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**Effect of fenbendazole in water on pigs infected with *Ascaris suum* in finishing pigs
under field conditions**

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ABSTRACT:

The husbandry of pigs for meat production is a constantly developing industry. Most studies on the effects of *Ascaris suum* infection in pigs and its prevention with anthelmintics are over a decade old. We examined the effect of 2.5 mg fenbendazole per kg bodyweight administered in drinking water for two consecutive days on *A. suum* infection 1 and 6 weeks after pigs arrived to fattening units. We hypothesised that the treatment would reduce the presence of *A. suum*-infections, improve the average daily weight gain of pigs, reduce the percentage of liver rejections in pens by 50% and increase the lean meat percentage at slaughter by 1%.

The study included a placebo group (427 pigs) and a treatment group (420 pigs) spanning four different farms previously reporting $\geq 15\%$ liver rejection. The treatment was given for 2 consecutive days 1 and 6 weeks after the pigs arrived to the fattening unit. Faecal samples were collected during weeks 1, 6 and 12 from all pigs and examined for *A. suum* eggs. Blood was collected during weeks 1 and 12 from a subgroup of the pigs and examined for anti-*A. suum* antibodies and clinical blood parameters. Data on liver rejection and lean meat percentage were collected post-mortem.

The proportion of *Ascaris* seropositive pigs changed from 8.6% to 22.2% and 20.3% to 16.3% in the placebo and treatment group respectively. Fenbendazole reduced the presence of *A. suum* eggs in faeces the percentage of liver rejections by 69.8%. The treatment did not affect daily weight gain or lean meat percentage. Pigs with *A. suum* eggs in faeces at week 6 had a lower average daily weight gain of 61.8 grams/day compared with pigs without parasite eggs. Fenbendazole treatment may be a useful option for farms struggling with persistent *A. suum* problems and demonstrate a beneficial effect on the weight gain of the animals shedding eggs in faeces and result in fewer condemned livers at slaughter.

Keywords: *Ascaris suum*, swine, anthelmintic, milk spots, fenbendazole, liver rejection

Highlights

- *Ascaris suum* infections in pigs lowered their daily weight gain.
- Fenbendazole administered in water reduced *A. suum* eggs in faeces.
- Fenbendazole treatment of finishing pigs reduced the number of liver rejections.
- Fenbendazole treatment did not affect the lean meat percentage.

1. Introduction

Ascaris suum is a globally distributed helminth commonly infecting swine via the faecal-oral route (Roepstorff et al., 1998a; Roepstorff et al., 1999; Roepstorff et al., 2011). The larvae migrate from the intestine and back via the liver and lungs, often causing damage in these organs (Roepstorff et al., 1997). In the liver, the characteristic lesions caused by the inflammatory response to the larvae, hepatic white spots, are a major reason for liver rejection at slaughter. The lungs can also be damaged as the larvae move into the alveolar and bronchial spaces. The damage caused in the lungs can pave the way for opportunistic bacterial and viral infections (Curtis et al., 1987; Adedeji et al., 1989).

The combination of organ damage, opportunities for secondary infections, alterations to immunity and the potential effect on host growth makes *A. suum* an economically important pig pathogen (Urban et al., 1989; Southern et al., 1989; Roepstorff et al., 2011; Vlaminck et al., 2015).

The control of *A. suum* is currently relevant for a number of reasons. Organic pig farming is becoming more popular (Carstensen et al., 2002; Eijck and Borgsteede, 2005), the parasite has

zoonotic potential (Newell et al., 2010; Betson et al., 2014), there are measurable environmental effects of anthelmintic pharmaceuticals (Sim et al., 2013; Wagil et al., 2015) and the development of resistance to anthelmintic drugs creates challenges for sustainable farming (Bauer and Gerwert et al., 2002; Roepstorff et al., 2011). These concerns motivate interest in new control strategies, such as the use of bioactive feed (van Krimpen et al., 2010; Jensen et al., 2011; Kaplan et al., 2014), breeding for parasite resistance (Nejsum et al., 2009; Skallerup et al., 2014) and administration of egg-destroying fungi (Roepstorff et al., 2011). However, before new methods can be used to supplement the use of anthelmintic drugs, we need to understand how to prolong the effectiveness of existing drugs. Although fenbendazole is widely used in the pig industry, studies examining its effect on production parameters are few and old (Becker and Bradley 1981; Stewart et al., 1984; Marchiondo and Szanto, 1987; Urban et al., 1989; Southern et al., 1989; Jungersen et al., 1999). With the introduction of new methods for pathogen control, and the constant development of novel systems in pig production, new studies are needed.

We examined the effect of administering 2.5 mg fenbendazole per kg pig bodyweight in drinking water on *A. suum* infections, pig average daily weight gain (ADWG), and carcass quantity and quality at slaughter. We hypothesised that, under farming conditions, the treatment could 1) reduce the presence of *A. suum* eggs in faeces, 2) increase the ADWG by 20 g/day per pig, 3) reduce the rejection of livers by 50% and 4) increase the lean meat percentage (LMP) by 1%.

2. Methods

2.1. Ethics statement

The experiment was approved by the Southern Finland Regional State Administrative Agency (ESAVI/5547/04.10.07/2013). Farmers who participated in the study gave written informed consent to set up the study on their farm and could withdraw from the study at any time.

