner; IVM, ivermectin.

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727 **FIG 6** Mean concentration-time profiles (mean ± SD, ng/g) in skin following oral administration of

728 AFX or IVM in scabies-infested pigs[a]

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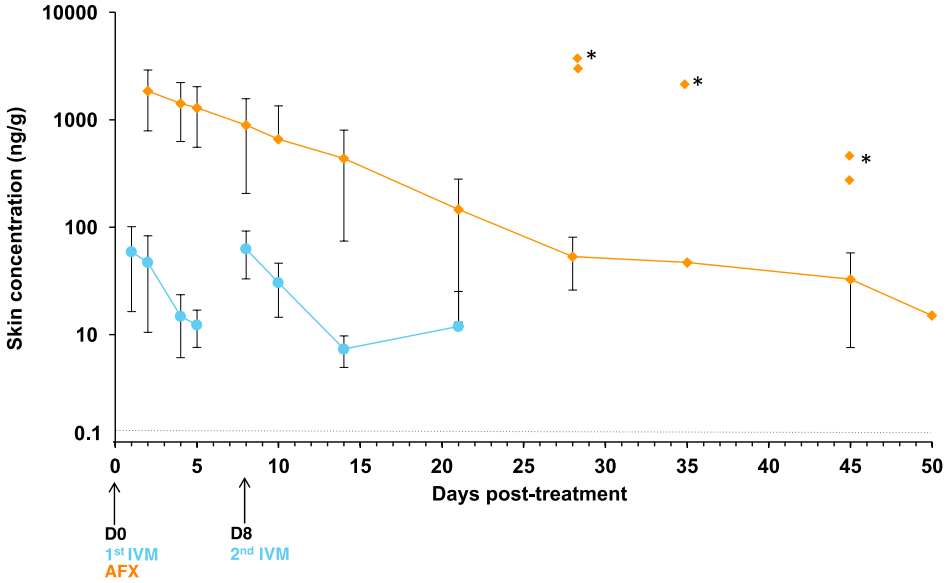

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733 [a]Concentrations measured on D1, 2, 4, 5, 8 (4 h after the second IVM dose), 10, 14, 21, 28, 35, 45, and 50 post-

734 treatment are depicted. * represents the outliers measured concentrations.

735 D, day; AFX, afoxolaner; IVM, ivermectin.

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742 **Table S1** Mite count values and efficacies for all groups over time[a]

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Day of Study	Afoxolaner Group (n=4) No. of mites (mean mite counts ± SD) Count range (n) Reduction % (range) Efficacy % (range)	Ivermectin Group (n=4) No. of mites (mean mite counts ± SD) Count range (n) Reduction % (range) Efficacy % (range)	Control Group (n=4) No. of mites (mean mite counts ± SD) Count range (n) Reduction % (range) Efficacy % (range)
Day 0	277 (69.3 ± 61.9) 4–127	252 (63 ± 38.8) 13–98	226 (56.5 ± 40.7) 15–106
Day 2	78 (19.5 ± 12.9) 7–33 71.8% (65.5–94%) 63% (37.4–86.7%)	256 (64 ± 33.8) 21–103 NA NA	211 (52.8 ± 42.7) 11–93 NA NA
Day 4	19 (4.8 ± 8.2) 0–17 93.1% (75–100%) 93.5% (76.9–100%)	95 (31.7 ± 22.7) 13–57 49.7% (35.9–75%) 56.9% (22.4–82.3%)	294 (73.5 ± 52.3) 8–124 NA NA
Day 8	0 0–0 100% 100%	31 (10.3 ± 15.4) 0–28 83.6% (53.6–100%) 81.3% (66–100%)	221 (55.3 ± 43.7) 9–105 NA NA
Day 10	0 0–0 100% 100%	9.3 (11.8 ± 4.5) 5–14 85.2% (61.5–89.9%) 87.6% (49.3–88%)	301 (75.3 ± 79.2) 1–151 NA NA
Day 14	0 0–0 100% 100%	10 (3.3 ± 4.9) 0–9 94.7% (82.7–100%) 95.4% (87.7–98.6%)	293 (73.3 ± 43.8) 22–124 NA NA
Day 21	0 0–0 100%	1 (0.33 ± 0.6) 0–1 99.5% (98.1–100%)	250 (62.5 ± 59.3) 18–144 NA

	100%	99.5% (98.4−100%)	NA
	0	6 (2 ± 3.5)	219 (54.8 ± 50.1)
Day	0−0	0−6	5−117
28	100%	96.8% (88.5−100%)	NA
	100%	96.3% (89−100%)	NA
	0	1 (0.3 ± 0.6)	227 (56.8 ± 74.5)
Day	0−0	0−1	3−167
35	100%	99.5% (98.1−100%)	NA
	100%	99.4% (98.2−100%)	NA
	0	2 (0.7 ± 1.2)	244 (61 ± 61.1)
Day	0−0	0−2	7−148
45	100%	98.9% (96.2−100%)	NA
	100%	98.9% (96.7−100%)	NA

[a]The table shows the mite count values (number of mites, mean and SD) in the AFX, IVM-treated and control groups of pigs on the various assessments days. It also presents the percentages of reduction in *S. scabiei* mite count and efficacies of AFX and IVM treatments over time. SD, Standard deviation; NA, not applicable.

760 **Table S2** Clinical score values and percentage of improvement for all groups over time[a]

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Day of Study	Afoxolaner Group (n=4) Mean clinical score ± SD Score range Improvement %	Ivermectin Group (n=4) Mean clinical score ± SD Score range Improvement %	Control Group (n=4) Mean clinical score ± SD Score range Improvement %
Day 0	60.5 ± 3 57−63 na	60.3 ± 1.70 58−62 na	61.3 ± 2.06 59−63 na
Day 2	55.5 ± 4.7 54−61 8.3%	58.8 ± 2.22 56−61 2.5%	65.3 ± 2.06 63−67 na
Day 4	34.5 ± 9.95 22−46 43%	40.3± 12.66 29−54 33.1%	62.8 ± 1.71 61−65 na
Day 8	19.5 ± 1.73 18−21 67.8%	27 ± 4.36 22−30 55.2%	61.8 ± 1.26 60−63 na
Day 10	12.8 ± 0.96 12−14 78.9%	23.3 ± 4.04 19−27 61.3%	62.5 ± 3.87 58−67 na
Day 14	10.3 ± 2.22 7−12 83.1%	17.7 ± 1.53 16−19 70.6%	57.5 ± 3.11 54−61 na
Day 21	7 ± 2.16 5−10 88.4%	14.3 ± 3.06 11−17 76.3%	57.3 ± 3.40 53−60 na
Day 28	6.5 ± 1.29 5−8 89.3%	13 ± 2.65 10−15 78.4%	58 ± 2.83 54−60 na
Day 35	3.5 ± 1.29 2−5	7 ± 2.0 5−9	57.8 ± 3.40 53−61

	94.2%	88.4%	na
Day 45	0 0–0 100%	4.3 ± 3.79 0–7 92.9%	56.8 ± 2.87 53–60 na

[a]The table shows the clinical score values (score, mean and SD) in the AFX, IVM-treated and control groups of pigs on the various assessments days. It also presents the percent improvement of AFX and IVM-treated pigs clinical scores over time from assessment at baseline (D0) to study-end.

SD, Standard deviation; NA, not applicable.