Research paper

First detection of ivermectin resistance in oesophagostomum dentatum in pigs

Michele Macrelli[a,*], Susanna Williamson[a], Sian Mitchell[b], Richard Pearson[c], Leigh Andrews[d], Alison A. Morrison[d], Mandy Nevel[e], Richard Smith[f], Dave J. Bartley[d]

[a] Animal and Plant Health Agency (APHA), Rougham Hill, Bury St Edmunds, Suffolk, IP33 2RX, United Kingdom
[b] Animal and Plant Health Agency (APHA), Job's Well Road, Carmarthen, Carmarthenshire SA31 3EZ, United Kingdom
[c] George Pig Practice, High Street, Malmesbury, Wiltshire, SN16 9AU, United Kingdom
[d] Moredun Research Institute (MRI) Pentlands Science Park, Edinburgh EH26 0PZ, United Kingdom
[e] Agriculture and Horticulture Development Boards (AHDB) Pork, Stoneleigh Park, Kenilworth, Warwickshire CV8 2TL, United Kingdom
[f] Animal and Plant Health Agency (APHA), Woodham Lane, Addlestone, Surrey, KT15 3NB, United Kingdom

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ABSTRACT

In 2016 suspected reduced ivermectin (IVM) efficacy in Oesophagostomum species in pigs was reported in England. Following this initial report, APHA raised awareness amongst private pig veterinary practitioners of the need to monitor the efficacy of the worm control on pig units. In 2017 another veterinary practitioner highlighted a potential in-field lack of IVM efficacy in treating Oesophagostomum species in sows on another breeder-finisher unit. In this trial, the efficacy of IVM against Oesophagostomum species worms has been investigated to determine whether suspected reduced efficacy (52% reduction in mean faecal egg count 14 days post ivermectin administration) on a mixed indoor and outdoor breeder-finisher pig farm in England reflected true IVM resistance under controlled experimental conditions. On days 0 and 40 of the trial, twenty helminth-naive pigs were artificially infected per os with 5000 Oesophagostomum L3 obtained from the farm under investigation. The pigs were allocated to treatment or control groups (n = 10 per group). Treatment group pigs received IVM (0.3 mg kg body weight) by sub-cutaneous injection as per manufacturer's instructions on day 44. Control group animals were left untreated. Faecal worm egg counts were monitored throughout the trial from day 15 post infection to determine time to patency. On day 50 all pigs were euthanased to assess the worm burdens. Resistance to IVM was confirmed in Oesophagostomum dentatum based on the results of a faecal egg count reduction test (FECRT) and a controlled efficacy test (CET). Efficacy based on mean reduction in faecal egg count of IVM-treated pigs compared to untreated control pigs was 86%. Mean reduction in IVM-treated pig worm burdens was 5% against an adult worm population and 94% against an L3/L4 population. The apparent discrepancy between FECRT and CET efficacy results appears to be due to egg development and/or oviposition suppression in IVM-treated female worms. The detection of IVM resistance in Oesophagostomum species worms for the first time in UK pigs is particularly important considering the global situation where resistance to pyrantel, levamisole and benzimidazole anthelmintics in Oesophagostomum species in pigs have already been reported. The results also provide an opportunity to discuss the wider issue of anthelmintic usage and efficacy on pig farms and highlight the need for wider surveillance for the occurrence of anthelmintic resistance in pigs.

1. Introduction

In the UK there is estimated to be approximately 30,000 premises with pigs, including those with pet pigs (APHA, 2017b), and a pig population of approximately 4.5 million. The UK pig industry has a variety of production systems including indoor and outdoor units, and straw-based and slatted accommodation with straw systems predominating. In England, around 40 per cent of the commercial pig breeding herd is kept outdoors (PHWC, 2017). In such systems, pigs may be more vulnerable to helminth infection (Mercy et al., 1989; Pattison et al., 1980; Roepstorff et al., 2011) and there is potential for greater clinical and economic impact of endoparasitism unless effective control is implemented. Oesophagostomum dentatum represents one of the most common intestinal parasites that affect pigs in the UK and

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* Corresponding author.
 E-mail address: Michele.macrelli@apha.gov.uk (M. Macrelli).

world-wide (Pattison et al., 1980; Taylor et al., 2016). The clinical significance of *O. dentatum* has been debated; although, clinical disease is infrequent and most *O. dentatum* infections in pigs remain subclinical, production losses have been reported (Daugschies, 2013).