2.2. *Experimental setup*

The study was set-up on five commercial pig farms and included 1,100 finishing pigs (Landrace / Yorkshire / Duroc): 550 in the placebo group (110 pigs per herd) and 550 in the treatment group (110 pigs per herd). The criteria for farm inclusion were as follows: a 1 year history of rejecting at least 15% of pig livers at slaughter, that piglets originated from only one farm, the presence of concrete semi-slatted floors, that at least 220 animals could be accommodated in one room of the finishing unit and that this room contained two rows of equal-sized pens divided by a central service corridor. The farms were recruited from southern Finland and sampled between September 2013 and April 2015. All finishing units bought weaner pigs at weights of 25–30 kg and used liquid feeding. The farms bought the pigs from the same producer, but not from the same location. The piglet producers are normally recommended to treat the piglets when 7 weeks of age with an anthelmintic effective against *A. suum*.

Prior to the start of the study and arrival of the pigs, the pens were cleaned using high-pressure water and allowed to dry for at least 1 week. Two Dosatron (Dosatron International, Tresses, France) dispensers were installed on each water line in the two parallel lines of pens, one for each experimental group inside each farm.

Upon arrival of the pigs, the farmers were instructed to allocate them to the pens of the experiment in two homogenous groups according to the estimated weights of the animals. The

farmers also had to agree to not relocate the animals after the start of the experiment. In all farms, the pigs were kept in a single room. The farmers were instructed to first do the daily cleaning in the pens belonging to one experimental group and then to wash their boots before starting the cleaning of the pens belonging to the other group. Different colour-marked shovels were used for the cleaning of the two parallel lines of pens of each group.

One week (week 1) after the pigs arrived to the finishing unit, 220 pigs per herd were individually weighed (DV203E digital, Danvaegt, Hinnerup, Denmark; Weight 1) and ear-tagged.

Approximately half of the pigs from each pen were randomly caught and restrained using a snout snare. Faeces were then collected directly from their rectum. Blood samples were taken from the jugular vein of the first two pigs caught in each pen and from each treatment group (~20 pigs) on farms 1, 2 and 3. Each sample was transferred to serum tubes (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) for analysis of haptoglobin (Hp) and anti-*Ascaris* antibodies and one EDTA tube (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria) for blood cell count. Serum tubes were centrifuged within 1 day of collection, and the sera were stored at -18 °C until analysis.

The sum weight of the pigs and their estimated water consumption over an 8 hour period were used to calculate the dosage of 2.5 mg/kg fenbendazole (Panacur AquaSol, MSD Animal Health, NJ, USA) to administer with the dispenser system in the pens of the treatment group (calculated using the application Aquaposo, Intervet International, USA). Similarly, a placebo solution was prepared for the dispenser systems in the pens of the control group. For all pens, water consumption was assumed to be the same for all pigs. The dispenser systems were

checked before use to verify that all water nipples were working. Before starting the administration of the drug, the last water nipple of both water lines was flushed until the white solution (medication or placebo) could be seen, ensuring that the water line was filled. The administration of the treatment was repeated once more the following day. The researchers and the farmers were blinded to the groups receiving placebo or treatment by using identical containers for both and providing a code with a serial number and either a green or red label.

A second faecal sample was collected from the same pigs as in week 1, 40–42 days after their arrival (week 6). The administration of two new doses of placebo or fenbendazole over 2 days was repeated based on new visual estimates of weight and water consumption taken over 8 hours in each pen.

One week before slaughter, which was about 12–13 weeks after arriving at the finishing unit (week 12), the pigs were individually weighed again (Weight 2). Faecal and blood samples were collected from the same animals as in week 1. Each animal was given a tattoo that allowed the slaughterhouse data to be collected post-mortem and to connect the animal to its farm and treatment group. The meat inspection data collected in the slaughterhouse included liver rejections due to more than five milk spots (yes/no) and LMP (measured by AutoFom III, Carometec A/S, Smørum, Denmark).

2.3. Blood sample analysis

Blood samples were analysed on the day of the collection using an automated multiparameter analyser with software for animal samples (CELL-DYN 3700 System, Abbott Diagnostics Division, Abbott Park, IL, USA). Measurements included white blood cell count (WBC), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), platelet count (PLT), mean

corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). *Ascaris* antibodies were evaluated at the laboratory of Parasitology and Immunology, Ghent University, Belgium, using an ELISA as described by Vlaminck et al. (2012). The Hp concentration in serum (mg/L) was determined using a HGB-binding assay (Makimura and Suzuki, 1982) and modified using tetramethylbenzidine as chromogen (Alsemgeest et al., 1994).

2.4. Faecal sample analysis

Faeces samples were collected in 50 mL containers, marked with a code, and shipped cooled to the Estonian University of Life Sciences for analysis. The treatment group and the identity of the farm were unknown to the person analysing the samples. A concentration flotation method described by Roepstorff and Nansen (1998b) was used. Eggs were floated in sucrose-salt flotation liquid and counted in a McMaster reading chamber using both chambers under a light microscope at $200\times$ magnification. The number of *A. suum* eggs per gram of faeces (EPG) was calculated by multiplying the egg count by 20.

2.5. Statistics

Power analysis

The sample size necessary for statistical interference (with confidence level 95% and study power 80%) was calculated based on the following hypotheses: treatment with fenbendazole will I) increase the ADWG by at least 20 g/day (SD = 115 g/day), II) increase the LMP by 1% (SD = 3.5%) and III) decrease the liver rejection rate by 50% (from 15% to 7%). This yielded sample sizes of 520, 193 and 264 pigs per group per hypothesis, respectively.

