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1 **Efficacy and Pharmacokinetics Evaluation of a Single Oral Dose of Afoxolaner against**
 2 ***Sarcoptes scabiei* in the Porcine Scabies Model for Human Infestation**

3
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24
 25 **Running title:** Evaluation of afoxolaner in a porcine scabies model

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31 ABSTRACT

32

33 Scabies is a major and potentially growing public health problem worldwide with an unmet
34 need for acaricidal agents with greater efficacy and improved pharmacological properties for
35 its treatment. The objective of the present study was to assess the efficacy and describe the
36 pharmacokinetics profile of a novel acaricide, afoxolaner (AFX), in a relevant experimental
37 porcine model. Twelve pigs were experimentally infested and treated either with 2.5 mg/kg
38 single dose oral AFX ($n = 4$), 0.2 mg/kg two-doses 8 days apart oral ivermectin (IVM, $n = 4$),
39 or no treatment against scabies ($n = 4$). Response to treatment was assessed by reduction of
40 mite counts in skin scrapings as well as clinical and pruritus scores over time. Plasma and
41 skin pharmacokinetic profiles for both AFX and IVM were evaluated. AFX efficacy was
42 100% at days 8 and 14 post-treatment and remained unchanged until the study-end (day 45).
43 IVM efficacy was 86% and 97% on days 8 and 14, respectively, with a few mites recovered at
44 study-end. Clinical and pruritus scores decreased in both treated groups and remained
45 constant in the control group. Plasma mean residence times (MRT) were 7.1 ± 2.4 and 1.1 ± 0.2
46 days for AFX and IVM, respectively. Skin MRT values were 16.2 ± 16.9 and 2.7 ± 0.5 days for
47 AFX and IVM, respectively. Overall, a single oral dose of AFX was efficacious for the
48 treatment of scabies in experimentally infested pigs and showed a remarkably long MRT in
49 plasma and notably in the skin.

50

51

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

53

54

55 Introduction

56 Scabies is an epidermal infestation caused by the mite *Sarcoptes scabiei* in humans (1). It is
57 increasingly recognised as a large and potentially growing public health problem worldwide
58 with a significant burden (2, 3). Prevalence is estimated to be around 100-130 million
59 cases/year (4–7). Scabies is often perceived erroneously as causing a simple itch but over the
60 past decade, studies emphasised its important morbidity, mostly caused by secondary
61 bacterial infections (4). Opportunistic pathogens like *Streptococcus pyogenes* (group A
62 streptococcus) and *Staphylococcus aureus* are commonly associated with human scabies.
63 Especially in tropical and sub-tropical countries, these can lead to invasive bacterial infection
64 and post-infection complications, such as post-streptococcal glomerulonephritis, acute
65 rheumatic fever and rheumatic heart disease (8–10). The psycho-social and economic impact
66 caused by scabies through school absenteeism or loss of work productivity due to pruritus and
67 lack of sleep is considerable and leads to an exacerbation of poverty in affected populations
68 (5, 11).

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

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

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

91 We recently optimised the experimental porcine model developed by Mounsey et al. in
92 2010 (24) and demonstrated its usefulness for preclinical assessment of drug candidates for
93 the treatment of scabies (18). Here, we assessed drug efficacies and pharmacokinetic profiles
94 of a single oral dose of AFX compared with two oral doses of IVM in experimentally-infested
95 pigs.

96

97 **Results**

98 **Study design.** Twelve 3-week old pigs were randomly assigned into 3 groups in January 2015
99 and were infested with *Sarcoptes scabiei* var. *swis* two weeks after their arrival (Fig. 1).
100 Dexamethasone (0.2 mg/kg) was used daily during the entire study to promote initial
101 infestation, and to increase intensity and duration of the infestation. Pigs were blinded-treated
102 nine weeks post-infection, at day 0 (D0), in end-March 2015. The first group of pigs ($n = 4$)
103 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 \pm 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

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

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

145
146 **Clinical assessment of drug efficacy.** The mean clinical scores over time in the three groups
147 are presented in Figure 3 and Supplementary Table 2. Clinical lesions disappeared completely
148 in all AFX-treated pigs whereas two out of three IVM-treated pigs still had lesions at the end
149 of the study, albeit 93% of improvement (Table S2). After treatment, the mean clinical scores
150 of both treated groups were statistically different from those of the control group ($P = 0.0001$)
151 and statistically different from each other ($P = 0.023$). No clinical signs of drug intolerance
152 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

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

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

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

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

235 The pharmacokinetics profile of AFX orally administered at a dose of 2.5 mg/kg
236 exhibited a long elimination profile, with drug present for approximately 7 days in plasma and
237 16 days in skin, i.e. ~7-fold longer than that of IVM. Previous investigations in dogs with
238 AFX orally administered at the same dosage demonstrated similar results. AFX was rapidly
239 absorbed (around 2–4 h), with a high initial plasma peak (C_{\max} 1,655 \pm 332 ng/mL). The
240 terminal plasma half-life was remarkably long at up to 15 days (15.5 \pm 7.8 d) (23), consistent
241 with the lipophilic and unionized properties of the small AFX molecule, and with its high
242 affinity to plasma proteins shown in dogs (> 99%) (23). Our study is the first one to address
243 AFX pharmacology parameters in the skin and further studies are needed to investigate in
244 which layer of the skin AFX is accumulating.

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

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

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

260

261 **Materials and Methods**

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

271 Twelve three-week old *Sus scrofa domesticus* « Large white » breed, female from the
272 same pig farm (Gambais, France) were housed at the Centre de Recherche Biomédicale in the
273 Ecole nationale vétérinaire d'Alfort, France ([http://www.vet-alfort.fr/web/fr/103-centre-de-](http://www.vet-alfort.fr/web/fr/103-centre-de-recherche-biomedicale.php)
274 [recherche-biomedicale.php](http://www.vet-alfort.fr/web/fr/103-centre-de-recherche-biomedicale.php)). The mean weight (\pm SD) at the arrival was 7.15 kg (\pm 0.64). Pigs
275 were healthy and initially free of sarcoptic mange and they had never received any
276 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 were placed in similar experimental
279 climate-controlled units by group (temperature of $21^{\circ}\text{C} \pm 2^{\circ}$, humidity $50\% \pm 10\%$, 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

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

310

311 **Parasitological and clinical assessment - data scoring.** The first experimental phase was the
312 progression of the scabies-infestation for 9 weeks after infection. At week 9 post-infection
313 (day 0), treatments were administered. The second experimental phase was the assessment of
314 drug efficacy and the pharmacokinetics study from day 0 to day 50 after treatment. Figure 1
315 illustrates the study design. The primary outcome was based on the reduction in the number of
316 live mites counted in the skin scrapings after treatment. The end-point was the complete
317 absence of live mites at day 14 post-treatment. Mites were collected and counted in skin
318 scrapings, taken on day 0 (just before treatment) and subsequently on days 2, 4, 8, 10, 14, 21,
319 28, 35, and 45 post-treatment to estimate the percentage efficacy of treatment and the
320 percentage of mite count reduction. Skin scrapings were obtained from each pig, around 0.2 g
321 of crusts were scraped using a scalpel blade from the ears until blood seeped from the
322 abrasion. Samples were examined in a Petri dish within 2 h after collection. Under a light heat
323 source, mites were encouraged to crawl out of the crusts. The mites were examined under a
324 stereomicroscope (Nikon®, SMZ645). Only live mites were counted and the number of life
325 stages (adult or immature stages) was noted. Immature stages included larvae and nymphs.
326 A clinical score (Fig. 3) was used based on the skin surface affected by scabies lesions (scale

327 from 0 to 6: 0, 0%; 1, <10%; 2, 10–29%; 3, 30–49%; 4, 50– 69%; 5, 70–89%; 6, 90–100%), the
328 intensity of the erythema of the skin (from 0 to 4: 0, no erythema; 1, mild; 2, moderate; 3, severe;
329 4, extremely severe), and the intensity of the encrustment (from 0 to 4: 0, no crust; 1, grey to
330 white, thin and irregular 1–2 mm crust; 2, 2–5 mm crust; 3, grey-brown >5 mm crust; and 4, >5 mm,
331 hard crust). The score was calculated for five anatomic sites (ears, legs, tail, back, and head)
332 and added up. Clinical examination and scoring of animals were carried out weekly after
333 infestation and on day 0 (just before treatment) and subsequently on days 2, 4, 8, 10, 14, 21,
334 28, 35, and 45 post-treatment. All animals were individually examined. Photographs were
335 taken from each pig.

