Effect of fenbendazole on shedding and embryonation of *Ascaris suum* eggs from naturally infected sows

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**Summary**

**Objectives:** To determine reduction of *Ascaris suum* egg shedding and ovicidal effects in naturally infected commercial female breeding swine treated with fenbendazole.

**Materials and methods:** Five shedding and three embryonation experiments across three commercial sow farms were conducted. *Ascaris suum*-infected sows were allocated to four treatments: untreated controls; 545.5 mg fenbendazole, 1 day (Treatment 1); 545.5 mg fenbendazole, 3 consecutive days (1636.5 mg total) (Treatment 2); and 1636.5 mg fenbendazole, 1 day (Treatment 3). Fecal samples were collected and evaluated by a standard flotation method (shedding study) or eggs were isolated and incubated (embryonation study) to determine embryonation rates. Groups were compared for time-to-negative (Kaplan-Meier survival analysis); percent negative (chi-square analysis); environmental burden (analysis of variance); and embryonation rates (analysis of variance).

**Results:** Time-to-negative ranges were 9.3-13.1, 8.9-13.1, and 9.8 days post treatment (DPT) for treatments 1, 2, and 3, respectively; control ranges were 13.4-28.2 DPT. Treatment sows were 90%-100% negative, compared to 0.0%-28.6% of controls. Environmental burden ranges were 7.0%-60.9%, 13.9%-60.8%, and 29.3% (treatments 1, 2, and 3, respectively) and 60.4%-219.0% for controls. All treatment values differed from controls (P < .05), but not from each other. Embryonation rates were lower for treatments than controls at 6 and 8 DPT (P < .001).

**Implications:** Fenbendazole at various dosages is effective against *A suum* infections in sows. Treatment should begin 14 days prior to movement into clean farrowing facilities. Under the conditions of this study, fenbendazole demonstrates ovicidal activity against *A suum* at 4-8 DPT.

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**Resumen - Efecto del fenbendazol en la excreción de huevos del *Ascaris suum* y la formación del embrión en hembras naturalmente infectadas**

**Objetivos:** Determinar la reducción de la excreción de huevos *Ascaris suum* y de los efectos ovicida en hembras comerciales infectadas tratadas con fenbendazol.

**Materiales y métodos:** Se realizaron cinco experimentos de excreción y tres de formación de embrión en tres granjas comerciales de hembras. Las hembras infectadas con el *Ascaris suum* fueron asignadas a cuatro tratamientos: control sin tratamiento; 545.5 mg de fenbendazol por 1 día (Tratamiento 1); 545.5 mg de fenbendazol por 3 días consecutivos (1636.5 mg en total) (Tratamiento 2); y 1636.5 mg de fenbendazol por 1 día (Tratamiento 3). Se recolectaron muestras fecales y se evaluaron por medio del método de flotación estándar (estudio de excreción) o se aislaron los huevos y se incubaron (estudio de formación de embrión) para determinar los índices de formación de embrión. Se compararon los grupos con el control por tiempo a negativo (análisis de supervivencia Kaplan-Meier); porcentaje negativo (análisis de chi-cuadrado); carga medioambiental (análisis de varianza); e índices de formación de embrión (análisis de varianza).

**Resultados:** Los rangos de tiempo a negativo fueron 9.3-13.1, 8.9-13.1, y 9.8 días post tratamiento (DPT) para los tratamientos 1, 2, y 3, respectivamente; los índices de control fueron 13.4-28.2 DPT. Las hembras en tratamiento fueron 90%-100% negativas, comparadas con un 0.0%-28.6% del grupo control. Los índices de carga medioambiental fueron 7.0%-60.9%, 13.9%-60.8%, y 29.3% para los tratamientos 1, 2, y 3, respectivamente, y 60.4%-219.0% para el control. Todos los valores de los tratamientos disfrybiel del control (P < .05), pero no entre ellos. Los índices de formación de embrión fueron más bajos en los tratamientos que en control a 6 y 8 DPT (P < .001).

