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A comparison of the efficacy of two ivermectin formulations against larval and adult *Ascaris suum* and *Oesophagostomum dentatum* in experimentally infected pigs

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Abstract

A study was conducted to evaluate and compare the efficacy of two injectable formulations of ivermectin (IVM-1 and IVM-2) at a dose rate of 0.3 mg/kg bodyweight versus placebo in the treatment and control of larval and adult stages of *Ascaris suum* and *Oesophagostomum* spp. in experimentally infected pigs. Seventy helminth free pigs were allocated on a liveweight basis to 7 groups each comprising 10 pigs (A–G). Group A served as an untreated control group. Groups B and C were used to investigate the efficacy of both formulations against adult stages of *A. suum* and *Oesophagostomum* spp., Groups D and E for efficacy against larval stages of *A. suum* and Groups F and G for efficacy against larval stages of *Oesophagostomum* spp. Pigs of groups A, B, C, D and E were infected on Day-0 with 1000 infective *A. suum* eggs each. Infective larvae of *Oesophagostomum* spp. (10,000/pig) were given on Day-0 to pigs of Groups F and G and on Day-21 to pigs of Groups A, B and C. Treatment was given to pigs of Group A (saline as placebo) on Day-7 and -28, IVM-1 to pigs of Group F on Day-7, pigs of Group D on Day-14 and pigs of Group B on Day-49. IVM-2 was given to pigs of Group G on Day-7, Group E on Day-28 and Group C on Day-49. Pigs of Groups F and G were sacrificed on Day-28, pigs of Groups A, D and E on Day-49 and pigs of Groups B and C on Day-56. Post mortem worm counts showed the following efficacies: (IVM-1) against larval *A. suum* 100%, against adult *A. suum* 94.4%, against larval *Oesophagostomum* spp. 52.0% and against adult *Oesophagostomum* spp. 83.0%. (IVM-2) against larval *A. suum* 100%, against adult *A. suum* 90.3%, against larval *Oesophagostomum* spp. 94.0% and against adult *Oesophagostomum* spp. 94.7%.
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1. Introduction

Since ivermectin (IVM) was introduced into the market, it has been demonstrated in many reports that it possessed excellent activity against the common endoparasites of swine such as *Ascaris suum* and *Oesophagostomum* spp. (for a review see Campbell, 1989). At present, this is still the case and fortunately, unlike the situation in sheep, there are no clearly demonstrated cases of resistance of swine parasites to IVM, although it was suggested that IVM showed a poor efficacy against a laboratory isolate of *Oe. quadrispinulatum* (Dangolla, Ph.D. thesis 1994). Later studies with this strain demonstrated that this was not because of resistance (Várady et al., 1996).

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In many countries, the anthelmintic market was flooded with generic ivermectins after the patent of the original IVM elapsed. Lifschitz et al. (2004) have shown that major differences can be observed in IVM kinetic behaviour when using different generic formulations. However, they did not investigate the consequences of these differences on the efficacy of a treatment. From time to time, veterinarians received complaints from farmers about possible lower efficacies of these generic products but in most cases, these complaints were neither investigated thoroughly nor published. An exception is the paper by Čerňanská et al. (2006) where a possible relationship between a lower efficacy of a generic IVM and anthelmintic resistance in sheep is suggested.

The aim of the present study was to compare the efficacy of a formulation of the original 1% injectable formulation of IVM with a generic 1% injectable IVM, in pigs experimentally infected with the two most common helminths in pigs namely *A. suum* and *Oesophagostomum* spp., the latter being recognised as the dose-limiting species for ivermectin.

2. Materials and methods

The study was conducted in accordance with VICH Guideline 9, Good Clinical Practice (GCP), VICH GL7 Efficacy of Anthelmintics General Requirements and VICH GL16 Efficacy of Anthelmintics: Specific Recommendations for Porcines and approved by the Ethical Commission for experimental studies with animals.

2.1. Pigs

The study was carried out on the Pig Research Station in Raalte, the Netherlands where earlier studies (Eijck and Borgsteede, 2005) had shown pigs to be free from helminth parasites. After weaning, 70 healthy piglets (females and castrated males) were selected and allocated to 7 groups of 10. Each group was balanced for weight and allocation of the groups to a treatment regime was at random. The groups were housed separately, fed the normal diet for their age and had unlimited access to water. Faecal examinations of pooled group faecal samples before the start of the study showed that they were negative for helminth eggs.

Body weights at the start of the study varied from 12.4 to 15.0 kg.