Control of nematodes in the UK, as elsewhere in the world, is achieved through a combination of management practices, particularly good hygiene or land management to interrupt transmission and the judicious use of anthelmintics. In the UK, the anthelmintics used in pigs are from one of two broad spectrum anthelmintic classes (benzimidazoles and avermectins). Avermectins are also used in the control of ectoparasites, namely lice or mange mites. Although no anthelmintic resistance has been demonstrated in pigs in the UK to date, cases of resistance to benzimidazoles, levamisole (Bjorn et al., 1990; Gerwert et al., 2002; Varady et al., 1996), and pyrantel (Roepstorff et al., 1987), in *Oesophagostomum* species have been reported in Europe (European Medicines Agency, 2017). The general subclinical nature of *Oesophagostomum* species infection in pigs may explain why drug resistance has not been generally revealed on clinical grounds. In 2016, concern was raised to the Animal Plant Health Agency (APHA) about suspected reduced ivermectin (IVM) efficacy in *Oesophagostomum* species on a pig farm in England (APHA, 2016). Faecal egg count reduction tests indicated reduced efficacy, but unfortunately it was not possible to investigate more fully at that time. This initial report raised awareness amongst private pig veterinary practitioners of the need to monitor the efficacy of the worm control on pig units. In 2017 another veterinary practitioner highlighted a potential in-field lack of ivermectin efficacy in treating *Oesophagostomum* species in sows on a breeder-finisher unit in England on which dry sows and gilts have free access to pasture over the summer months (APHA, 2017a). The on-farm FECRT gave rise to the suspicion of ivermectin resistance (52% reduction in mean faecal egg counts 14 days post ivermectin administration) which was then investigated in a controlled efficacy trial.

2. Materials and methods

2.1. Farm and herd details

The enterprise, on which the initial study took place, is a 300-sow breeder-finisher unit on which dry sows and gilts have free access to pasture between March and October and are housed over winter in straw yards. Sows farrow indoors all year round. The land where the sows and gilts are kept had been used by pigs each year for approximately 50 years.

The herd is open; replacement breeding gilts are introduced every eight weeks from a single nucleus herd with a quarantine period of four weeks before gradual introduction to the main herd. During this period the replacement breeding gilts are observed daily and vaccinated against *Erysipelas*, parvovirus, leptospirosis and *Mycoplasma hyopneumoniae*. The approximate replacement rate is 45%. Although artificial insemination represents the main method of breeding, there are also nine boars on site. The herd is positive for *Mycoplasma hyopneumoniae* and *Streptococcus suis* and free from PRRS and swine dysentery. Sows and gilts enter in the farrowing room a week prior to farrowing and they remain until the piglets are weaned, which is around 28 days of age. In the farrowing room the sows and gilts are kept in individual farrowing crates on a fully-slatted floor. The farrowing room is cleaned and disinfected between batches.

2.2. Historic parasite management

Parasite control in sows on the farm over the last ten years has involved IVM administration (Ivomec® 1% injectable; 0.3 mg/kg bodyweight) to sows on entry to the farrowing rooms. Estimation of the IVM dose rate is based on the farmer's experience and sows are not weighed prior to treatment. Lice control in the gilts is achieved by in-feed IVM (Ecomectin® 6 mg/g premix for medicated feed; 0.1 mg/kg bodyweight)

for one week every six to eight weeks. Ivermectin (Ivomec® 1%) is also administered by injection to the gilts one week before their first farrowing. The investigation was prompted when faecal monitoring performed by the attending veterinary surgeon raised suspicious of inefficacy.