Average individual daily weight gain (g/day) was calculated as (Weight 2 – Weight 1) /

(Days between Weight 1 and Weight 2) for each pig. A dichotomous variable of presence or absence of *A. suum* eggs in the pig faeces was created from the EPG counts. Continuous variables used in models were checked for a normal distribution.

Four types of model were built to test the hypotheses. The hierarchical structure of the data included farm as a forced fixed factor. Pen was considered a random factor in models that used individual pig data.

Hypothesis I: a logistic model was built with pig as the unit of analysis to test the association between the dependent variable *A. suum* presence/absence in faeces after first treatment (week 6) and treatment as the independent variable. Hypothesis IIa: a mixed model was built with pig as the unit of analysis to test the association between the dependent variable ADWG and independent variable treatment. Hypothesis IIb: a mixed model was built with pig as the unit of analysis to test the association between the dependent variable ADWG and the independent variables *A. suum* antibodies (weeks 1 and 12), *A. suum* presence/absence in faeces and EPG (weeks 6 and 12). Treatment was included as fixed effect. Hypothesis III: a random logistic model was used to study the effect of treatment on the proportion of liver rejections at the pig level. Hypothesis IV: a random linear regression model was used to test the association between the LMP of pigs recorded at slaughter and treatment.

At the beginning of the study (week 1) and at week 12, mixed linear regression models were used to explore associations between blood parameters and treatment and the presence of *A. suum* in faecal samples. Farm was included as a fixed variable and pen as a random variable to control for a possible clustering effect. A square-root transformation was used to normalise Hp values.

Variables with confounding effects in models were identified and retained if they changed the regression coefficient of the model by at least 10%. *P*-values ≤ 0.05 were considered significant. STATA 13.0 (Stata Corporation, TX, USA) software was used for the statistical analyses.

3. Results

3.1. Experimental groups

One farm had to be excluded from the study because the pigs had been relocated during the experiment and the slaughterhouse data could not be obtained at the end of the study. Before the first sampling, one pig from the placebo group in farms 1 and 3, and one pig from the fenbendazole group in farm 4, left the study. The final sample included 847 pigs.

At the beginning of the sampling in week 1 (Table 1), the 427 pigs in the placebo group weighed an average of 38.0 ± 7.5 kg (mean $\pm$ SD) and there were 10.4 ± 3.1 animals per pen. The fenbendazole group included 420 pigs with a mean weight of 37.5 ± 7.6 kg, and there were 10.2 ± 2.2 per pen. By the second weighing, 63 animals had left the study (Table 1).

Overall, the proportions of faecal samples containing *A. suum* eggs were 0.0%, 8.1% and 12.5% of the pigs examined at weeks 1, 6 and 12, respectively. At week 6, the group given placebo had 30 pigs with *A. suum* eggs in the faeces. This increased to 49 pigs at week 12. The group given fenbendazole had two pigs with eggs (EPG = 20) in the faeces at week 6, but no eggs were found at week 12 (Table 2). The number of eggs shed by pigs in the placebo group increased throughout the study, but remained low in the fenbendazole group (Table 2).

Oesophagostomum dentatum eggs were found in one pig in the fenbendazole group at weeks 1 and 6 and in one pig from the placebo group at week 1.

Clinical blood parameter results can be found in Table 1S. During week 1, five pigs (8.6%) in the placebo group and 12 pigs (20.3%) in the fenbendazole group had an OD value higher than 0.5, which is considered the cut-off point for positive *Ascaris* samples (Vlaminck et al., 2012). Corresponding figures for week 12 were 12 pigs (22.2%) in the placebo group and nine pigs (16.4%) in the fenbendazole group. The difference between the treatment groups was not statistically significant at either sampling ($P = 0.10$ at week 1, and $P = 0.39$ at week 12). Fig. 1S shows the mean optical density of anti-*A. suum* antibodies in both treatment groups during weeks 1 and 12.

Of the pigs in the placebo group three (60.0%) of the seropositive pigs and 8 (15.7%) of the seronegative pigs at week 1 shed eggs in the faeces five weeks later (week 6). At the end of the study (week 12), 11 (91.7%) of the seropositive pigs and eight (19.1%) of the seronegative pigs in the placebo group had *Ascaris* eggs in the faeces. In the treatment (fenbendazole) group none of the pigs in the tested for anti-*Ascaris* antibodies shed eggs in faeces, regardless of antibody status. Ten (58.8%) of the 17 animals seropositive at week 1 in the treatment group remained so at week 12.

The results of mixed models showed that higher MCHC was associated with the fenbendazole group at week 1 (coefficient = 3.00, 95% CI: 1.45-4.54, $P < 0.001$). A lower HGB was associated with the presence of *Ascaris* eggs in faeces at week 12 (coefficient = -6.63, 95% CI: -12.41-(-0.85), $P = 0.025$).

3.2.1. Effect of treatment on the shedding of *Ascaris suum* in faeces

The administration of fenbendazole reduced the presence of *A. suum* eggs in the faeces compared to the placebo group (Table 2, Table 3).

3.3.1. Effect of treatment on average daily weight gain

Administering fenbendazole in water to groups of pigs did not result in a lower ADWG compared to pigs given placebo (Table 2S).