2.2. Helminth infections

Fresh eggs of *A. suum* were obtained from female worms collected at a local abattoir. Eggs were incubated for 6 weeks at 27 °C until the infective stage was reached; thereafter they were stored at 4 °C until used. Doses of 1000 eggs each were prepared for individual pig infections. On the day of infection, the eggs were less than 2 months old. To obtain infective larvae of *Oesophagostomum* spp., faeces were collected from sows from an organic farm where the pigs were known to be heavily infected (Eijck and Borgsteede, 2005). Faeces were cultured for 8 days at 27 °C and infective larvae collected by the method of Roberts and O'Sullivan (1950). Infective doses of 10,000 larvae per pig were prepared. The pigs were infected by oral administration of fluid containing the infective dose. The fluid containing the infective dose was sucked up into a tube using a syringe which had been previously half-filled with water. This tube was then placed at the back of the tongue and the water in the syringe used to flush out the infective material into the mouth. The vial containing each individual infective dose was then rinsed with some water and the above procedure repeated to ensure that each pig received the complete dose of infective material.

2.3. Treatments

The ivermectin formulations used for treatment were IVM-1 (Ivomec 1% injection, Merial Animal Health) and IVM-2 (Ecomectin 1% injection, ECO Animal Health) both given at a dose rate of 0.3 mg/kg bodyweight. Pigs were weighed to calculate the correct dose volume and immediately thereafter treated. The treatment was given by subcutaneous injection in the neck behind the ear. Control animals were injected with sterile saline. The efficacy of the treatment against larval and adult stages was investigated in different groups by selecting treatment dates to coincide with the expected development stage of the parasite at that time. This was based on an assumed pre-patent period of *A. suum* of at least 6 weeks and of *Oesophagostomum* spp of at least 21 days. Table 1 gives the complete design of the study.

The injection sites for both ivermectin formulations and the saline placebo were examined before treatment, at 3, 8 and 24 h post treatment and at necropsy to assess any possible local reactions associated with either treatment.

2.4. Post mortem procedures

Pigs were euthanased by electrocution followed by immediate exsanguination. The entire intestinal tract was removed from each animal. For counting the

Table 1
Study design

Group	No. of pigs	Infection	Date(s) of infection	Treatment	Date(s) of treatment	Sacrifice
A	10	*A. suum Oe.* spp.[a]	D-0, D-21	Saline	D-7, D-28	D-49
B	10	*A. suum Oe.* spp.[a]	D-0, D-21	IVM-1[b]	D-49	D-56
C	10	*A. suum Oe.* spp.[a]	D-0, D-21	IVM-2[c]	D-49	D-56
D	10	*A. suum*	D-0	IVM-1[b]	D-14	D-49
E	10	*A. suum*	D-0	IVM-2[c]	D-14	D-49
F	10	*Oe.* spp.[a]	D-0	IVM-1[b]	D-7	D-28
G	10	*Oe.* spp.[a]	D-0	IVM-2[c]	D-7	D-28

The infective dose was 1000 *Ascaris suum* embryonated eggs and/or 10,000 *Oesophagostomum* spp. 3rd stage larvae per pig.
[a] Mainly *Oesophagostomum dentatum*.
[b] IVM-1: Ivomec 1% injection (Merial Animal Health).
[c] IVM-2: Ecomectin 1% injection (ECO Animal Health).

number of *A. suum*, the small intestine was isolated, stripped from the mesentery and divided into three parts. In separate buckets, tepid water was run into an end of each part and the contents flushed out by drawing the intestine through the fingers. The washings and contents in the bucket were then poured over a wire sieve (mesh 2.0 mm) and worms thus recovered were collected for counting and determination of stage of development and sex.

For *Oesophagostomum* counts, the colon and caecum were opened with scissors, washed with water and the contents and washings collected in a bucket. More water was added to the material in the bucket to adjust the volume to 10 l. Two samples of 100 ml were taken while thoroughly mixing with a motor driven propellor. The samples were sieved (mesh 0.74 mm) and the material from the sieve collected, fixed with a 4% formalin solution and stored for further examination. Worms were counted using a dissecting microscope and species were identified after clearance in chlorallactophenol at 100× magnification according to Várady and Čorba (2000).