**Implicaciones:** El fenbendazol en diferentes dosis es efectivo contra las infecciones de *A suum* en hembras. El tratamiento debe comenzar 14 días antes del cambio a instalaciones de maternidad limpias. Bajo las condiciones de este estudio, el fenbendazol demuestra actividad ovicida contra el *A suum* entre 4-8 DPT.

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Résumé - Effet du fenbendazole sur l'excrétion et l'embryonnation d'œufs d'Ascaris suum provenant de truies naturellement infectées

Objectifs: Déterminer la réduction d'excrétion d'œufs d'Ascaris suum et les effets ovicides chez des truies d'élevages commerciaux naturellement infectées traitées avec du fenbendazole.

Matériels et méthodes: Cinq essais en lien l'excrétion et trois avec l'embryonnation ont été réalisés dans trois fermes commerciales de reproduction. Des truies infectées par A suum ont été réparties dans quatre groupes de traitement: témoin non-traité; 545,5 mg de fenbendazole pour 1 journée (Traitement 1); 545,5 mg de fenbendazole pour 3 jours consécutifs (1636,5 mg au total) (Traitement 2); et 1636,5 mg de fenbendazole pour 1 journée (Traitement 3). Des échantillons de fèces furent prélevés et évalués par la méthode standard de flottaison (étude sur l'excrétion) ou les œufs furent isolés et incubés (étude sur l'embryonnation) afin de déterminer les taux d'embryonnation. Les groupes ont été comparés quant au délai pour devenir négatifs (analyse de survie Kaplan-Meier); le pourcentage d'animaux négatifs (chi-carré); la charge environnementale (analyse de variance); et taux d'embryonnation (analyse de variance).

Résultats: L'écart des délais pour devenir négatif étaient de 9,3-13,1; 8,9-13,1; et 9,8 jours post-traiteme (JPT) pour les traitements 1, 2, et 3, respectivement; l'écart pour le groupe témoin était de 13,4-28,2 JPT. Les taux de négativité variaient entre 90%-100% pour les truies traitées, comparativement à 0%-28,6% pour les témoins. Les charges environnementales variaient entre 7,0%-60,9%; 13,9%-60,8%; et 29,3% pour les traitements 1, 2, et 3 respectivement, et 60,4%-219,0% pour les témoins. Les valeurs des groupes traités différaient toutes significativement (P < 0,05) de celles du groupe témoin mais pas entre elles. Les taux d'embryonnation étaient moindres à 6 et 8 JPT pour les groupes traités comparativement aux témoins (P < 0,001).

Implications: L'administration de fenbendazole à différents dosages est efficace contre une infection par A suum chez les truies. Le traitement devrait débuter 14 jours avant le déplacement dans des installations de mise-bas propres. Dans les conditions expérimentales de cette étude, le fenbendazole a montré une activité ovicide contre A suum à 4-8 JPT.

Ascaris suum, the large roundworm of swine, is the most common and important parasite of swine, with worldwide distribution.1,2 The main economic impacts of A suum on swine are reduced feed efficiency,3,4 decreased average daily gain,5,7 liver condemnations at slaughter due to larval migration and organ pathology (“milk spots”),8,10 and increased medication costs related to treatment.11,12

Additional A suum impacts include suboptimal vaccine efficacy associated with migrating larvae;13,14 organ damage from larval migration, predisposing the host to secondary diseases;15–24 morbidity and mortality associated with acute infection,25–27 and impacts on carcass and meat quality.28,29 Ascaris suum may be a zoonotic infection in areas where humans and swine cohabit.30–33