3.3.2. Effect of Ascaris suum on average daily weight gain

If *A. suum* eggs were found in the animal's faeces at week 6, the pig gained an average 61.8 grams/day less ($P = 0.02$) than pigs without eggs in the faeces (Table 3S). This corresponds to a 7.3% lower ADWG in the group with *A. suum* eggs in the faeces for the 11–12 week period. The model predicted no association between ADWG and eggs in the faeces at week 12 ($P = 0.36$). The number of *A. suum* eggs in faeces at 6 and 12 weeks did not affect the ADWG ($P = 0.14$ and $P = 0.37$, respectively). Also, the presence of anti-*A. suum* antibodies at week 1 or 12 did not predict changes in ADWG ($P = 0.27$ and $P = 0.17$, respectively).

3.4. Effect of treatment on liver rejections

Fewer livers were rejected at slaughter in pens where the pigs were given fenbendazole than in pens with pigs given placebo (OR: 0.18, 95% CI: 0.09–0.37, $P < 0.001$) (Table 4S). The proportion of rejected livers in pigs given placebo was 23.6% ($N = 96$, 95% CI: 19.7–28.0), while in pigs given fenbendazole it was 7.3% ($N = 29$, 95% CI: 5.0–10.1; Fig. 1). The reduction in the proportion of liver rejections between groups was 69.8% (95% CI: 60.1–78.3).

3.5. Effect of treatment on lean meat percentage

Treatment did not affect the LMP, but there were differences between farms (Table 5S, Fig. 2S).

4. Discussion

We tested whether administering 2.5 mg fenbendazole per kg pig bodyweight in drinking water for 2 consecutive days during weeks 1 and 6 after arriving at the finishing unit will reduce the presence of *A. suum* in the faeces, increase ADWG, reduce the number of livers rejected and increase LMP at slaughter.

4.1. Effect of treatment on *A. suum* in faeces

Treating pigs in the finishing unit with an anthelmintic at a 5 week interval was expected to expel adult *A. suum* and lower the infection pressure in the farm environment. No eggs were found in the faeces of pigs in either of the groups at the start of the study, and so the treatments mainly would have had an effect on non-adult larvae present rather than deworming existing adult *Ascaris* worms producing eggs. In the placebo group, the number of pigs with *A. suum* eggs in the faeces increased over time, indicating that without effective treatment infections would occur (Table 2). This was supported by an increase in the number of pigs with a humoral response to *A. suum* infection in the placebo group, mainly in farm 1 and 2 (Fig. 1S). It is however not clear whether the seroconversion is attributed to exposure to the parasite prior to the study or due to exposure from new infections. Overall, the results support the hypothesis that fenbendazole treatment in the water supply will reduce the presence of *A. suum* eggs in faeces.

4.3. Effect of treatment on average daily weight gain

Treatment with fenbendazole did not improve the ADWG. The assumption of lower weight gain would depend on the presence of adult worms parasitising the pigs' guts. Since *A. suum* eggs were first found in pig faeces at week 6, it is possible that the adult worms were not present long enough in the placebo group and at sufficient numbers to produce an effect on weight gain between the treatment groups. However, we did find that *A. suum* can affect weight gain. When using the presence of *A. suum* eggs in the faeces of individual pigs as a predictor for ADWG at week 6, there was a negative association. Pigs with eggs in the faeces had an ADWG that was 61.8 grams/day lower for the 12 week period than pigs without eggs in the faeces. The mean weight at slaughter was 7.3% lower for pigs with *A. suum* in the faeces at week 6. The finding is in contrast to results from a study that experimentally infected pigs three times with 2,000 *A. suum* eggs 11 days apart followed by fenbendazole treatment (Stewart et al., 1985). That study found no differences in ADWG, feed-conversion efficiency or in histology of the lungs and livers. Support for our study can be found in investigations demonstrating improved growth of naturally infected pigs treated with fenbendazole during the growing phase (Urban et al., 1989). The effect was explained as a result of permanent damage caused by the parasite in animals without timely treatment. A second study concluded that the effect of treating *A. suum* infections with fenbendazole could improve the feed conversion of weanling pigs, but that naturally infected pigs were less successful at mounting a protective immune response compared to artificially infected animals (Southern et al., 1989).

In pigs previously exposed to *A. suum* under experimental conditions, 90% fewer larvae reached the small intestine because of acquired immunity (Jungersen et al., 1999). However, the presence of adult larvae in the intestine did not affect the protective immunity. We found humoral evidence of infection throughout the study. The placebo group had more pigs

seroconverting during the experiment, while no new pigs seroconverted in the fenbendazole group. It appears that pigs in the fenbendazole group did not end up hosting adult nematodes within the observation period, with the exception of two pigs during week 6. These two pigs were both from the same farm and pen, and it is possible these pigs did not get effective treatment during week 1. We did not find evidence that previous exposure to *A. suum* could be associated with differences in ADWG. This is in contrast to a recent study using the same anti-*A. suum* serological test as ours that reported a connection between the presence of antibodies and ADWG in pigs from 20 finishing units in Belgium 1 week before slaughter (Vlaminck et al., 2015). Treatment with fenbendazole did not increase ADWG 20 g/day per pig as predicted, but the absence of *A. suum* eggs in the faeces equated to an increase in ADWG of 61.8 g/day per pig. The hypothesis was thus partly satisfied.

4.4. Effect on liver rejections

By limiting the number of new *A. suum* infections, and thus the number of migrating larvae in the pigs, the damage to the pigs' livers should be reduced and any existing damage would have time to heal before slaughter. The data showed that pigs in both groups were exposed to parasites during the study (Table 1S, Fig. 1S), but eggs were almost exclusively found in the placebo group (Table 2). This meant that larvae migration likely did occur in both groups, but the difference was likely the continuous infection pressure in the placebo group.