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

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

345

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

352 Germany) to extract a piece of epidermis and dermis from the neck region of the pigs on day
353 0 (just before treatment) and subsequently on days 1, 2, 4, 5, 8 (4 h after the second
354 administration of drug), 10, 14, 21, 28, 35, 45, and 50 post-treatment. Plasma and tissue
355 samples were stored at -20°C until drug analysis. IVM concentrations were measured in
356 plasma and skin by high performance liquid chromatography (HPLC) with fluorescence
357 detection using a procedure previously described and validated (18, 40). The procedure was
358 performed in the Toxalim laboratory, INRA, Toulouse, France. AFX concentrations were
359 measured in plasma and skin by liquid chromatography-mass spectrometry (LC-MS) in the
360 Merial/Boehringer laboratories in Missouri (plasma analyses) and New Jersey (skin analyses),
361 USA. The extracted analyses were chromatographed by reverse-phase HPLC and quantified
362 by a triple quadrupole mass spectrometer system using the electrospray interface (41, 42). For
363 IVM concentrations, the linearity was similar in the plasma and in the skin ($r = 0.99$ over a
364 0.1–100 ng/mL concentration range) and the limit of quantitation (LOQ) were 0.05 ng/mL in
365 the plasma, and 0.1 ng/g in the skin (18). For AFX concentrations, the lower LOQ was 1
366 ng/mL in plasma and in skin. The pharmacokinetics parameters were determined using a non-
367 compartmental analysis (Kinetica computer program version 4.2, InnaPhase®, Philadelphia,
368 PA). The area under the concentration–time curve (AUC) and the mean residence time (MRT)
369 were calculated from the time of administration to the time of the last measurable
370 concentration (t_{last}), using the arithmetic trapezoidal rule. The peak plasma concentration
371 (C_{max}) and time of peak plasma concentration (T_{max}) were read from the plotted concentration
372 versus time for each pig.

373

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

377 calculated according to the following formula: Efficacy (%) = [(C – T)/C] x 100 where C was
378 the arithmetic mean number of live mites for the control group and T was the arithmetic mean
379 number of live mites for the treated group for each time point. The percentage reduction of
380 the mite count was calculated according to the formula: Reduction (%)= [(Mpre – Mpost) /
381 Mpre] x 100 where Mpre was the arithmetic mean number of live mites at baseline (day 0),
382 and Mpost the arithmetic mean number of live mites post-treatment (days 2, 4, 8, 10, 14, 21,
383 28, 35, and 45). The decrease over time in mite count and in clinical and pruritus scores
384 within each group of pigs was tested for significance ($P < 0.05$) by repeated measures in a
385 mixed model with a robust variance estimate using STATA version12® software. We use a
386 negative binomial regression model to assess the relationship between parasites (variable to
387 explain), treatments and time (40, 41). Pharmacokinetics parameters obtained in the different
388 groups were compared by a non-parametric Mann-Whitney test at a significance level of
389 $P < 0.05$.

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411

412 **Competing Interests.**

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421 afoxolaner only. Merial had no role in the study design, data collection and interpretation, or
422 decision to submit the work for publication. No other potential conflict of interest relevant to
423 this article was reported.

424 **References**

- 425 1. Chosidow O. 2006. Clinical practices. Scabies. *N Engl J Med* 354:1718–1727.
426
- 427 2. Chosidow O, Fuller LC. 2017. Scratching the itch: is scabies a truly neglected disease?
428 *Lancet Infect Dis*.
429
- 430 3. WHO | 10th meeting of the Strategic and Technical Advisory Group for Neglected
431 Tropical Diseases. WHO.
432
- 433 4. Engelman D, Kiang K, Chosidow O, McCarthy J, Fuller C, Lammie P, Hay R, Steer
434 A, Members Of The International Alliance For The Control Of Scabies. 2013. Toward the
435 global control of human scabies: introducing the International Alliance for the Control of
436 Scabies. *PLoS Negl Trop Dis* 7:e2167.
437
- 438 5. Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ, Marks R,
439 Naldi L, Weinstock MA, Wulf SK, Michaud C, J L Murray C, Naghavi M. 2014. The global
440 burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. *J*
441 *Invest Dermatol* 134:1527–1534.
442
- 443 6. Karimkhani C, Dellavalle RP, Coffeng LE, Flohr C, Hay RJ, Langan SM, Nsoesie EO,
444 Ferrari AJ, Erskine HE, Silverberg JI, Vos T, Naghavi M. 2017. Global Skin Disease
445 Morbidity and Mortality: An Update From the Global Burden of Disease Study 2013. *JAMA*
446 *Dermatol*.
447
- 448 7. Karimkhani C, Colombara DV, Drucker AM, Norton SA, Hay R, Engelman D, Steer
449 A, Whitfeld M, Naghavi M, Dellavalle RP. 2017. The global burden of scabies: a cross-
450 sectional analysis from the Global Burden of Disease Study 2015. *Lancet Infect Dis*.
451
- 452 8. Reid HF, Bassett DC, Gaworzewska E, Colman G, Poon-King T. 1990. Streptococcal
453 serotypes newly associated with epidemic post-streptococcal acute glomerulonephritis. *J Med*
454 *Microbiol* 32:111–114.
455
- 456 9. Hoy WE, White AV, Dowling A, Sharma SK, Bloomfield H, Tipiloura BT, Swanson
457 CE, Mathews JD, McCredie DA. 2012. Post-streptococcal glomerulonephritis is a strong risk
458 factor for chronic kidney disease in later life. *Kidney Int* 81:1026–1032.
459
- 460 10. Steer AC, Jenney AWJ, Kado J, Batzloff MR, La Vincente S, Waqatakirewa L,
461 Mulholland EK, Carapetis JR. 2009. High burden of impetigo and scabies in a tropical
462 country. *PLoS Negl Trop Dis* 3:e467.
463
- 464 11. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K,
465 Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK,
466 Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim
467 AN, Barker-Collo S, Barrero LH, Bartels DH, Basáñez M-G, Baxter A, Bell ML, Benjamin
468 EJ, Bennett D, Bernabé E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G,
469 Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R,
470 Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-
471 Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R,
472 Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F,

- 473 Chen H, Cheng AT-A, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE,
474 Condon J, Connor MD, Cooper LT, Corriere M, Cortinovia M, de Vaccaro KC, Couser W,
475 Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J,
476 Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J,
477 Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER,
478 Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P,
479 Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fèvre EM, Finucane
480 MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FGR, Franklin R, Fransen
481 M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F,
482 Gillum RF, Gmel G, Gosselin R, Grainger R, Groeger J, Guillemin F, Gunnell D, Gupta R,
483 Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R,
484 Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE,
485 Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan
486 G, Kassebaum N, Kawakami N, Keren A, Khoo J-P, King CH, Knowlton LM, Kobusingye O,
487 Koranteng A, Krishnamurthi R, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh
488 J, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R,
489 Ma J, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes
490 W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM,
491 McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M,
492 Mensah GA, Merriman TR, Meyer A-C, Miglioli V, Miller M, Miller TR, Mitchell PB,
493 Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A,
494 Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan
495 KMV, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R,
496 O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A,
497 Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N,
498 Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA,
499 Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiah KD, Ranganathan D,
500 Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T,
501 Robinson C, De Leòn FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S,
502 Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz
503 S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA,
504 Sliwa K, Smith E, Smith JL, Stapelberg NJC, Steer A, Steiner T, Stolk WA, Stovner LJ,
505 Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas
506 B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbaris
507 MK, Ubeda C, Undurraga EA, van der Werf MJ, van Os J, Vavilala MS, Venketasubramanian
508 N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG,
509 Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC,
510 Williams SRM, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh P-H, Zaidi AKM, Zheng Z-J,
511 Zonies D, Lopez AD, Murray CJL, AlMazroa MA, Memish ZA. 2012. Years lived with
512 disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic
513 analysis for the Global Burden of Disease Study 2010. *Lancet Lond Engl* 380:2163–2196.
514
515 12. Strong M, Johnstone P. 2007. Interventions for treating scabies. *Cochrane Database*
516 *Syst Rev* CD000320.
517
518 13. Salavastru CM, Chosidow O, Boffa MJ, Janier M, Tiplica GS. 2017. European
519 guideline for the management of scabies. *J Eur Acad Dermatol Venereol J EADV* 31:1248–
520 1253.
521
522 14. Mounsey KE, Bernigaud C, Chosidow O, McCarthy JS. 2016. Prospects for