Significant changes in the way most modern swine are managed, such as housing pigs indoors, separation of ages and production phases, improved sanitation, and effective anthelmintic use, have either eliminated or significantly reduced parasite incidence and severity. Due to reduced prevalence and overdispersion within populations,34 parasites cause mainly a subclinical disease and not often considered to be of major importance in modern swine production.12,35 However, due to several A suum characteristics, the parasite still persists in modern production systems.1,36–39 Those characteristics are a highly fecund adult female (estimated to produce 1 to 2 million eggs per day for up to 55 weeks),40,41 eggs that are highly resistant to environmental conditions and disinfectants,42–44 a direct life cycle involving extra-intestinal migration,45 and a relatively short pre-patent period.45 The high environmental contamination level and egg resistance make it nearly impossible to eradicate A suum from contaminated facilities, thus continuous monitoring and implementation of control measures are required.1,35,44

Recent husbandry requirements driven by concerns other than parasite control, such as group sow housing, required provision of nesting or bedding materials, and drug use restrictions, are re-introducing known risk factors and creating exposures that promote A suum transmission, resulting in increased prevalence and clinical severity within those farm types.35,46–49 In addition, niche production systems (eg, organic, pasture-raised, differentiated markets) and swine associated with regional and national exhibition show circuits provide environments for the parasite’s maintenance.50–54

The goal for most parasite control programs is to minimize clinical disease and production impact through preventing transmission and reducing environmental contamination.35,55–58 This is accomplished by improved sanitation, management of pig flow, and use of anthelmintics at key times in the parasite’s life cycle and host’s production cycle. On breeding farms, sanitizing the farrowing facilities before loading sows and removal of organic material on the skin of sows by washing prior to farrowing are often implemented as control measures.44,55–57 Another common practice in the swine industry is to administer anthelmintics to pregnant sows prior to entering clean farrowing facilities.35,44 This reduces transmission of eggs to the farrowing environment (eg, stall, pasture) and from the sow to piglets. Treatment timing prior to movement to farrowing may vary considerably, and if the period between treatment and moving into farrowing area is too short, egg shedding into the farrowing environment is not prevented.

Fenbendazole is a broad-spectrum benzimidazole (Class I) anthelmintic approved for use in swine in North America and elsewhere around the world. Fenbendazole has a high safety margin59,60 and is highly effective against the adult and larval stages of A suum.60–65 The benzimidazole class of anthelmintics has ovicidal activity against parasites of a number of species.66–70 This activity results from the benzimidazole molecule binding with embryonic tubulin at the leading edge of polymerization, which prevents microtubule formation.68,69,71

Fenbendazole is currently available in North America as a feed additive (Safe-Guard Medicated Dewormer for Swine; Merck Animal Health, Summit, New Jersey) or as an individual feed top-dress (Safe-Guard...
The use of fenbendazole in swine has been studied extensively, and its use in sow herds is common. However, little information is available regarding fenbendazole's impact on shedding dynamics and embryonation of *A. suum* eggs post treatment under common commercial conditions. Judicious drug use would be supported by evidence of the magnitude of impact on shedding, shedding duration, and time to negative shedding post treatment under commercial conditions. Understanding the dynamics of reduced environmental contamination is of benefit in developing effective control measures for *A. suum* in commercial swine operations worldwide. Further knowledge on controlling *A. suum* in swine may be used to model *Ascaris lumbricoides* control in humans.

The series of experiments presented herein were conducted to characterize the impact of fenbendazole (Safe-Guard EZ Scoop) on *A. suum* egg shedding and embryonation rates for *A. suum* eggs that were shed from naturally infected gestating sows under commercial conditions that included commonly used anthelmintic protocols.

### Materials and methods

All animals were cared for in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (http://www.fass.org/docs/agguide3rd/Aguide_3rd_ed.pdf) and Pork Quality Assurance Plus (PQA Plus) guidelines (http://www.pork.org/Certification/2341/pqaPlusMaterials.aspx).