2.3. On-farm faecal egg count reduction test

The on-farm faecal egg count reduction test (FECRT) was conducted on two groups of 10 individually identified sows (from parity one to parity six). One group (Group 1) was treated with injectable IVM (Ivomec® 1% injectable; 0.3 mg/kg bodyweight)) subcutaneously and the second group (Group 2) was treated with oral benzimidazole (Fenbendazole; Pigfen® 5 mg fenbendazole/kg bodyweight in feed, once) and injectable IVM (Ivomec® 1%); the IVM treatment still being required for control of lice. These anthelmintic treatments were administered when the sows entered in the farrowing house, around one week before farrowing. Although the sows were not weighed before the anthelmintic administration the dose administered was sufficient to treat a 300 kg sow. The farmer regularly reviewed sow weights based on those recorded in cull sows from the farm, and the sows' bodyweights would not have exceeded 300 kg. However, the on-farm FECRT was not performed according to all the guidelines of the WAAVP (Coles et al., 2006).

Individual sow faeces were collected for faecal egg counts just prior to treatment and one and two weeks post-treatment and were examined at APHA to establish the individual percentages for faecal egg count reduction. These sows were kept in individual farrowing crates on a fully-slatted floor in the farrowing rooms during the period of assessment. No control (untreated) group of sows was possible. Faecal egg counts were determined using a modified improved McMaster method (Ministry of Agriculture, Fisheries and Food, 1986) with a detection limit of 50 eggs per gram (EPG) of faeces.

Percentage faecal egg count reduction (FECR) was determined according to the formula $(1 - [T2/T1]) \times 100$. Where T1 and T2 are the arithmetic mean eggs counts in the group (McKenna, 1990) treated with Ivomec® and Ivomec® + Pigfen® on the days of treatment (day 0) and on day 14 post treatment respectively.

2.4. Culture of larvae and morphological larval identification

The strongyle type eggs (Fig. 1a) detected during the on-farm FECRT were further identified by APHA. Following APHA standard operating procedures (Ministry of Agriculture, Fisheries and Food, 1986) the strongyle-type eggs were cultured to third stage larvae from 100 g of pooled sow faeces collected prior to IVM treatment and two weeks post- treatment. The pooled samples were incubated at 27 °C for 7 days. The larvae were then recovered using a Baermann apparatus. Subsequently 100 L3 were examined and morphologically identified as *Oesophagostomum* species (Fig. 1b) according to Thienpont et al. (1979).

2.5. Controlled efficacy trial

Twenty helminth naive Duroc cross pigs, from a high health status herd, were divided into two groups which were balanced for sex (seven male and three female pigs) and weight (average weight of the groups were 18 kg and 19 kg for control and treated groups respectively with individual weights ranging from 13 to 24 kg).This allocation was made on arrival to minimise welfare issues (aggression and stress) associated with mixing established groups (Peden et al., 2018). The allocation of the groups to be either treatment or control (n = 10 per group) was random. Pigs were housed on straw bedding with free access to water and fed on a concentrate pig diet.

All pigs were individually infected at around two months of age with 5000 *Oesophagostomum dentatum* larvae (L3) *per os* each on day 0 and a further 5000 L3 *per os* each on day 40. The larvae were generated
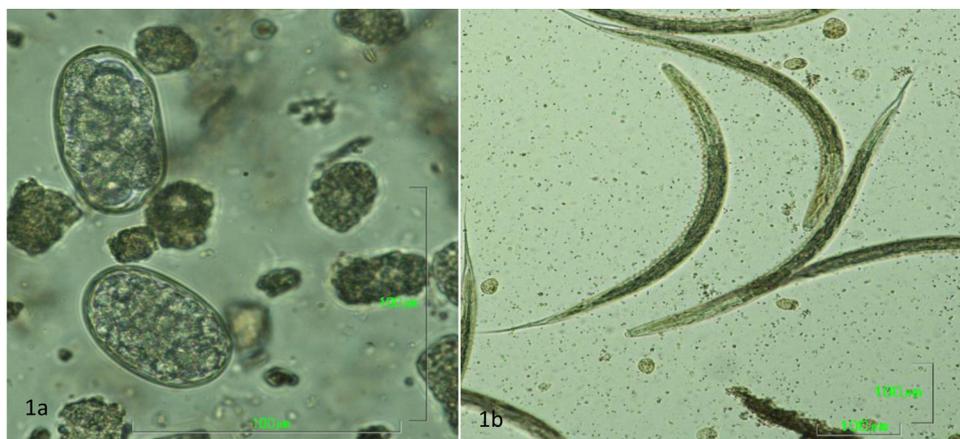
Fig. 1. (a and b) Images of Strongyle-type eggs (a) identified prior to ivermectin administration, (b) *Oesophagostomum* sp. third stage larvae after faecal material coproculture.
(Images kindly provided by Claire Corfield, APHA Shrewsbury)

from eggs in pre-treatment faeces samples from the farm under investigation using the coproculture techniques described by Coop et al. (1982).