- 523 Moxidectin as a New Oral Treatment for Human Scabies. *PLoS Negl Trop Dis* 10:e0004389.
524
- 525 15. Currie BJ, Harumal P, McKinnon M, Walton SF. 2004. First documentation of in vivo
526 and in vitro ivermectin resistance in *Sarcoptes scabiei*. *Clin Infect Dis Off Publ Infect Dis Soc*
527 *Am* 39:e8-12.
528
- 529 16. Mounsey KE, Holt DC, McCarthy JS, Currie BJ, Walton SF. 2009. Longitudinal
530 evidence of increasing in vitro tolerance of scabies mites to ivermectin in scabies-endemic
531 communities. *Arch Dermatol* 145:840–841.
532
- 533 17. Chosidow O, Giraudeau B. 2012. Topical ivermectin--a step toward making head lice
534 dead lice? *N Engl J Med* 367:1750–1752.
535
- 536 18. Weber T, Selzer PM. 2016. Isoxazolines: A Novel Chemotype Highly Effective on
537 Ectoparasites. *ChemMedChem* 11:270–276.
538
- 539 19. Kunkle BN, Drag MD, Chester TS, Larsen DL. 2014. Assessment of the onset of
540 action of afoxolaner against existing adult flea (*Ctenocephalides felis*) infestations on dogs.
541 *Vet Parasitol* 201:204–206.
542
- 543 20. Otranto D. 2014. NEXGARD®. Afoxolaner, a new oral insecticide-acaricide to
544 control fleas and ticks in dogs. Editorial. *Vet Parasitol* 201:177–178.
545
- 546 21. Shoop WL, Hartline EJ, Gould BR, Waddell ME, McDowell RG, Kinney JB, Lahm
547 GP, Long JK, Xu M, Wagerle T, Jones GS, Dietrich RF, Cordova D, Schroeder ME, Rhoades
548 DF, Benner EA, Confalone PN. 2014. Discovery and mode of action of afoxolaner, a new
549 isoxazoline parasiticide for dogs. *Vet Parasitol* 201:179–189.
550
- 551 22. Letendre L, Huang R, Kvaternick V, Harriman J, Drag M, Soll M. 2014. The
552 intravenous and oral pharmacokinetics of afoxolaner used as a monthly chewable antiparasitic
553 for dogs. *Vet Parasitol* 201:190–197.
554
- 555 23. Mounsey K, Ho M-F, Kelly A, Willis C, Pasay C, Kemp DJ, McCarthy JS, Fischer K.
556 2010. A tractable experimental model for study of human and animal scabies. *PLoS Negl*
557 *Trop Dis* 4:e756.
558
- 559 24. Bernigaud C, Fang F, Fischer K, Lespine A, Aho LS, Dreau D, Kelly A, Sutra J-F,
560 Moreau F, Lilin T, Botterel F, Guillot J, Chosidow O. 2016. Preclinical Study of Single-Dose
561 Moxidectin, a New Oral Treatment for Scabies: Efficacy, Safety, and Pharmacokinetics
562 Compared to Two-Dose Ivermectin in a Porcine Model. *PLoS Negl Trop Dis* 10:e0005030.
563
- 564 25. Arlian LG, Runyan RA, Achar S, Estes SA. 1984. Survival and infectivity of
565 *Sarcoptes scabiei* var. *canis* and var. *hominis*. *J Am Acad Dermatol* 11:210–215.
566
- 567 26. Van Neste DJ, Staquet MJ. 1986. Similar epidermal changes in hyperkeratotic scabies
568 of humans and pigs. *Am J Dermatopathol* 8:267–273.
569
- 570 27. Meyer W, Schwarz R, Neurand K. 1978. The skin of domestic mammals as a model
571 for the human skin, with special reference to the domestic pig. *Curr Probl Dermatol* 7:39–52.
572

- 573 28. Beugnet F, de Vos C, Liebenberg J, Halos L, Larsen D, Fourie J. 2016. Efficacy of
574 afoxolaner in a clinical field study in dogs naturally infested with *Sarcoptes scabiei*. *Parasite*
575 *Paris Fr* 23:26.
576
- 577 29. Taenzler J, Liebenberg J, Roepke RKA, Frénais R, Heckerth AR. 2016. Efficacy of
578 fluralaner administered either orally or topically for the treatment of naturally acquired
579 *Sarcoptes scabiei* var. *canis* infestation in dogs. *Parasit Vectors* 9:392.
580
- 581 30. Romero C, Heredia R, Pineda J, Serrano JA, Mendoza GD, Trápala P, Cordero AM.
582 2016. Efficacy of fluralaner in 17 dogs with sarcoptic mange. *Vet Dermatol* 27:353-e88.
583
- 584 31. Becskei C, De Bock F, Illambas J, Cherni JA, Fourie JJ, Lane M, Mahabir SP, Six
585 RH. 2016. Efficacy and safety of a novel oral isoxazoline, sarolaner (Simpatica™), for the
586 treatment of sarcoptic mange in dogs. *Vet Parasitol* 222:56–61.
587
- 588 32. Mounsey KE, Murray HC, Bielefeldt-Ohmann H, Pasay C, Holt DC, Currie BJ,
589 Walton SF, McCarthy JS. 2015. Prospective study in a porcine model of *sarcoptes scabiei*
590 indicates the association of Th2 and Th17 pathways with the clinical severity of scabies.
591 *PLoS Negl Trop Dis* 9:e0003498.
592
- 593 33. Swe PM, Zakrzewski M, Kelly A, Krause L, Fischer K. 2014. Scabies mites alter the
594 skin microbiome and promote growth of opportunistic pathogens in a porcine model. *PLoS*
595 *Negl Trop Dis* 8:e2897.
596
- 597 34. Currie BJ, McCarthy JS. 2010. Permethrin and ivermectin for scabies. *N Engl J Med*
598 362:717–725.
599
- 600 35. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdtts V. 2012. The pig: a model
601 for human infectious diseases. *Trends Microbiol* 20:50–57.
602
- 603 36. Drag M, Saik J, Harriman J, Larsen D. 2014. Safety evaluation of orally administered
604 afoxolaner in 8-week-old dogs. *Vet Parasitol* 201:198–203.
605
- 606 37. Ozoe Y, Asahi M, Ozoe F, Nakahira K, Mita T. 2010. The antiparasitic isoxazoline
607 A1443 is a potent blocker of insect ligand-gated chloride channels. *Biochem Biophys Res*
608 *Commun* 391:744–749.
609
- 610 38. Casida JE. 2015. Golden Age of RyR and GABA-R Diamide and Isoxazoline
611 Insecticides: Common Genesis, Serendipity, Surprises, Selectivity, and Safety. *Chem Res*
612 *Toxicol* 28:560–566.
613
- 614 39. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. 2010. Improving
615 bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS*
616 *Biol* 8:e1000412.
617
- 618 40. Lifschitz A, Virkel G, Sallovitz J, Sutra JF, Galtier P, Alvinerie M, Lanusse C. 2000.
619 Comparative distribution of ivermectin and doramectin to parasite location tissues in cattle.
620 *Vet Parasitol* 87:327–338.
621
- 622 41. Lifschitz A, Virkel G, Imperiale F, Sutra JF, Galtier P, Lanusse C, Alvinerie M. 1999.