### Farms

The farms in which all experiments (five shedding and three embryonation experiments) were conducted were sow farms in a commercial swine production company, using Large White-Landrace cross maternal genotypes, between 2010 and 2013. Farms A, B, and C were selected because they had each been previously diagnosed with *A. suum* infection in breeding sows as part of a system surveillance study reported by Pittman et al. Farm A was a 1000-sow, one-site, breed-to-market operation located in southeastern Virginia. Farms B and C were each 2000 breed-to-feeder-pig operations (feeder pigs 10 weeks of age), both located in northeastern North Carolina. All farms were managed with weekly batches of gestating, multiparous sows in which sows were housed in individual gestation and farrowing stalls, weaning pigs at approximately 3 weeks of age. Replacement gilts, purchased from an off-site multiplication flow, were housed in groups of four to six until bred, when they were moved into individual gestation stalls. All farms utilized fenbendazole (Safe-Guard EZ scoop) as a feed top-dress weekly for gestating sow groups 2 weeks prior to farrowing. All routine fenbendazole use was suspended prior to starting and for the duration of the experiments. Animals were fed individually through automated drop boxes once daily, and individual water nipples were available for each animal at all times.

### Experiments

**Egg shedding.** Five separate shedding experiments were conducted among the three farms between February 2011 and December 2013: one each at Farm A and Farm B and three at Farm C. Each experiment differed in number of subjects, sample collection dates, and treatments, as detailed in Table 1. All subject enrollment, sample collection, sample processing, and application of treatments were consistent among shedding experiments. No animals were used in more than one shedding experiment.

**Embryonation.** Three separate embryonation experiments were conducted between January 2012 and December 2013. Each experiment differed in number of subjects, sample collection dates, and treatments, as detailed in Table 2. All subject enrollment, treatment applications, sample collection, sample processing, and embryonation evaluation were consistent among experiments. No animals were used in more than one embryonation experiment.

**Sow inclusion and exclusion criteria.** Inclusion and exclusion criteria were consistent for all experiments. On the basis of reported literature and previous observations in the herd, younger animals (gilts through second-parity sows) were initially screened for *A. suum* infection status, because of a higher expected prevalence of infection. The goal was to identify gestating adult females shedding *A. suum* eggs in their feces. In order to screen a large number of animals in an efficient and rapid manner, a modified fecal flotation method was utilized. Approximately 1 gram of feces was collected directly from the rectum of selected gilts and sows. A new clean nitrite glove was used for each animal to prevent sample cross-contamination. Each fecal sample was placed in a 15-mL centrifuge tube, pre-filled with 5 mL of a concentrated sugar solution (Sheather’s solution). Samples were processed immediately on site. Fecal samples and sugar solution were manually homogenized within the 15-mL tube by vigorous shaking. Supplemented homogenization, if required, was accomplished using a disposable wooden stirrer, discarded after a single use. A volume of sugar solution was added to each tube sufficient to create a reverse meniscus at the top of the tube. A 22 × 22-mm glass coverslip was placed on top of each tube and allowed to sit for 10 minutes minimum. Coverslips were removed, placed on glass microscope slides, and examined under 40× magnification for *A. suum* eggs. Each coverslip was examined until confirmation of at least one egg was observed or no ova were visualized on the entire coverslip, ie, the sample was determined to be negative. In all experiments, animals providing positive test samples were enrolled in the study and randomly allocated using a random number generator to treatment and control groups, while animals with negative test samples were excluded from enrollment. For all enrolled animals, a day 0 fecal sample was collected and eggs per gram were quantified using the modified Wisconsin sugar flotation technique. Animals with a negative day 0 fecal test were considered to have been “false positives” on initial screening and were excluded from the remainder of the experiment.