Individual data on liver rejections and LMP would have allowed us to link the slaughterhouse data with the infection status of the individual animal, but this proved to not be possible. As a consequence, the data were analysed at pen level for the two groups.

The study farms had a history of at least 15% liver rejections because of *A. suum* liver spots. In pens that were administrated fenbendazole in the water, 69.8% fewer livers were rejected than in placebo pens. This is similar to what has been described previously (Stewart et al.,

1984). The result of this study thus proved the hypothesis that the treatment could lower the liver rejection rate at slaughter by at least 50%.

4.5. Effect on lean meat percentage

The meat production industry has changed over time and with it the criteria for evaluating the value of pigs. The LMP is a method that was recently introduced in the EU to ensure comparable quality standards and pricing (Commission Implementing Decision, 2016). The LMP measured in this study was calculated from variables including thickness of the skin, fat and muscle depth (Commission Implementing Decision, 2016). The value of the pig carcass is determined by the quantity (carcass weight) and quality (LMP). Over 30 years ago a connection was demonstrated between the presence of adult *A. suum* worms in pigs and slaughterhouse variables (Steward et al., 1984). We found, however, that treatment with fenbendazole did not affect LMP, and the hypothesis was rejected. Our results were likely affected by the proportion of pigs that hosted adult, egg-shedding helminths. At all three time points in this study, 13.9% of the pigs had *A. suum* eggs in their faeces.

When investigating the potential benefits of treatments in pigs, the technical aspects of how the outcome is measured should be considered that determine the economic benefit to the farmer. In the case of LMP, the value is generated by an instrument with a tolerated error of measurement of 2.5% r.m.s.e. based on a sample of at least 60 carcasses, including outliers (Commission Regulation, 2008). Fatter pigs are graded with poorer quality because fat subtracts from the LMP value. This means that past results demonstrating an effect of fenbendazole treatment on factors determining quality, and thus the value of the pig, would be assessed differently in our present production system.

4.6. Study design

We chose to administer fenbendazole in the water because this is more practical for the farmer than administering it as powder in the feed. It is also a new way of delivering anthelmintics to pigs. Our results indicate that the mode of administration and the effect of the drug had the desired effect (Table 2), and can be an option if pig liver rejection rates on a farm are higher than the average.

Conducting our study under conditions similar to natural situations had benefits and drawbacks. The success of treatment strategies under conditions with natural infection makes this relevant for the actual situations where it would be implemented. The downside is that other factors at both the animal and management level could have influenced our results. We took measures to control these differences between farms in the model by ensuring a large sample size, randomisation, blinding of the analysis and accounting for clustering.

To account for factors that could influence the groups, the pigs were of the same breed and were raised at the same time and in the same rooms. Weight and blood data (Table 1S) indicated that we were successful in creating two similar treatment groups. A fifth farm was excluded from the study because slaughterhouse data could not be obtained and because the criteria that were set for the study were not met. This meant that the planned sample size was not met and, for the hypothesis examining the effect of treatment on ADWG, the study power was reduced to 68%. The remaining sample size goals were met.

5. Conclusion

Treatment of pigs with 2.5 mg fenbendazole per kg bodyweight for 2 consecutive days 1 and 6 weeks after arriving at the finishing unit reduced the presence of *A. suum* eggs in faeces. In

pens receiving the treatment, 69.8% fewer livers were rejected at slaughter. Pigs with *A. suum* in faeces 6 weeks after arriving at the finishing unit exhibited ADWGs that were 61.8 g/day lower than those exhibited by pigs with no eggs over a 12 week period. Treatment with fenbendazole could not be associated with a change in ADWG and did not have a measurable effect on LMP.

Conflict of interest

The authors do not have any financial or personal conflicts of interest that could bias the study.

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Author contributions

Study design (CO, MH, BL, TO, EJ, TL), sampling (CO, MH, TL, MHH), analysis (BL, CO, MH, TO), writing the manuscript (BL), final approval of the manuscript (BL, CO, TO, MH, EJ, TL, MHH).

References

1. Adedeji, S.O., Ogunba, E.O., Dipeolu, O.O., 1989. Synergistic effect of migrating *Ascaris* larvae and *Escherichia coli* in piglets. J. Helminthol. 63, 19-24.
2. Alsemgeest, S.P., Kalsbeek, H.C., Wensing, T., Koeman, J.P., van Ederen, A.M., Gruys, E., 1994. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. Vet. Q. 16, 21-23.
3. Bauer, C., Gerwert, S., 2002. Characteristics of a flubendazole resistant isolate of *Oesophagostomum dentatum* from Germany. Vet. Parasitol. 103, 89-97.
4. Becker, H.N., Bradley, R.E., 1981. Fenbendazole as a therapy for naturally acquired *Stephanurus dentatus* and gastrointestinal nematodes in feral swine. Vet. Parasitol. 9, 111-115.
5. Betson, M., Nejsum, P., Bendall, R.P., Deb, R.M., Stothard, J.R., 2014. Molecular epidemiology of ascariasis: a global perspective on the transmission dynamics of *Ascaris* in people and pigs. J. Infect. Dis. 210, 932-941.
6. Carstensen, L., Vaarst, M., Roepstorff, A., 2002. Helminth infections in Danish organic swine herds. Vet. Parasitol. 106:253-264.
7. Crompton, D.W.T., 2001. *Ascaris* and ascariasis. Adv. Parasitol. 48, 286-346.
8. Curtis, S.E., Tisch, D.A., Todd, K.S., Simon, J., 1987. Pulmonary bacterial deposition and clearance during ascarid larval migration in weanling pigs. Can. J. Vet. Res. 51, 525-527.
9. Eijck, I.A., Borgsteede, F.H., 2005. A survey of gastrointestinal pig parasites on free-range, organic and conventional pig farms in The Netherlands. Vet. Res. Commun. 29, 407-414.
10. Commission Implementing Decision, 2016. (EU) 2016/170 of 5 February 2016 authorising methods for grading pig carcasses in Finland (notified under document

C(2016) 658) Available: <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32016D0170>

11. Commission Regulation, 2008. (EC) No 1249/2008 of 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcasses and the reporting of prices thereof.