- 623 Moxidectin in cattle: correlation between plasma and target tissues disposition. *J Vet*
624 *Pharmacol Ther* 22:266–273.
625
- 626 42. Lespine A, Alvinerie M, Sutra J-F, Pors I, Chartier C. 2005. Influence of the route of
627 administration on efficacy and tissue distribution of ivermectin in goat. *Vet Parasitol*
628 128:251–260.
629
- 630 43. Hilbe JM. 2011. *Negative Binomial Regression, 2nd Edition* Cambridge University
631 Press. New York.
632
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640 **TABLE 1** Pharmacological parameters for AFX and IVM in plasma and in skin after oral
641 administration to scabies-infested pigs^a

642

| | 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* |

643

644

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

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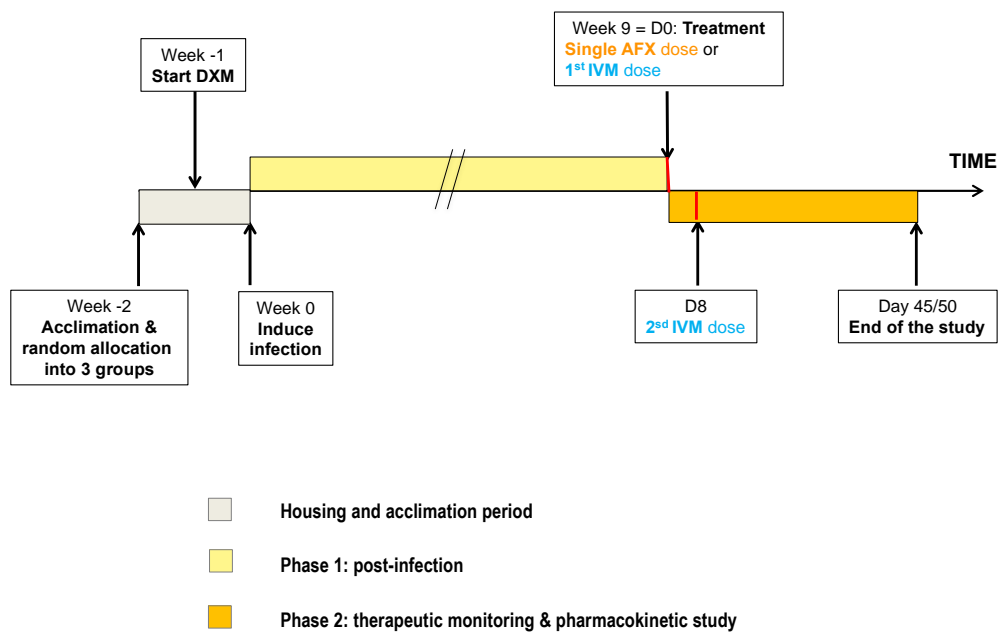
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659 **FIG 1** Study design showing the three experimental phases^a

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662 ^aDXM, dexamethasone; D, day; AFX, afoxolaner; IVM, ivermectin

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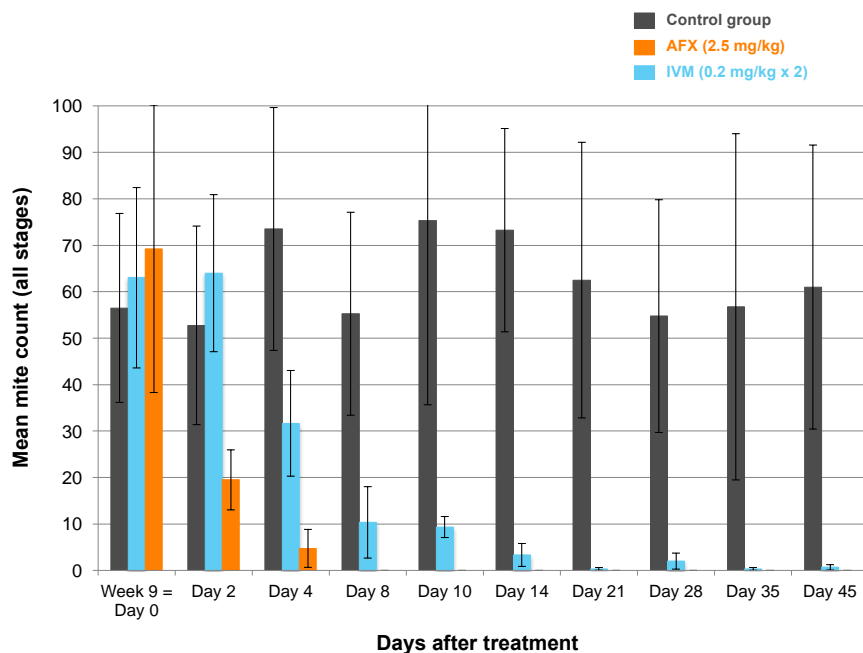
Bernigaud, C. (Auteur de correspondance), Fang, F., Fischer, K., Lespine, A., Aho, L. S., Mullins, A. J., Teclé, B., Kelly, A., Sutra, J.-F., Moreau, F., Lilin, Beugnet, F., Botterel, F., Chosidow, O., Guillot (2018). Efficacy and pharmacokinetics evaluation of a single oral dose of afoxolaner against *Sarcoptes scabiei* in the porcine Scabies model for human infestation.

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

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

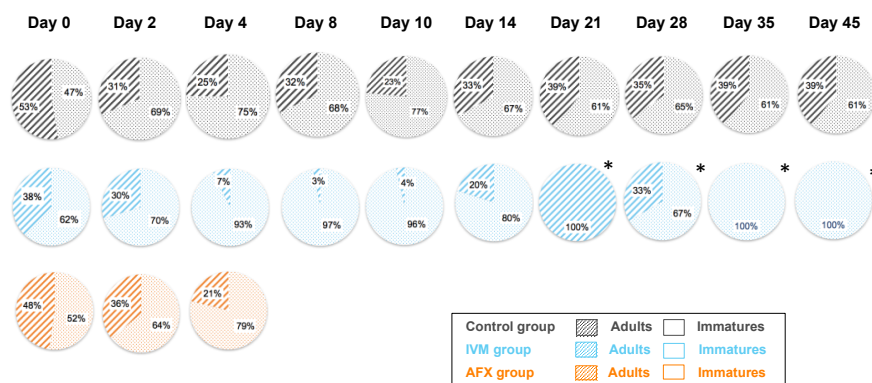
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679 **A.**