Treatment group animals received IVM (0.3 mg kg body weight) by sub-cutaneous injection as per manufacturer's instructions on day 44. All pigs were individually weighed using calibrated electronic scales. Control group animals were left untreated. Faecal worm egg counts were monitored throughout the trial from day 15 post infection to determine time to patency and as a proxy measure of patent worm burdens and worm fecundity. All pigs had zero egg counts at the beginning of the trial. A modification of the salt flotation faecal egg count method as detailed by Jackson and Christie (1972) was used, with a sensitivity of one egg per gram. On day 50 all pigs were euthanized and the stomach, small and large intestines were removed and separated. To obtain estimations of the total worm burdens each section was washed through in warm physiological saline and then incubated in saline at 38 °C for a minimum of four hours. Then the gastrointestinal mucosae were scraped off and collected. Ten percent aliquots were examined to estimate worm burdens for the large intestine samples whereas 2% were examined for the stomach and small intestines. Enumerated nematodes were morphologically examined to determine species using criteria described by Nosal et al. (2013). The infection strategy was designed to investigate the efficacy of ivermectin against adult and 4-day post-infection (L3/L4) larvae. The protocol is based on the WAAVP recommendations (Duwel et al., 1986; Hennessy et al., 2006) and VICH (VICH GL16 (ANTHELMINTIC: PORCINE), 2001) guidelines. Adult female worms were identified to assess the presence/absence of eggs *in utero*. Due to the large numbers of eggs generated by female worms, quantitative counts were not undertaken, but all individual female worms were subjectively ranked between 1 and 3, with 1 indicating no eggs observed, 2 indicating small numbers of eggs observed and 3 indicating the uterus was packed with eggs.

Nematode burdens, FECs and eggs *in utero* scores were square-root transformed to normalize for variance. Burdens were compared using one way ANOVA (Minitab version 17). The percentage efficacy (PE) of

the IVM treatment was calculated using the standard formulae (Dash et al., 1988), (1 − [T2/T1][C1/C2]) x 100 where C and T are the faecal egg counts of untreated control and post treatment animals and 1 and 2 represents pre and post treatment faecal egg counts respectively. Efficacies based on worm burden were calculated using the equation ([C–T]/C) x 100. A different equation was chosen from the on-farm FECRT in order to best utilise the data available from the control (untreated) group. Anthelmintic resistance was deemed to be present when the PE in reducing nematode burden or FEC was < 90%, (Coles et al., 2006).

2.6. Ethical approval

All experimental procedures described here were assessed and received approval from the Moredun Research Institute Experiments and Ethics Committee and were conducted under the legislation of a UK Home Office License in accordance with the Animals (Scientific Procedures) Act of 1986.

3. Results

3.1. On-farm faecal egg count reduction test

Faecal eggs were detected in all sows prior to administration of IVM, group arithmetic mean count was 1400 eggs per gram (EPG) ranging between 100 and 3400 EPG (Table 1). Following ivermectin administration group 1 FECs averaged 665 EPG, ranging between 0 and 2850 EPG at day 14 post treatment. Following Ivermectin + fenbendazole treatment (group 2), no eggs were detected in any animal at day 14 post treatment. In Groups 1 and 2 efficacies based on faecal egg count reduction were 52% (95% confidence intervals −31, 82) and 100% respectively.

Table 1
Arithmetic mean faecal egg counts of two groups of pigs (day 0, 7 and 14) and treatment efficacy estimations 14 days post ivermectin (IVM) or IVM + fenbendazole administration.