Available: <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32008R1249>

12. Jensen, A.N., Mejer, H., Mølbak, L., Langkjær, M., Jensen, T.K., Angen, Ø., Martinussen, T., Klitgaard, K., Baggesen, D.L., Thamsborg, S.M., Roepstorff, A., 2011. The effect of a diet with fructan-rich chicory roots on intestinal helminths and microbiota with special focus on *Bifidobacteria* and *Campylobacter* in piglets around weaning. *Animal* 5, 851-860.

13. Jungersen, G., Eriksen, L., Roepstorff, A., Lind, P., Meeusen, E.N., Rasmussen, T., Nansen, P., 1999. Experimental *Ascaris suum* infection in the pig: protective memory response after three immunizations and effect of intestinal adult worm population. *Parasite Immunol.* 21, 619-630.

14. Kaplan, R.M., Storey, B.E., Vidyashankar, A.N., Bissinger, B.W., Mitchell, S.M., Howell, S.B., Mason, M.E., Lee, M.D., Pedroso, A.A., Akashe, A., Skrypec, D.J., 2014. Antiparasitic efficacy of a novel plant-based functional food using an *Ascaris suum* model in pigs. *Acta Trop.* 139, 15-22.

15. van Krimpen, M.M., Binnendijk, G.P., Borgsteede, F.H., Gaasenbeek, C.P., 2010. Anthelmintic effects of phytogenic feed additives in *Ascaris suum* inoculated pigs. *Vet. Parasitol.* 168, 269-277.

- 493 16. Makimura, S., Suzuki, N., 1982. Quantitative determination of bovine
 494 serumhaptoglobin and its elevation in some inflammatory diseases. Jpn. J. Vet. Sci.
 495 44, 15–21.
- 496 17. Marchiondo, A.A., Szanto, J., 1987. Efficacy of dichlorvos, fenbendazole, and
 497 ivermectin in swine with induced intestinal nematode infections. Am. J. Vet. Res. 48,
 498 1233-1235.
- 499 18. Nejsum, P., Roepstorff, A., Jørgensen, C.B., Fredholm, M., Göring, H.H., Anderson,
 500 T.J., Thamsborg, S.M., 2009. High heritability for *Ascaris* and *Trichuris* infection
 501 levels in pigs. Heredity (Edinb) 102, 357-364.
- 502 19. Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H.,
 503 Opsteegh, M., Langelaar, M., Threlfall, J., Scheutz, F., van der Giessen, J., Kruse, H.,
 504 2010. Food-borne diseases - the challenges of 20 years ago still persist while new ones
 505 continue to emerge. Int. J. Food. Microbiol. 139, S3-15.
- 506 20. Roepstorff, A., Eriksen, L., Slotved, H.-C., Nansen, P., 1997. Experimental *Ascaris*
 507 *suum* infection in the pig: worm population kinetics following single inoculations with
 508 three doses of infective eggs. Parasitology 115, 443-452.
- 509 21. Roepstorff, A., Nilsson, O., Oksanen, A., Gjerde, B., Richter, S.H., Ortenberg, E.,
 510 Christensson, D., Martinsson, K.B., Bartlett, P.C., Nansen, P., Eriksen, L., Helle, O.,
 511 Nikander, S., Larsen, K., 1998a. Intestinal parasites in swine in the Nordic countries:
 512 prevalence and geographical distribution. Vet. Parasitol. 76, 305-319.
- 513 22. Roepstorff, A., Nansen, P., 1998b. Epidemiology, Diagnosis and Control of Helminth
 514 Parasites of Swine. Animal Health Manual. FAO. 171 P.
- 515 23. Roepstorff, A., Nilsson, O., O'Callaghan, C.J., Oksanen, A., Gjerde, B., Richter, S.H.,
 516 Ortenberg, E.O., Christensson, D., Nansen, P., Eriksen, L., Medley, G.F., 1999.

Intestinal parasites in swine in the Nordic countries: multilevel modelling of *Ascaris suum* infections in relation to production factors. Parasitology 119, 521-534.

24. Roepstorff, A., Mejer, H., Nejsum, P., Thamsborg, S.M., 2011. Helminth parasites in pigs: new challenges in pig production and current research highlights. Vet. Parasitol. 180, 72-81.

25. Sim, W.J., Kim, H.Y., Choi, S.D., Kwon, J.H., Oh, J.E., 2013. Evaluation of pharmaceuticals and personal care products with emphasis on anthelmintics in human sanitary waste, sewage, hospital wastewater, livestock wastewater and receiving water. J. Hazard. Mater. 248-249, 219-227.

26. Skallerup, P., Thamsborg, S.M., Jørgensen, C.B., Enemark, H.L., Yoshida, A., Göring, H.H., Fredholm, M., Nejsum, P., 2014. Functional study of a genetic marker allele associated with resistance to *Ascaris suum* in pigs. Parasitology 141, 777-787.