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681 **B.**

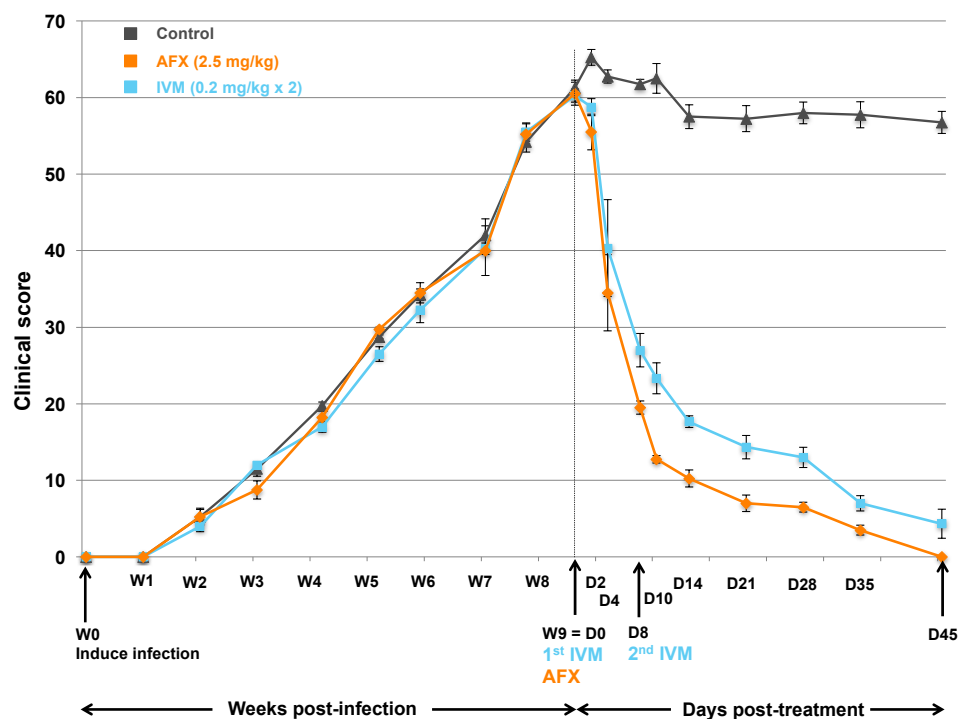


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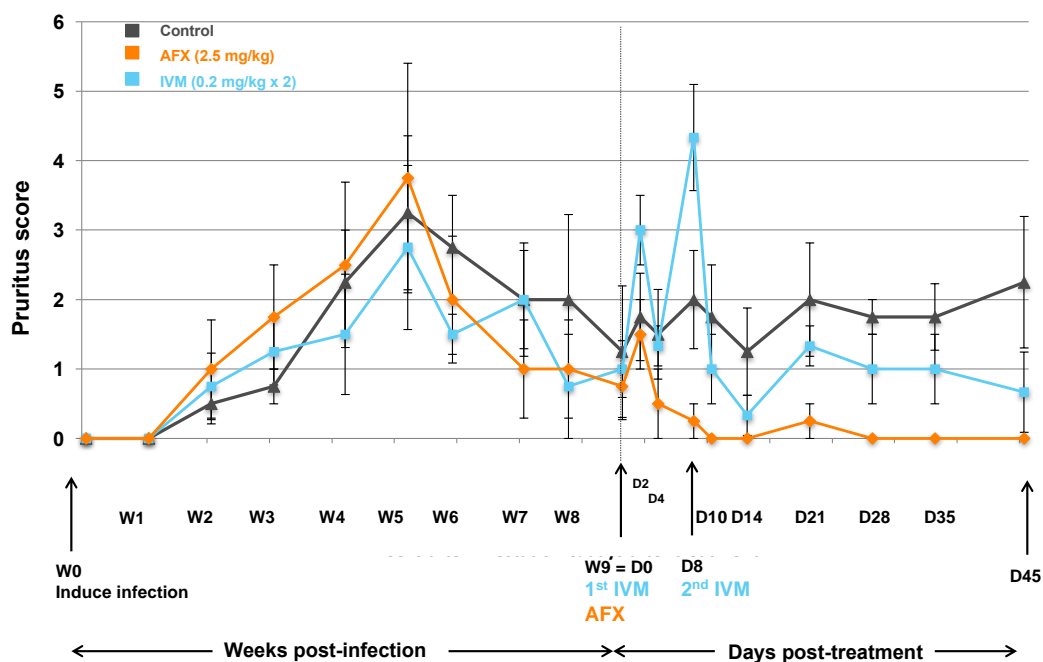
684 ^a*, less than 10 mites were recovered in total from the skin scrapings; AFX, afoxolaner; IVM, ivermectin.

685 **FIG 3** Clinical scores (mean \pm SD) for the AFX- or IVM-treated and control pigs over time from
 686 scabies-infestation to study-end^a
 687



688
 689
 690 ^aThe 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.
 698

699 **FIG 4** Pruritus scores (mean \pm 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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704 ^aD, day; W, week; AFX, afoxolaner; IVM, ivermectin.

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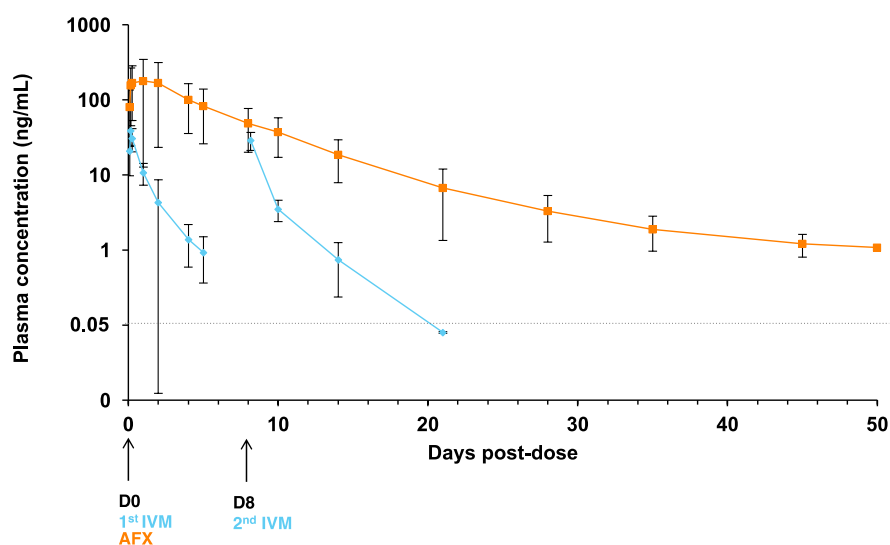
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712 **FIG 5** Mean concentration-time profiles (mean \pm SD, ng/ml) in plasma following oral administration
 713 of AFX or IVM in scabies-infested pigs^a
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718 ^aConcentrations measured on hour 2, 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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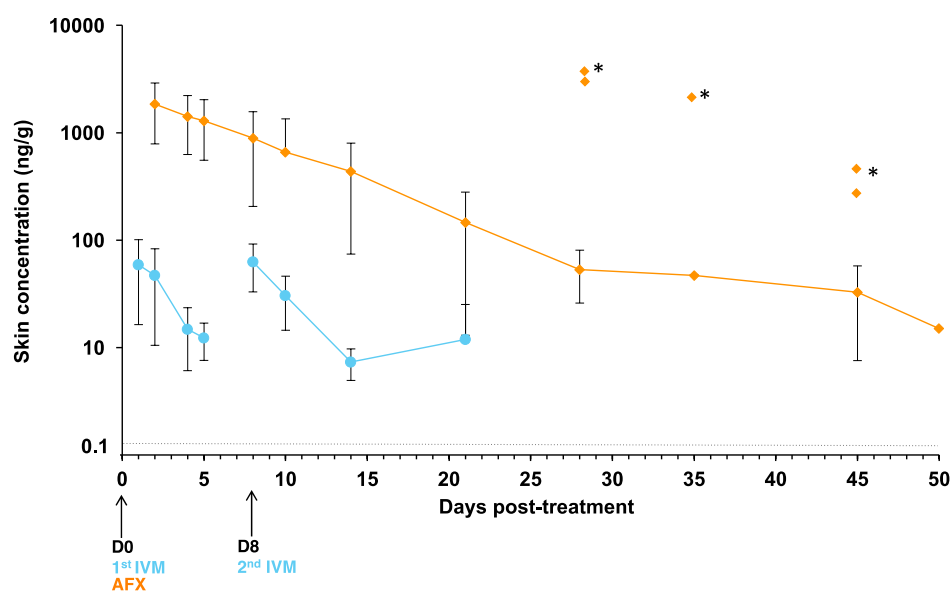
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727 **FIG 6** Mean concentration-time profiles (mean \pm SD, ng/g) in skin following oral administration of
 728 AFX or IVM in scabies-infested pigs^a
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733 ^aConcentrations measured on D1, 2, 4, 5, 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) | Ivermectin Group (n=4) | Control Group (n=4) |
|--------------------|--|---|---|
| | No. of mites (mean mite counts \pm SD) | No. of mites (mean mite counts \pm SD) | No. of mites (mean mite counts \pm SD) |
| | Count range (n) | Count range (n) | Count range (n) |
| | Reduction % (range) | Reduction % (range) | Reduction % (range) |
| | Efficacy % (range) | Efficacy % (range) | Efficacy % (range) |
| Day 0 | 277 (69.3 \pm 61.9) 4–127 | 252 (63 \pm 38.8) 13–98 | 226 (56.5 \pm 40.7) 15–106 |
| Day 2 | 78 (19.5 \pm 12.9) 7–33 71.8% (65.5–94%) 63% (37.4–86.7%) | 256 (64 \pm 33.8) 21–103 NA NA | 211 (52.8 \pm 42.7) 11–93 NA NA |
| Day 4 | 19 (4.8 \pm 8.2) 0–17 93.1% (75–100%) 93.5% (76.9–100%) | 95 (31.7 \pm 22.7) 13–57 49.7% (35.9–75%) 56.9% (22.4–82.3%) | 294 (73.5 \pm 52.3) 8–124 NA NA |
| Day 8 | 0 0–0 100% 100% | 31 (10.3 \pm 15.4) 0–28 83.6% (53.6–100%) 81.3% (66–100%) | 221 (55.3 \pm 43.7) 9–105 NA NA |
| Day 10 | 0 0–0 100% 100% | 9.3 (11.8 \pm 4.5) 5–14 85.2% (61.5–89.9%) 87.6% (49.3–88%) | 301 (75.3 \pm 79.2) 1–151 NA NA |
| Day 14 | 0 0–0 100% 100% | 10 (3.3 \pm 4.9) 0–9 94.7% (82.7–100%) 95.4% (87.7–98.6%) | 293 (73.3 \pm 43.8) 22–124 NA NA |
| Day 21 | 0 0–0 100% | 1 (0.33 \pm 0.6) 0–1 99.5% (98.1–100%) | 250 (62.5 \pm 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 |