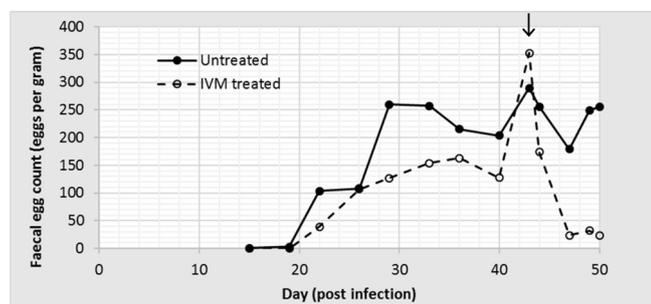| Treatment group	Mean EPG ± SEM (Range)			Estimated percentage efficacy (95% Confidence Intervals)
	day 0	day 7	day 14	
Ivermectin (IVM)	1400 ± 318 (100 -3400)	1825 ± 1136 (50-11600)	665 ± 319 (0-2850)	52 (−31, 82)
IVM + fenbendazole	1331 ± 385 (300–3400)	12.5 ± 12.5 (0-100)	0 ± 0 (0–0)	100 (100, 100)

Fig. 2. Arithmetic mean faecal egg counts of the two groups of pigs (untreated control (●) and ivermectin treated group (IVM; ○)) 15–50 days post infection with 5000 infective larvae (Note a second challenge of 5000 L3 was administered on day 40 but will not have added to the egg counts as larvae would not have reached maturity/patency). Treatment was on day 44 post first infection (↓).

3.2. Controlled efficacy trial

3.2.1. Faecal egg counts

A small number of animals (n = 2) showed positive FECs on day 15 post infection (PI) but FECs in the majority of animals did not become positive until day 22 post first infection (Figs. 2 and 3). Group mean egg counts increased from first detection and peaked on day 43 post first infection at ~290 and 350 eggs per gram for the IVM treated and untreated control groups respectively. Following the administration of IVM, mean FEC significantly reduced in treated animals (p = 0.001) compared to untreated animals by 6 days after treatment. The faecal egg count reduction based on comparison of counts in control and treated animals was 86% seven days post IVM administration.

3.2.2. Worm burdens

The average percentage establishment rate of nematodes, based on the sum of adult (means count 63 worms) and L3/L4 population (means count 33 worms) was 1.9% in the control pigs (Table 2). All animals from both groups had positive adult and L3/L4 worm burdens at post-mortem examination.

Significant reductions in nematode burdens were only observed against the L3/L4 populations in the IVM treated animals compared to the control burdens (p < 0.001). No significant difference was observed between adult worm populations of the two groups.

All worms found in the large intestine were identified as *Oesophagostomum dentatum*. Four animals (two untreated and two IVM treated) had 50 *Hyostrongylus* spp each in the stomach. This could have
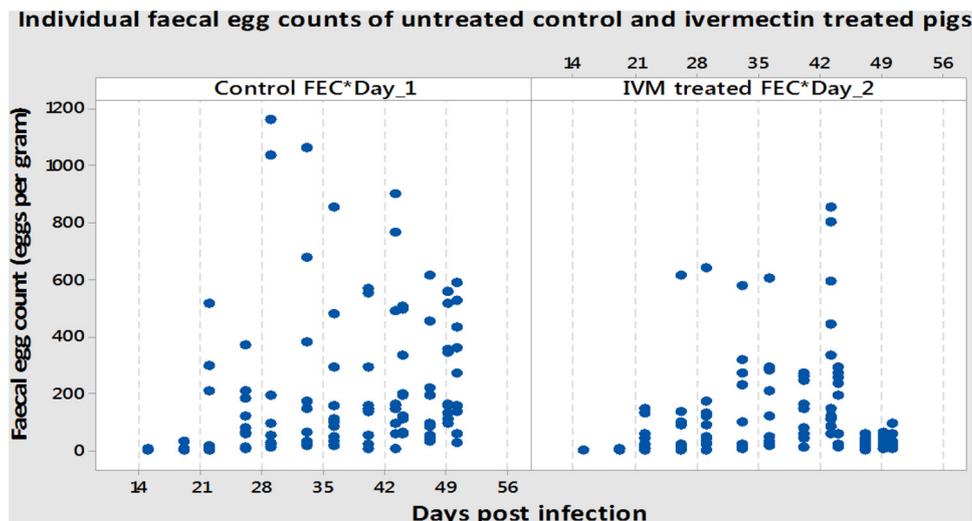
originated in small numbers from the faeces samples taken from the farm under investigation. No worms were found in the small intestines.