27. Southern, L.L., Stewart, T.B., Bodak-Koszalka, E., Leon, D.L., Hoyt, P.G., Bessette, M.E., 1989. Effect of fenbendazole and pyrantel tartrate on the induction of protective immunity in pigs naturally or experimentally infected with *Ascaris suum*. J. Anim. Sci. 67, 628-634.

28. Stewart TB, Bidner TD, Southern LL, Simmons LA., 1984. Efficacy of fenbendazole against migrating *Ascaris suum* larvae in pigs. Am. J. Vet. Res. 45, 984-986.

29. Stewart, T.B., Southern, L.L., Gibson, R.B., Simmons, L.A., 1985. Immunization of pigs against *Ascaris suum* by sequential experimental infections terminated with fenbendazole during larval migration. Vet. Parasitol. 17, 319-326.

30. Urban, J.F.Jr., Romanowski, R.D., Steele, N.C., 1989. Influence of helminth parasite exposure and strategic application of anthelmintics on the development of immunity and growth of swine. J. Anim. Sci. 67, 1668-1677.

- 541 31. Vlaminck, J., Nejsun, P., Vangroenweghe, F., Thamsborg, S.M., Vercruysse, J.,
542 Geldhof, P., 2012. Evaluation of a serodiagnostic test using *Ascaris suum*
543 haemoglobin for the detection of roundworm infections in pigpopulations. Vet.
544 Parasitol. 189, 267–273.
- 545 32. Vlaminck, J., Düsseldorf, S., Heres, L., Geldhof, P., 2015. Serological examination of
546 fattening pigs reveals associations between *Ascaris suum*, lung pathogens and
547 technical performance parameters. Vet. Parasitol. 210, 151-158.
- 548 33. Wagil, M., Białk-Bielińska, A., Puckowski, A., Wychodnik, K., Maszkowska, J.,
549 Mulkiewicz, E., Kumirska, J., Stepnowski, P., Stolte, S., 2015. Toxicity of
550 anthelmintic drugs (fenbendazole and flubendazole) to aquatic organisms. Environ.
551 Sci. Pollut. Res. Int. 22, 2566-2573.
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Table 1.

Number of animals and samples at the beginning (week 1) and end (week 12) of the study. P = N pigs in study, F = N faecal samples collected, B = N blood samples collected.

	Herd 1	Herd 2	Herd 3	Herd 4	Total
	P/F/B	P/F/B	P/F/B	P/F/B	P/F/B
Population week					
1					
Placebo group	110/43/19	108/55/20	110/53/20	99/50/0	427/201/59
Fenbendazole	102/46/20	107/53/20	111/53/20	100/48/0	420/200/60
group					
Total	212/89/39	215/108/40	221/106/40	199/98/0	847/401/119
Pigs culled during the study, died/slaughtered*					
Placebo group	17	4	4	6	31
Fenbendazole	9	13	4	6	32
group					
Total	26	17	8	12	63
Population (week					
12)					
Placebo group	93/42/17	104/55/20	106/53/18	93/50/0	396/200/55
Fenbendazole	93/38/19	94/53/20	107/53/18	94/48/0	388/192/57
group					
Total	186/80/36	198/108/40	213/106/36	187/98/0	784/392/112

* Animals died or were slaughtered by the farmer during the study period.

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Table 1S.

Mean $\pm$ SD of clinical blood parameters in pigs receiving placebo (P) or fenbendazole (T) at weeks 1 and 6 after arrival into the finishing herd. The blood samples were taken during weeks 1 and 12.

	Week 1		Week 1		Week 12		Week 12	
	N	Group P	N	Group T	N	Group P	N	Group T
WBC ($10^9/l$)	58	19.5 ± 10.3	60	20.2 ± 12.4	37	24.3 ± 10.0	39	25.0 ± 10.6
RBC ($10^{12}/l$)	58	6.8 ± 0.5	60	6.7 ± 0.4	37	7.0 ± 0.5	39	7.1 ± 0.4
HGB (g/l)	58	106.2 ± 16.4	60	106.8 ± 8.2	37	106.5 ± 8.2	39	106.1 ± 8.3
HCT (l/l)	58	0.33 ± 0.03	60	0.33 ± 0.03	37	0.38 ± 0.03	39	0.38 ± 0.03
PLT ($10^9/l$)	58	416.5 ± 191.4	60	438.1 ± 113.3	37	396.9 ± 71.9	39	440.6 ± 110.7
MCV (fl)	58	48.4 ± 7.1	60	48.9 ± 3.9	37	48.5 ± 3.5	39	48.4 ± 3.2
MCH (pg)	58	15.8 ± 1.1	60	15.8 ± 1.1	37	15.6 ± 1.3	39	15.6 ± 1.3
MCHC (g/l)	58	325.8 ± 6.9	60	$328.8 \pm 7.4^*$	37	323.7 ± 6.81	39	325.2 ± 7.3
Haptoglobin (mg/l)	57	2154.7 ± 712.6	58	2041.7 ± 702.3	53	1840.8 ± 505.8	55	2069.9 ± 804.6

* Significant difference ($P \leq 0.001$) between treatment groups according to the linear mixed model. WBC = white blood cell count, RBC = red blood cell count, HB = haemoglobin, HCT = haematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.

Table 2.

The presence and mean egg counts of *A. suum* eggs in faeces 1, 6 and 12 weeks after entering the fattening unit. P = placebo group, T = treatment group, EPG = eggs per gram faeces.