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

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

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

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764 "The table shows the clinical score values (score, mean and SD) in the AFX, IVM-treated and control

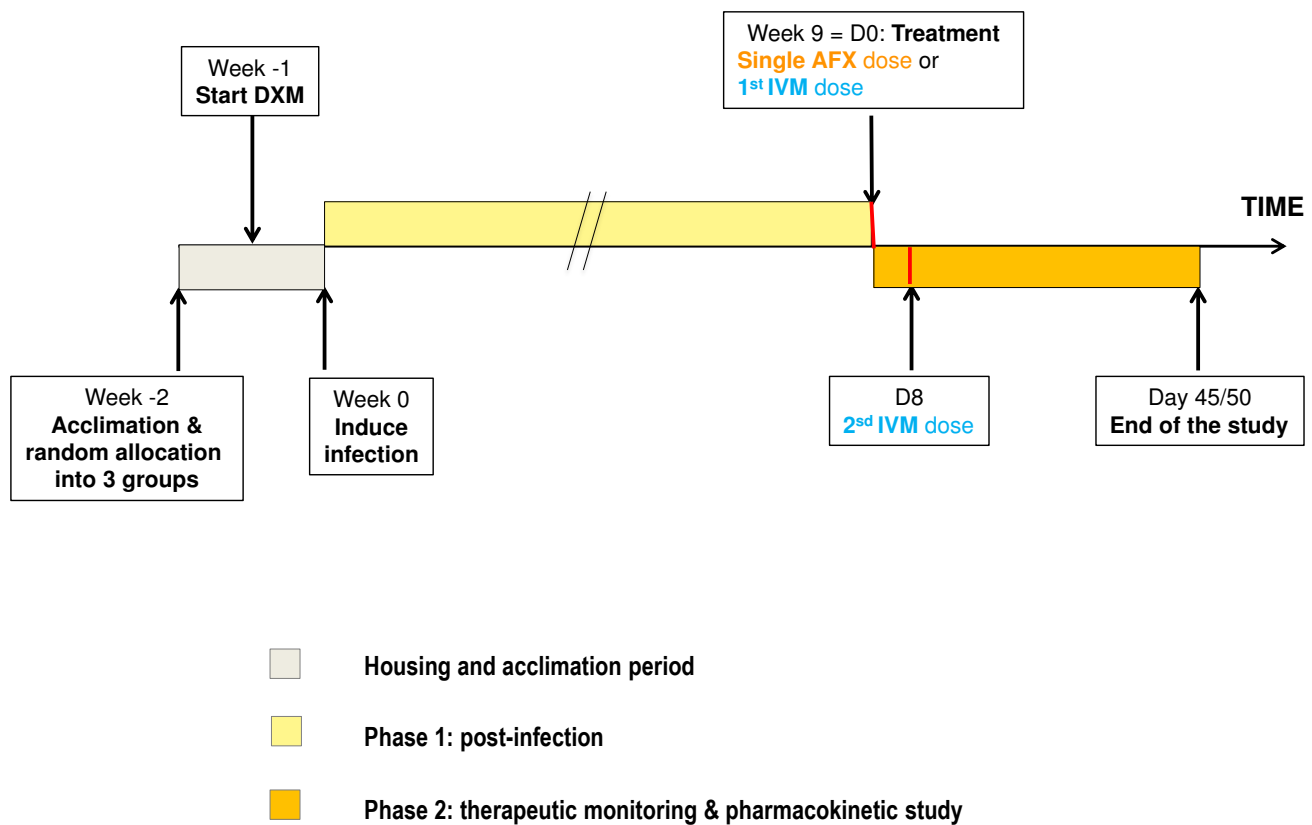
765 groups of pigs on the various assessments days. It also presents the percent improvement of AFX and