### Treatments

Treatments. Treatments were applied in a manner consistent with existing on-farm processes and were consistent for all experiments. Safe-Guard EZ Scoop was used for all fenbendazole treatments, and the scoop provided in the package was used for subject dose allotments. The scoop when level full provides approximately 545.5 mg of fenbendazole, sufficient to provide 3 mg per kg bodyweight to a 181.8-kg animal. In common field application, a level full scoop is provided to each sow, regardless of bodyweight, and treatments were based on this.
Table 1: Five shedding experiments comparing fenbendazole treatment dosages and treatment periods for *Ascaris suum* in naturally infected gestating sows*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Farm</th>
<th>Treatment</th>
<th>n</th>
<th>Fecal sample collection days</th>
<th>No. negative (%)</th>
<th>Time-to-negative (days)</th>
<th>Mean</th>
<th>SE</th>
<th>Range</th>
<th>Mean BURD (%)</th>
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<td>A</td>
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<td>136.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>7</td>
<td>10</td>
<td>13.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4-18</td>
<td>36.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>TX2</td>
<td>10</td>
<td>10</td>
<td>100.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4-18</td>
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<td>79.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>8-14</td>
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<td></td>
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<td>10</td>
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<td>10.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8-14</td>
<td>60.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>CNT</td>
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<td></td>
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<td>1.5</td>
<td>14-37</td>
<td>60.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.7</td>
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<td>4-22</td>
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<td>2 (28.6)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>9.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6</td>
<td>6-14</td>
<td>32.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>29.3&lt;sup&gt;b&lt;/sup&gt;</td>
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* In five studies across three commercial sow farms, gestating sows (F1 Large White × Landrace, multiparous; n = 185) positive for *A. suum* were each randomly allocated to one of four treatment groups: untreated controls (CNT); 545.5 mg fenbendazole (Safe-Guard EZ Scoop; Merck Animal Health, Summit, New Jersey) given on a single day (TX1); 545.5 mg fenbendazole given on each of 3 consecutive days (TX2); and 1636.5 mg fenbendazole given on a single day (TX3). Fecal samples were collected at various time points within each experiment for evaluation by fecal flotation and egg quantification. Treatment groups were compared by number negative at end of study period; time-to-negative (time to stop shedding) by survival analysis; and environmental burden (BURD), a measure of total egg shedding during the study.

<sup>a,b</sup> Values with different superscripts within a category and within an experiment are statistically significant (P < .05): number negative, chi-square with Fisher's exact test; mean time-to-negative, Kaplan-Meier log-rank test; mean environmental burden, analysis of variance.

n = number of sows by experiment-treatment; SE = standard error; NA = not applicable.

Methodology. Therefore, a single scoop was estimated to provide 545.5 mg fenbendazole for each animal.

Treatment groups for all experiments were as follows: non-treated control; a single 545.5-mg scoop of fenbendazole for 1 day (Treatment 1); a single 545.5-mg scoop of fenbendazole daily for 3 consecutive days, total 1636.5 mg (Treatment 2); or three 545.5-mg scoops of fenbendazole on a single day, total 1636.5 mg (Treatment 3).

Treatment was applied to sows' individual feed drop boxes the day prior to feed being dispensed the following morning. Anthelmintic intake by each sow was monitored as complete intake of feed prior to the next feeding, and all animals in the studies consumed their entire daily ration.

Sample collection and processing. Egg shedding experiments. At each sample day, approximately 5 grams of feces was collected per rectum of each enrolled sow. If a fecal collection from a sow failed after two attempts, that sow was excluded from the day's collection. After collection, fecal samples were placed into 50-mL screw-top centrifuge tubes and held at 4°C until processing within 24 to 72 hours after collection. Samples were processed using the modified Wisconsin sugar flotation method. Slides were evaluated using 40× magnification, and the number of *A. suum* eggs on the entire slide was counted. A maximum egg count per slide (“cutoff”) of 500 eggs was utilized.

Embryonation experiments. Samples in Experiment 1 were collected on day 8 after the start of treatment. Samples in Experiment 2 were collected prior to treatment (day 0) and on day 8 after the start of treatment. Samples in Experiment 3 were collected on day 0, and on days 2, 4, and 6 after the start of treatment.