	Farm 1	Farm 2	Farm 3	Farm 4	Total
	P/T	P/T	P/T	P/T	P/T
	(mean EPG)	(mean EPG)	(mean EPG)	(mean EPG)	(mean EPG)
Week 1					
Negative	43/46	55/53	53/53	50/48	201/200
	(0)	(0)	(0)	(0)	(0)
Positive	0/0	0/0	0/0	0/0	0/0
	(0)	(0)	(0)	(0)	(0)
Week 6					
Negative	17/44	51/50	53/53	50/46	171/193
	(773)	(0)	(0)	(1)	(181)
Positive	26/0	4/0	0/0	0/2	30/2
	(0)	(0)	(0)	(0)	(0)
Week 12					
Negative	10/38	42/53	53/53	46/48	151/192
	(1251)	(336)	(0)	(4)	(364)
Positive	32/0	13/0	0/0	4/0	49/0
	(0)	(0)	(0)	(0)	(0)

Table 3.

Logistic model predicting the effect of treatment with fenbendazole or placebo on the presence of *A. suum* eggs in faeces. Farm used as fixed effect and pen as random effect.

	Odds ratio	Standard error	Z	P-value	Upper 95% CI	Lower 95% CI
Treatment						
Placebo	Reference					
Fenbendazole	0.01	0.02	-2.60	0.009	0.00	0.33
Farm						
1	Reference					
2	0.01	0.02	-2.60	0.009	0.00	0.35
3	1					
4	0.01	0.02	-2.53	0.011	0.00	0.35
Constant	0.79	0.86	-0.21	0.830	0.09	6.69

Observations: 290, number of groups: 56, model P -value <0.01, Wald $\chi^2 = 11.77$.

Table 2S.

Mixed model predicting average daily weight gain (g) as a function of treatment. Farm was included as fixed effect and pen as random effect.

	Coefficient	Standard error	Z	P- value	Upper 95% CI	Lower 95% CI
Treatment						
Placebo	Reference					
Fenbendazole	-3.40	9.05	-0.37	0.708	-21.14	14.35
Farm						
1	Reference					
2	23.12	13.17	1.76	0.079	-2.69	48.92
3	58.71	12.95	4.53	0.000	33.33	84.09
4	-19.77	13.44	-1.47	0.141	-46.11	6.58
Constant	955.79	10.75	88.93	0.000	934.73	976.86

Observations: 784, number of groups: 79, model P -value <0.001, Wald $\chi^2 = 43.85$.

Table 3S.

Mixed model predicting average daily weight gain (g) as a function of the presence of *A. suum* in faeces at week 6. Farm and treatment were included as fixed effects and pen as random effect.

	Coefficient	Standard error	Z	P- value	Upper 95% CI	Lower 95% CI
<i>Ascaris suum</i>						
Negative	Reference					
Positive	-61.83	25.61	-2.41	0.016	-112.03	-11.64
Treatment						
Placebo	Reference					
Fenbendazole	-9.49	12.90	-0.74	0.462	-34.77	15.78
Farm						
1	Reference					
2	21.73	19.31	1.13	0.261	-16.12	59.58
3	51.37	19.60	2.62	0.009	12.96	89.77
4	-19.84	19.81	-1.00	0.316	-58.66	18.98
Constant	961.17	17.66	54.44	0.000	926.57	995.77

Observations: 394, number of groups: 79, model *P*-value <0.001, Wald $\chi^2 = 28.86$.

Table 4S.

Mixed model predicting the squared mean of proportion of liver rejections for pens given either fenbendazole or placebo. Farm was included as fixed effect.

	Odds ratio	Standard error	Z	P- value	Upper 95% CI	Lower 95% CI
Treatment						
Placebo	Reference					
Fenbendazole	0.18	0.07	-4.57	0.000	0.09	0.37
Farm						
1	Reference					
2	0.09	0.04	-5.29	0.000	0.04	0.22
3	0.02	0.01	-6.31	0.000	0.00	0.06
4	0.07	0.03	-5.43	0.000	0.03	0.18
Constant	1.58	0.52	1.40	0.162	0.83	3.02

Observations: 806, number of groups: 79, model P -value <0.001, Wald $\chi^2 = 64.21$.

Table 5S.

Mixed model predicting the mean of lean meat percentage for pigs in pens given either fenbendazole or placebo. Farm was included as fixed effect.

	Coefficient	Standard error	Z	P-value	Upper 95% CI	Lower 95% CI
Treatment						
Placebo	Reference					
Fenbendazole	-0.13	0.23	-0.58	0.559	-0.58	0.31
Farm						
1	Reference					
2	0.55	0.33	1.68	0.092	-0.09	1.20
3	2.51	0.33	7.64	0.000	1.86	3.15
4	2.05	0.34	6.04	0.000	1.39	2.72
Constant	58.69	0.27	215.46	0.000	58.16	59.22

Observations: 79, model P -value <0.001, Wald $\chi^2 = 81.55$.

Fig. 1S. Mean optical density of anti-*Ascaris suum* antibodies in two treatment groups at week 1 (58 pigs receiving placebo and 59 receiving fenbendazole) and week 12 (54 pigs receiving placebo and 55 receiving fenbendazole) after entering the finish unit in farm 1-3. The horizontal line indicates the cut-off for positive samples (Vlaminck et al., 2012).

Fig. 1. Percentage (%) of liver rejections in pigs from pens either receiving placebo (406 pigs) or fenbendazole (400 pigs) in four farms. Error bars indicate the 95% confidence intervals.