2.5. Data analysis

The pig was regarded as the experimental unit in this study and results for individual pigs were analyzed. From the seven treatment groups, each of the following four sets of treatment comparisons was analyzed as a one factor completely randomized design. *Ascaris*-comparisons—(1) against adult stages: Groups A, B and C. (2) Against larval stages: Groups A, D and E. *Oesophagostomum*-comparisons—(3) against adult stages: Groups A, B and C. (4) Against larval stages: Groups A, F and G. Group A served as a control group for all four sets of comparisons. Groups A and B provide *Ascaris* and *Oesophagostomum* counts and is thus associated with two sets of comparisons. Transformation of worm burdens (count + 1) were used for the calculations. Least square means statement was used to provide Tukey pair-wise comparisons of the treatments with a family wise error rate of 5%. If there were obvious outliers, the Bonferroni adjusted Wilcoxon *P*-values were calculated. Effectiveness of treatment was demonstrated if the percentage drop in geometric mean number of worms in treated pigs compared with untreated control pigs was at least 90% using the formula $[C - T]/C - 100$ where C = geometric mean of the control group and T = the geometric mean of the treated group. This is in accordance with VICH GL16 'Efficacy of anthelmintics: specific recommendations for Porcines' (VICH GL16, 2001).

3. Results

3.1. Clinical signs

Both ivermectin formulations were well tolerated with no systemic signs or local reactions at the injection site. No pigs had to be excluded from the study for any reason.

3.2. Worm counts

Faecal samples taken before infection were all negative for helminth eggs and all worms found after slaughter were therefore considered to be the result of the artificial infection.

The mean worm numbers of *A. suum* and the efficacy of the treatments are presented in Table 2 and those of *Oesophagostomum* spp. in Table 3.

3.3. Efficacy

Treatment against larval stages of *A. suum* was 100% effective for both formulations ($P < 0.02$). Both formulations were also highly effective against adult

Table 2
Post mortem worm counts of *Ascaris suum* (infective dose 1000 eggs)

	Group				
	A	B	C	D	E
Treatment	Placebo	IVM-1[a]	IVM-2[b]	IVM-1	IVM-2
Day of infection	D-0	D-0	D-0	D-0	D-0
Day of treatment	-	D-49	D-49	D-14	D-14
Day of sacrifice	D-49	D-56	D-56	D-49	D-49
No. pigs infected	8/10	2/10	4/10	0/10	0/10
Mean no. worms	12.1	0.8	0.9	0	0
Max.	48	7	4	0	0
Min.	0	0	0	0	0
Geom. mean	5.68	0.32	0.55	0	0
Efficacy	–	94.4% ($P = 0.0017$)	90.3% ($P = 0.0045$)	100% ($P = 0.02$)	100% ($P = 0.02$)

[a] IVM-1: Ivomec 1% injection (Merial Animal Health).
[b] IVM-2: Ecomectin 1% injection (ECO Animal Health).

Table 3
Post mortem worm counts of *Oesophagostomum* spp. (infective dose 10,000 larvae)

	Group				
	A	B	C	F	G
Treatment	Placebo	IVM-1[a]	IVM-2[b]	IVM-1	IVM-2
Day of infection	D-0	D-21	D-21	D-0	D-0
Day of treatment	-	D-49	D-49	D-7	D-7
Day of sacrifice	D-49	D-56	D-56	D-28	D-28
No. pigs infected	10/10	8/10	7/10	10/10	7/10
Mean no. worms	495, 50% ♂♂	200, 76.3% ♂♂	70, 57.1% ♂♂	270, 41.8% ♂♂	90, 33.3% ♂♂
Max.	850	600	250	800	250
Min.	50	0	0	50	0
Geom. mean	409.42	69.5	21.57	196.59	24.74
Efficacy	–	83.0% (Tukey $P = 0.1156$), (Wilcoxon $P = 0.0285$)	94.7% (Tukey $P = 0.0057$), (Wilcoxon $P = 0.0057$)	52.0% (Tukey $P = 0.5307$), (Wilcoxon $P = 0.0894$)	94.0% (Tukey $P = 0.0009$), (Wilcoxon $P = 0.0007$

[a] IVM-1: Ivomec 1% injection (Merial Animal Health).
[b] IVM-2: Ecomectin 1% injection (ECO Animal Health).

A. suum, 94.4% for IVM-1 ($P = 0.0017$) and 90.3% for IVM-2 ($P = 0.0045$), respectively, relative to the untreated controls There were no significant differences between IVM-1 and IVM-2 ($P = 0.9217$).