On each collection day, feces (approximately 100 to 500 g) was collected per rectum in a clean plastic sample bag in a manner consistent with the egg-shedding experiment. Samples were held at 4°C when not being processed. Samples were processed in a manner to obtain a large number of *A. suum* eggs, not for fecal quantification. Processing and sample embryonation was based on several reported methodologies and equipment availability. *Ascaris suum* eggs were isolated using a method similar...
Table 2: Three embryonation experiments on a sow farm comparing three fenbendazole treatment dosages and treatment periods for Ascaris suum in naturally infected gestating sows*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DPT</th>
<th>CNT</th>
<th>TX1</th>
<th>TX2</th>
<th>TX3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>11</td>
<td>95.4a (8.6)</td>
<td>29.3b (24.2)</td>
<td>10</td>
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<tr>
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<td>0</td>
<td>13</td>
<td>92.8a (13.3)</td>
<td>25</td>
<td>92.5a (17.4)</td>
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<tr>
<td>3</td>
<td>8</td>
<td>11</td>
<td>95.6a (8.6)</td>
<td>18</td>
<td>29.4b (24.0)</td>
</tr>
</tbody>
</table>

1 In three studies on a commercial sow farm, gestating sows (n = 137; described in Table 1) positive for A suum were each randomly allocated to one of four treatment groups (described in Table 1). Fecal samples were collected at various time points by experiment, and A suum eggs were isolated and incubated to determine embryonation rates. Treatment groups were compared by mean embryonation rates as a percent of ova reaching full larval development.

• Values within a row with different superscripts are statistically different (Tukey’s studentized range test).

DPT = days post treatment; ER = embryonation rate (percent); SD = standard deviation; ND = not done; NS = not significant (P > .05).

To the modified Wisconsin sugar flotation method, but adjusted for a large sample volume as follows. Samples were homogenized by hand within the collection bags, and a 100-gram sub-sample was weighed out and placed in a 1-L plastic container. Feces was then mixed with 200 mL of tap water and homogenized in the container using a kitchen potato masher. The fecal-water homogenate was then strained through a large tea strainer into a second 1-L plastic container to remove large-particle organic material. Up to 200 mL of the strained contents was then poured into 200-mL dilution bottles. The bottles were centrifuged at 145g for 10 minutes in a large bucket centrifuge. The pellet was re-suspended in Sheather’s solution to a volume of 200 mL, which was then centrifuged at 145g for 10 minutes. The suspension was allowed to stand for a minimum of 10 minutes, then the top 10 to 15 mL was poured off into 50-mL conical centrifuge tubes. Tap water was added up to 45 mL, and the suspension was homogenized manually by vigorous shaking. The tube was centrifuged at 145g for 10 minutes, then the pellet was re-suspended in 30 mL of 0.1 N H2SO4 and transferred into a 50-mL filtered top culture flask (item #10062-872, VWR Radnor, Pennsylvania). Egg concentration was evaluated by counting eggs in a 10-µL sub-sample at 40× magnification. Samples with > 25 eggs per µL were diluted with 0.1 N H2SO4 to achieve this maximum concentration, as it has been reported that egg density influences development. Culture flasks were held at 4°C until embryonation incubation was initiated for all samples within an experiment.

The embryonation period occurred independently for each experiment, once all samples from all collection days were processed. When all samples within an experiment had been processed, culture flasks were simultaneously incubated at room temperature (approximately 23°C to 25°C) in the dark for 60 days to ensure complete larval development. Flasks were agitated by hand three to four times per week for aeration. At the end of 60 days, flasks were held at 4°C until embryonation rates could be evaluated.

To calculate embryonation rates, culture flasks were shaken and a 10-mL subsample was poured into a 15-mL conical centrifuge tube and centrifuged at 145g for 10 minutes. The pellet was re-suspended in 5 mL of Sheather’s solution. A volume of Sheather’s solution was added in order to create a meniscus at the top of each tube. A 22 × 22 mm glass coverslip was placed on top of each tube for a minimum of 10 minutes. Coverslips were removed and placed on a glass microscope slide then examined under 100× magnification for A suum eggs presence and embryonation stage. The first 100 A suum eggs observed were evaluated and determined to be either fully embryonated (larvae visualized) or unembryonated, including any stage of development from one-cell to pre-larval stages. If fewer than 100 eggs were visualized on a coverslip, additional 10-mL sub-samples were processed until 100 total eggs were counted. Failure to count 100 total eggs from a culture flask resulted in exclusion of that sample from the experiment. Percent of embryonation was recorded as the number of eggs containing fully embryonated larvae out of 100 eggs.