766 IVM-treated pigs clinical scores over time from assessment at baseline (D0) to study-end.

767 SD, Standard deviation; NA, not applicable.

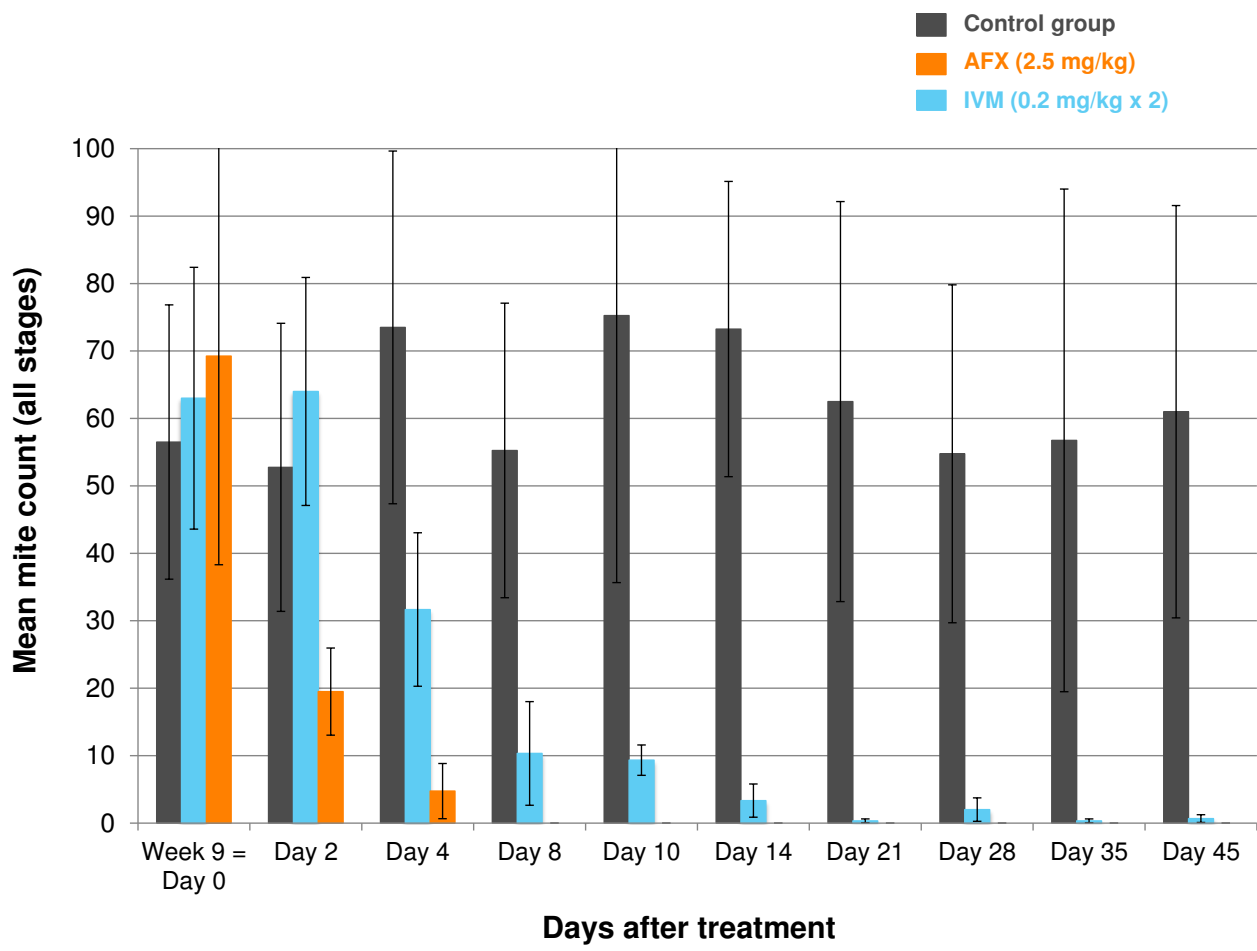
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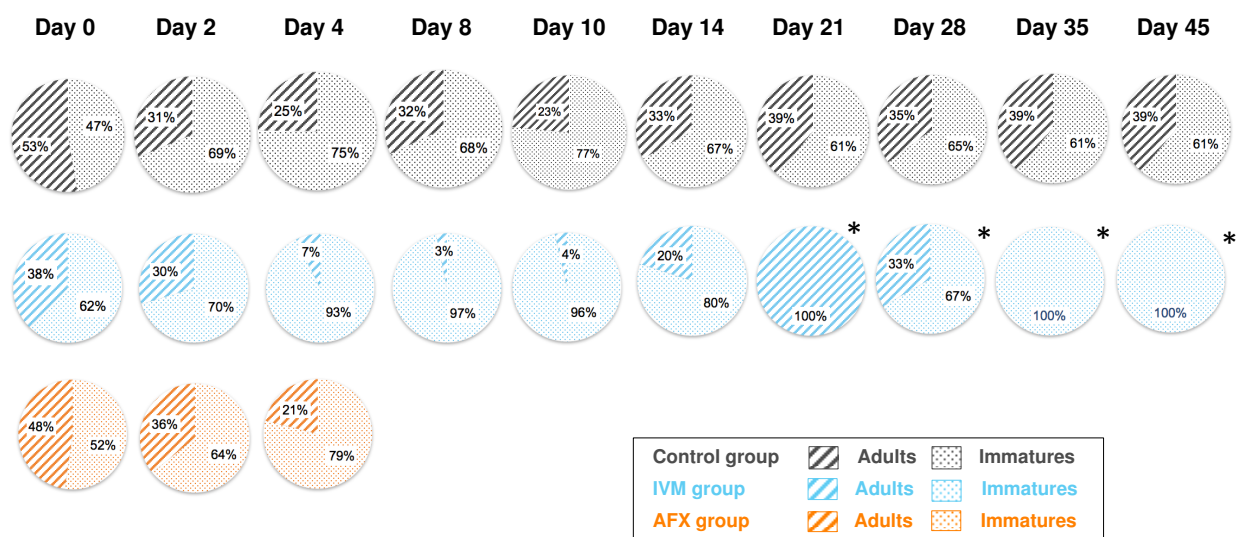
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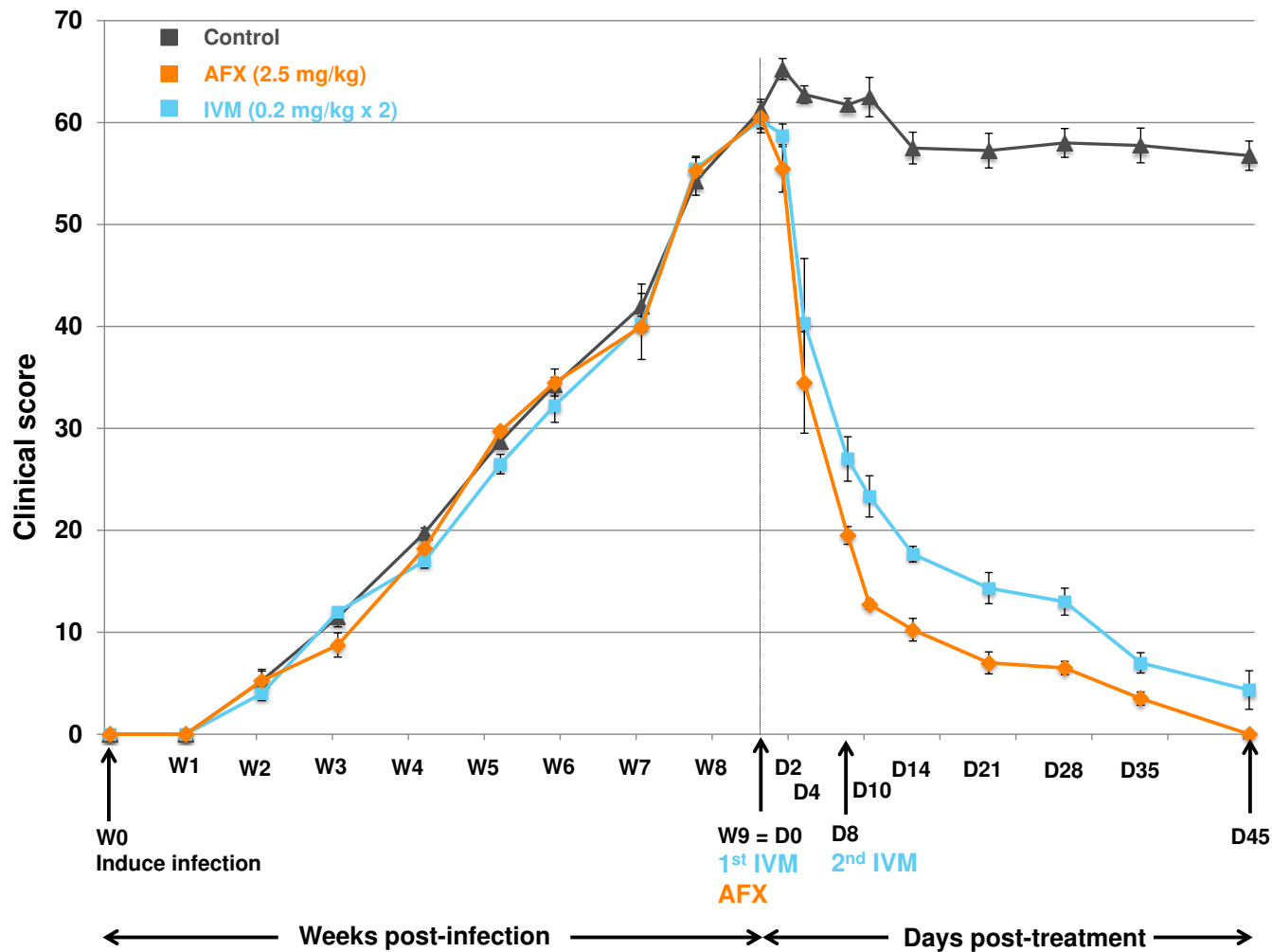
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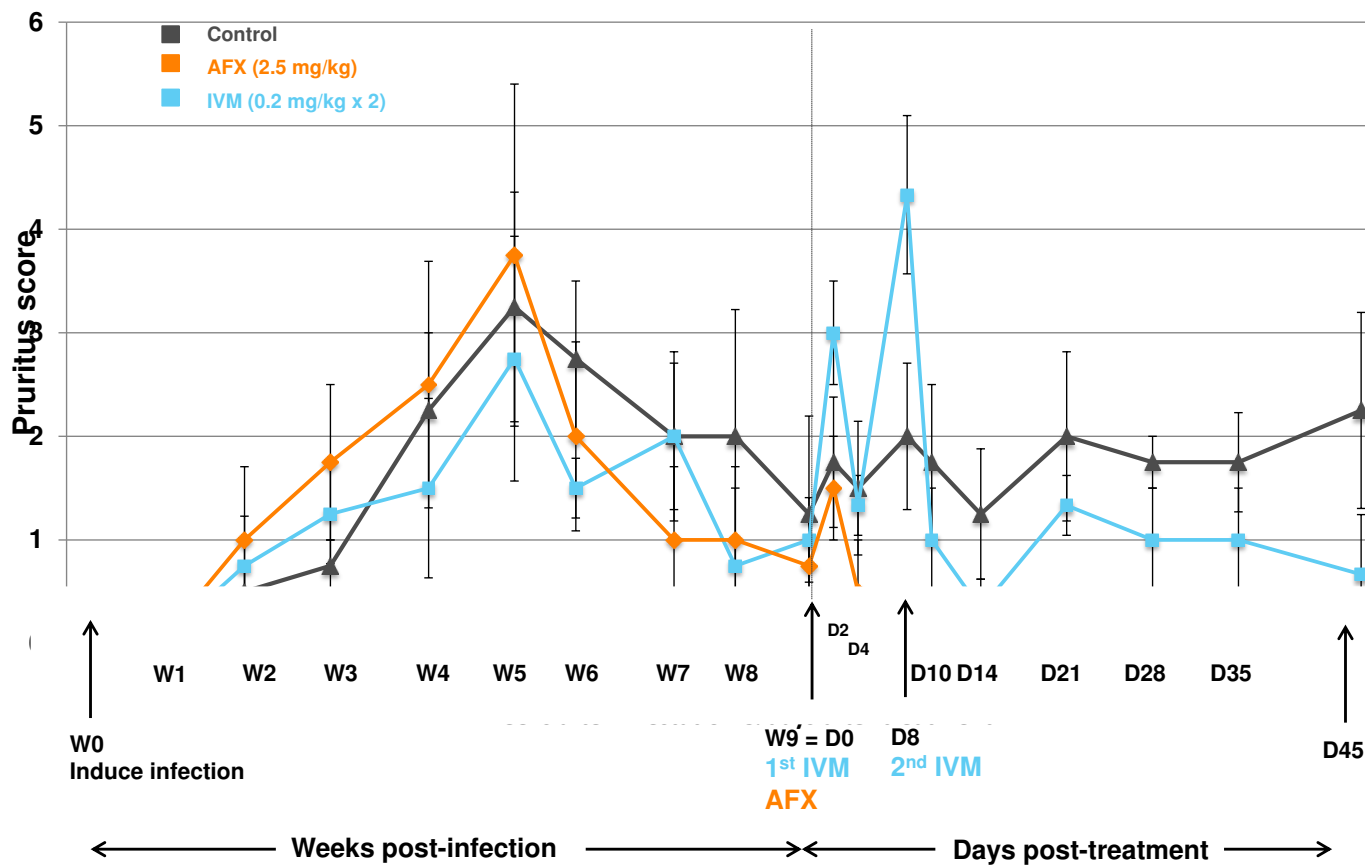
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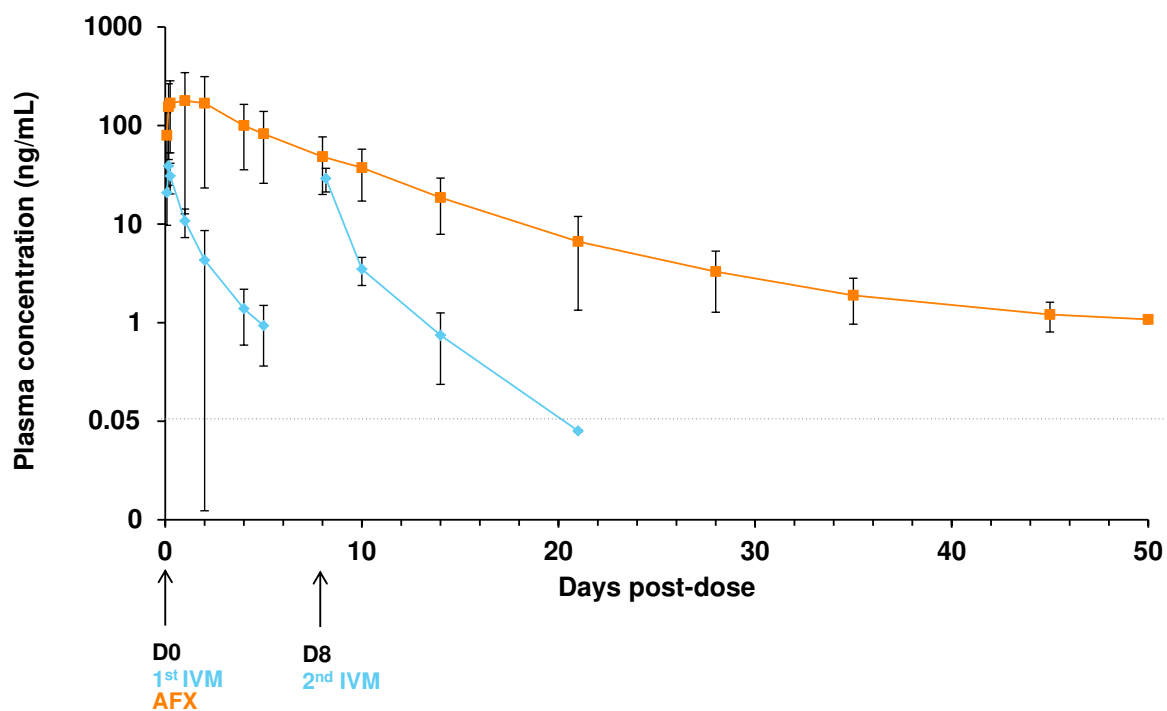