When compared to the untreated control group, mean reductions in *O. dentatum* adult and L3/L4 worm populations were 5% and 94% respectively following IVM administration (Table 2).

The individual average score of eggs *in utero* based on morphological analysis of all female adult worms from both the untreated and IVM treated animals is shown in Fig. 4. The average score was significantly lower in the IVM treated group worm compared to the control group worms (p = 0.035); 2.0 and 2.7 respectively.

4. Discussion

Ivermectin-resistance in adult *Oesophagostomum dentatum*, suspected on the basis of field evidence, was confirmed in this study under laboratory conditions based on both faecal egg count reduction and controlled efficacy results. Although, resistance of *O.dentatum* to ivermectin has been induced experimentally (H Bjorn, unpublished, cited in Nansen and Roepstorff (1999)) and suspected resistance to ivermectin in field isolates has been previously reported in Denmark during a survey to examine the prevalence of anthelmintic resistance in a Danish sow herd (Dangolla et al., 1997) this is the first time that IVM resistance in *Oesophagostomum* species worms in pigs has been confirmed.

The detection of ivermectin resistance is important considering the global situation where resistance to pyrantel (Roepstorff et al., 1987), levamisole and benzimidazoles anthelmintics (Bjorn et al., 1990; Gerwert et al., 2002; Varady et al., 1996) in *Oesophagostomum species* worms in pigs have already been reported (European Medicines Agency, 2017).

Furthermore, the development of resistance to MLs is of concern given that only two classes of anthelminthic products are available for use in the UK in pigs against *Oesophagostomum* spp: avermectins (ivermectin) and benzimidazoles (flubendazole and fenbendazole). Many outdoor breeding units in the UK undertake periodic anthelmintic treatment of sows and this detection of IVM resistance reinforces the need to evaluate the efficacy of worm control programmes for *Oesophagostomum* spp control and other nematodes of greater importance to UK pig production, namely *Hyostrongylus rubidus* and *Ascaris suum*.

Although this paper describes resistance on a single pig unit, low efficacy of ivermectin in treating *Oesophagostomum* was previously suspected in sow herds in England by APHA in 2016 and in Denmark (Dangolla et al., 1997). In the 2016 investigation, the practitioner noticed reduced weaning weights across litters possibly due to poor milk production and in-house testing of lactating sows revealed high

Fig. 3. Individual faecal egg counts of the two groups of pigs (untreated control and ivermectin (IVM) treated) from 15 to 50 days post first infection with 5000 infective larvae (Note a second challenge of 5000 L3 was administered on day 40 but will not have reached maturity/patency). Treatment was on day 44 post first infection (↓).

Table 2

Arithmetic mean (± S.E.M) large intestine worm counts, range of counts, differentiation of worm burdens into adult male, adult female and L3/L4 worms, and percentage efficacy (P.E.) of anthelmintic treated groups of pigs relative to untreated control pigs five days post-ivermectin administration.

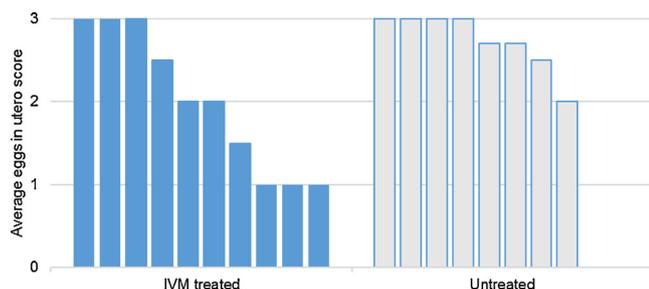| Treatment	Arithmetic mean worm burden (± S.E.M) [range]				Percentage efficacy		
	Male	Female	L3/L4	Total	L3/L4	Adult	Total count
Untreated	36 (± 9) [10–80]	27 (± 11) [0–120]	33 (± 1) [0–100]	96 (± 23) [10–280]	N/A	N/A	N/A
Ivermectin	38 (± 9) [0–90]	22 (± 4) [10–40]	2 (± 1) [0–10]	62 (± 13) [10–130]	94	5	35

Fig. 4. Bar chart of average eggs *in utero* scores from female worms derived from ivermectin (IVM) treated and untreated individual animals, note no females were detected in two of the untreated animals.

strongyle-type worm egg counts in spite of their earlier anthelmintic treatment. The larval differentiation identified the strongyle-type worms as *O. dendatum*. In all these cases FECRT was used as a screening tool for the investigation of in-field anthelmintic efficacy. Although it was not possible in this study to carry out the on-farm FECRT according to all the guidelines of the WAAVP (Coles et al., 2006), the results provided valuable evidence pointing to reduced IVM efficacy that justified further investigation and consideration of the controlled efficacy trial.