Statistical analysis
All statistical analyses were performed using Enterprise Guide 5.1 software (SAS Institute, Inc, Cary, North Carolina). In each case, sow was considered the experimental unit. The dependent variable in each shedding experiment was the duration of fecal A suum egg shedding, measured as the time to first negative fecal exam for experimental sows. Survival analysis with log-rank test (PROC LIFETEST) was used and the model included treatment group, farm (in the case of experiments), parity, and treatment-farm interaction fixed effects, and the random effect of sow within farm by treatment group. Through backward elimination, effects determined non-significant on the basis of analysis were dropped from further analysis. Comparisons between treatment groups were made using Kaplan-Meier methods as an
estimation for survival function from lifetime data. Mean survival times (± standard error) were estimated for each treatment. Additionally, the percentage of animals that produced a negative fecal exam by the end of the study was evaluated using chi-square and Fisher’s exact test methods. A calculated mean environmental burden value for each treatment group was evaluated using analysis of variance (ANOVA) methods to evaluate treatment effects, and treatment means were separated using Tukey’s studentized range test. The model implemented included treatment group, farm, parity, and treatment-farm interaction fixed effects. The environmental burden calculation was an attempt to compare observed eggs per gram of feces (EPG) excreted (EPGobs) for the duration of the study period compared to an expected eggs excreted (EPGexp) for each sow and between treatments as a measure of the potential environmental contamination provided by each group. The EPGexp value was that individual’s initial (day 0) EPG multiplied by the number of study days. The EPGobs value was calculated as the cumulative sum of EPG on one sampling day (D1) times the number of days until just prior to the next sampling day (D1+X) through the duration of the study \[\sum (EPG_{D1} \times (D_{1+X} - D_1))\]. For example, in an animal with sample day EPG values of 100, 80, and 20 on days 0, 4, and 8 respectively, the EPGexp for this animal is 900 [100 EPG_{Day0} × 9 total days], the EPGobs is 740 [(100 EPG_{Day0} × 4 days) + (80 EPG_{Day4} × 4 days) + (20 EPG_{Day8} × 1 day)], and the environmental burden is 0.822 (740 EPGobs per 900 EPGexp). This environmental burden estimate indicates that the individual shed 82.2% of the eggs over the study duration as was anticipated for that individual.

For embryonation rates, ANOVA methods were used to evaluate treatment effects. When ANOVA effects were significant, treatment means were separated using Tukey’s studentized range test. Analyses were conducted separately for each embryonation experiment because of differences in the sampling protocol followed. In each case, sow was considered the experimental unit. The dependent variable in each study was the percent embryonated A. suum eggs (mean embryonation rate), measured as the number of eggs with visible larvae from the 100 eggs evaluated. Independent variables considered were treatment sampling day, and a treatment-sampling day interaction.

Results

The number of sows in each experiment and between treatment groups varied considerably due to the method of enrollment described, in which a rapid and efficient survey of a large number of sows (e.g., 100 to 250) was conducted.

Egg-shedding experiments

In shedding Experiment 1, there were no recorded samples above 500 eggs per gram. In Experiment 2, two Treatment 1 samples and three Treatment 2 samples were above 500 eggs per gram at day 0. In Experiment 3, a single Control sample at days 8 and 10 and one Treatment 2 sample at day 0 exceeded 500 eggs per gram. In Experiment 4, eleven control samples exceeded 500 eggs per gram, with 10 of the 11 coming between days 8 and 22 of sampling, while 10 Treatment 1 and six Treatment 2 samples at day 0 exceeded 500 eggs per gram. One Treatment 1 day 8 sample exceeded 500 eggs per gram in Experiment 4. In Experiment 5, one Treatment 2 and one Treatment 3 day 0 sample each exceeded 500 eggs per gram, as did one Control sample on days 8, 10, and 21.