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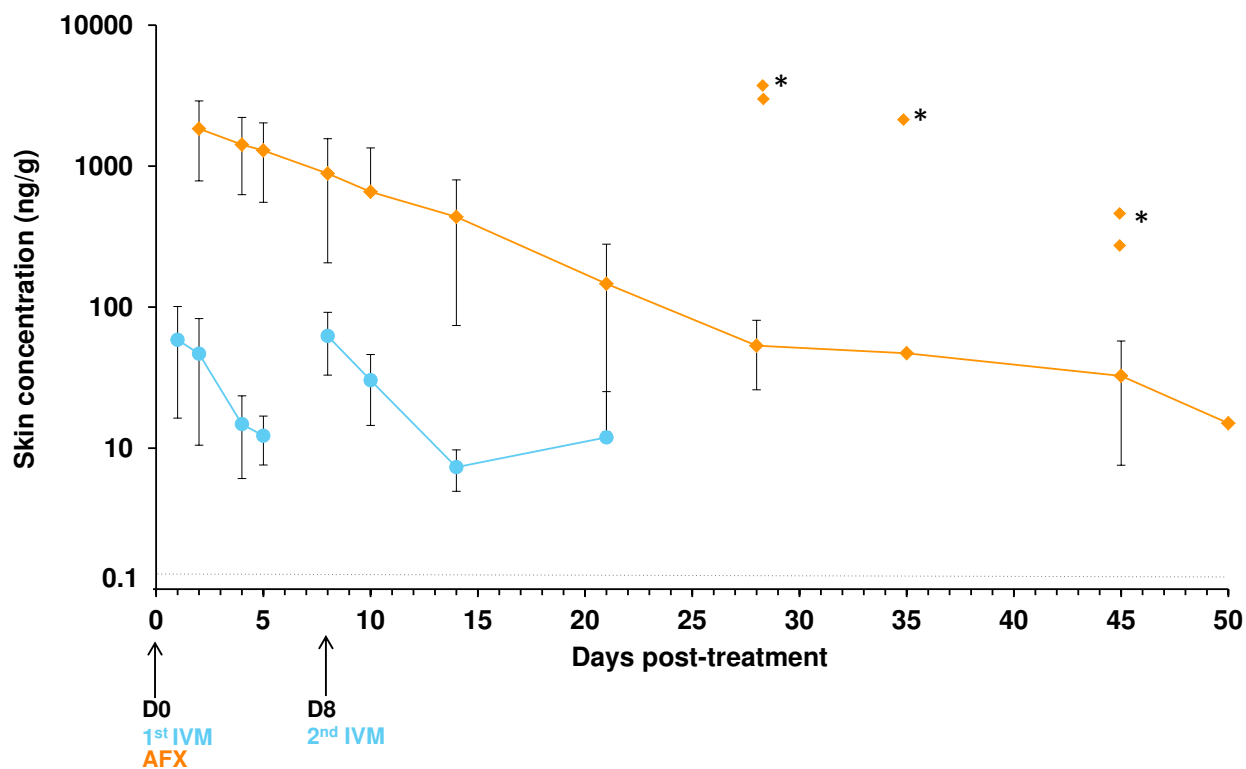


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