The use of FECRT in pigs should become a vital part of veterinary investigation and advice, as it is for small ruminants.

Predisposing factors for the development of anthelmintic resistance on this studied pig unit in 2017 include the regular use of the same anthelmintic, IVM, for over ten years. This is likely to have played a significant role in the development of ivermectin resistance. Regular drug treatments increase the selection pressure on the resistance alleles of the parasite population. The potential for *Oesophagostomum* spp. to develop anthelmintic resistance may be higher in comparison to other nematodes such as *A. suum* due to the high number of worms in the intestine upon which resistance may be selected and the relative short-lived free-living larvae, and the short generation time (Jackson, 1993).

This process has been observed in sheep where the frequent use of ivermectin without alternation with other drugs has been reported as the reason for the rapid development of resistance in Haemonchus contortus in South Africa and New Zealand (Shoop, 1993; Van Wyk et al., 1989).

Another risk factor considered was underdosing of pigs with ivermectin. Sub-therapeutic anthelmintic concentrations will allow more worms to survive the treatment, and increase the development rate of resistance (De Graef et al., 2013; Sargison, 2011). However, as described previously, the farmer regularly reviewed sow weights based on those recorded in cull sows from the farm, thus under dosing was not suspected to be a risk factor on this farm. Another potential risk factor on this farm is the continuous use of both the same pasture fields between March and October each year for over 50 years, and continuous occupation of strawed sow yards for a similar period of time. Previous studies in the UK have shown that larvae of *O. dentatum* may overwinter during mild winters (Rose and Small, 1980). However, the development of *O. dentatum* does not take place at 5 ˚C (Roepstorff et al., 1987) and low temperatures (≤ 4 ˚C) are lethal for eggs and pre-infective larvae (Rose and Small, 1980). Thus it may be that persistence of worm eggs in

the strawed accommodation may be as important, if not more so, than on the pasture.

It is important to highlight that the pasture management on this pig unit differs from most commercial outdoor pig units in the UK, where the pig herd is moved to new ground every two to three years to allow pigs to fit with crop rotation as part of land management, as well as having benefits for nematode and other disease control.

Analysis of the results obtained indicates that there is a significant difference in ivermectin efficacy against adult and L3/L4 worms (5% vs 94%, respectively). Similar findings were also obtained by Petersen et al., 1996 in their study. Other workers (Borgsteede et al., 2007; Petersen et al., 1996) also found that more male worms survived the ivermectin treatment (63.3%♂ vs 36.6%♀) as seen here. However, it is important to highlight that in this study, surviving females showed a significant reduction in fecundity. Suppression in oviposition and/or egg generation has been observed previously in sheep (McKellar et al., 1988) and cattle (Bartley et al., 2012) nematodes post ivermectin administration. Compared to a previous study (Christensen et al., 1995) the experimental infection with *Oesophagostomum dentatum* resulted in a low worm establishment in both control and treated groups. This may have had an impact on the size, development and the fecundity of the worm population in both groups, however the controlled study corroborated the FECRT suspicions.

Based on the results of this paper, more comprehensive studies are needed in the future to establish the clinical and production impact of *Oesophagostomum* species infestations within the pig production system.

In conclusion, the detection for the first time of IVM resistance in *Oespophagostomum species* worms in pigs represents an important opportunity to discuss the wider issue of anthelmintic usage and efficacy on pig farms. Veterinarians and farmers should be aware of the possibility of resistance development with long term application of ivermectin in pig production systems. Therefore, wider surveillance based on worm egg counts for worm prevalence, and faecal egg count reduction testing for nematode anthelmintic resistance in pigs is timely in order to mitigate possible risk factors for further anthelmintic resistance development.

Conflict of interests

The authors declare that they have no conflicts of interest.

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