Efficacy of the treatments against larval stages of *Oesophagostomum* spp. was 52.0% for IVM-1 (n.s., $P = 0.5307$) and 94.0% for IVM-2 ($P = 0.0009$). The Tukey *P*-value comparing the efficacy of both ivermectin formulations was 0.0143, but with the Wilcoxon test it was 0.0645. Against adult stages, the efficacy of the treatments was 83.0% for IVM-1 and 94.7% for IVM-2. Pigs treated against the adult stage of *Oesophagostomum* spp. with IVM-2 had significantly lower total numbers of worms compared to pigs given the placebo using both statistical methods ($P = 0.057$ with the Tukey and 0.0006 with the Wilcoxon). The worm counts for IVM-1 were not significantly lower compared to placebo using the Tukey method, but were using the Wilcoxon method ($P = 0.1156$ with the Tukey and 0.0285 with the Wilcoxon). The counts for the IVM-1 and IVM-2 groups were not significantly different using either statistical method. The percentage drop in the geometric mean count for IVM-2 treated pigs as compared with non-medicated pigs was well above the required 90% for a claim. No significant differences were present between both treatments ($P = 0.3880$ with the Tukey and 0.2226 with the Wilcoxon).

4. Discussion

The present study has shown that the generic ivermectin formulation was as effective as the original

product. In fact, the efficacy percentages against the ivermectin dose limiting parasite in pigs (*Oesophagostomum* spp.), were higher for the generic product, although differences between the formulations were not statistically significant.

The high efficacy of ivermectin against *A. suum* in experimentally and naturally infected pigs as an injection or as an in-feed preparation has been reported earlier (Stewart et al., 1981; Schillhorn van Veen and Gibson, 1983; Marchiondo and Szanto, 1987; Campbell, 1989; Primm et al., 1992). In the case of *Oesophagostomum* spp., the two main species in pigs are *Oe. dentatum* and *Oe. quadrispinulatum*. It is generally accepted that *Oe. dentatum* is the more dominant and most studies have been done with this species. In a number of such studies, Petersen et al. (1996) found a variable efficacy of ivermectin at 0.3 mg/kg against *Oe. dentatum* adults (69.1 and 96.2%) while against L4 efficacy of 90.9% was reported. One interesting finding in the their studies against adult worms was that mainly male worms survived the treatment (80.3 and 99.2%, respectively). This was less obvious in the present study, although in groups B and C, the survival of males was higher than that of females (76.3 and 57.1%, respectively).

Várady et al. (1996) reported a lower efficacy of ivermectin at the recommended dose against *Oe. quadrispinulatum* compared with *Oe. dentatum*. In the present study, we were not able to evaluate the efficacy of both ivermectin formulations against *Oe. quadrispinulatum* because relatively few worms of this species were found in the untreated controls.

The ranges of the infective doses used in this study were in accordance with VICH Guideline 16 (250–500 eggs of *A. suum* and 2000–15,000 larvae of *Oesophagostomum* spp.). Also the timings of treatments following infection, for testing the efficacy against larval stages, were within the guidelines, i.e. 7–9 days post infection (p.i.) for *Oesophagostomum* spp. and 11–15 days p.i. for *A. suum*. When evaluating efficacy against adult *Oesophagostomum* spp. treatment was within the recommended period of 28–45 days p.i. In the case of *A. suum* an interval of 65 days is recommended in the Guidelines but it is our experience that after experimental infection, *A. suum* can reach patency after a minimum of 42 days. We have also observed that pigs often lose their worm burden soon after patency is established and it was for these reasons that an infection to treatment interval for *A. suum* of 49 days was selected. To evaluate the efficacy of any anthelmintic, it is of the utmost importance that sufficient untreated control animals have adequate worm burdens to allow meaningful comparisons with treated groups. Fortunately, in our study 8 out of the 10 control pigs had worms and efficacy calculations were therefore possible.

According to VICH Guide Line 16, efficacy of 90% or more is required for a label claim. Based on the findings of the present study, treatment with IVM-1, the currently marketed pioneer Merial product, would be regarded as effective only against larval and adult *A. suum* whereas treatment with IVM-2, the generic ivermectin formulation, proved effective against larval and adult stages of both *A. suum* and *Oesophagostomum* spp. There is no obvious explanation for the apparent inferior efficacy of the pioneer ivermectin formulation against *Oesophagostomum* spp. It is unlikely that anthelmintic resistance could be a factor as the farm from which the isolate used for challenge originated, was an organic farm where there was no history of any regular treatments with ivermectin. If resistance should have played a role, it should have affected the results of the generic ivermectin also. Under the same experimental conditions, the generic formulation of ivermectin was highly effective against the same field strain of *Oesophagostomum* spp. Despite the unexplained variability in worm burdens found in this study, it does show that a quality generic anthelmintic can provide a similar or, in some cases superior level of parasite control as a pioneer product.

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