In the survival analyses, there were significant farm (experiment) effects \((P < .001)\) and evidence of a trend in farm-treatment interaction \((P = .054)\) in the full model; therefore, data was analyzed and reported independently by experiment in a reduced model. There was no parity effect, and parity was excluded from the reduced models. In each experiment, there was a high censoring rate in the control groups \((range 70.0% to 100.0\%)\, as many subjects remained positive throughout the period of testing. Kaplan-Meier survival analysis revealed a significant difference in time-to-negative egg shedding between controls and each treatment group. In all shedding experiments, Treatment 1, Treatment 2, and Treatment 3 did not differ in time-to-negative shedding. Complete mean time-to-negative, standard error, and range for each experiment are shown in Table 1. A graph of the survival analysis has been included (Figure 1) for the combined data from experiments 1 through 5. Note that due to the different durations of experiments 1 through 5, the control group contains multiple censored data points, while the largest number of sows censored in any treatment group by experiment was one. Therefore, this graph considerably underestimates the impact of fenbendazole treatments and is a conservative assessment.

When all shedding experiments were combined, only nine of 40 control animals \((22.5\%)\, were negative for A. suum eggs throughout the respective study periods. Among the treatment groups, 66 of 67 \((98.5\%)\, for Treatment 1, 65 of 67 \((97.0\%)\, for Treatment 2, and all of 11 \((100.0\%)\, for Treatment 3 were negative for A. suum eggs at completion of their respective experiments. The percent negative at end of study differences between control and each treatment was significant by Fisher’s exact test \((P < .001)\). There was no difference in percent negative at end of study among the fenbendazole treatments in any of the five experiments. The results for each experiment are shown in Table 1.

For environmental burden analysis, a significant effect of treatment \((P < .001)\) and farm \((P < .001)\) and a trend in the treatment-farm interaction \((P = .054)\) were observed; therefore, data was analyzed and reported by experiment. The environmental burden analysis demonstrated a significant difference \((P < .05)\) between control and all fenbendazole treatments in each experiment \((Tukey’s studentized range test)\). There were no differences among treatments in any of the five experiments. The environmental burden estimates for controls in experiments 1, 3, and 5 were greater than 100%, representing increasing eggs per gram counts on subsequent samplings from individual control sows. Average environmental burdens for each experiment are listed in Table 1.

It is valuable to note that during collection, most notably on days 6 and 8 post treatment, many of the treated animals were actively expelling adult ascarids (visually observed or manually extracted during rectal sample collection), presumably due to treatment effects of fenbendazole.

Embryonation experiments

In experiments 2 and 3, the independent variables of treatment, sampling day, and a treatment-sampling day interaction were significant sources of variation; therefore, analyses were conducted for each sampling day within each experiment. In all experiments, embryonation rates of controls ranged from 90.3% to 99.3%. Ovicial activity within treatments was realized on day 4 post treatment, with significant embryonation rate reduction in Treatment 3 \((P < .01)\) and numerical differences in Treatment 1 and Treatment 2 in Experiment 3. After day 4 post treatment \((day 6 in Experiment 3 and day 8 in experiments 1 and 2)\, all treatments
were significantly different ($P < .001$) from controls for each experiment. Complete embryonation rates and standard deviations for all embryonation experiments are shown in Table 2.

The only differences in embryonation rate between fenbendazole dosages were observed on day 4 post treatment in Experiment 3. A numerical difference in embryonation rate was observed in treatments 1 and 2, although it was not statistically significant when compared to controls. By day 6, a difference was no longer observed among treatments and all were different from controls.

It is important to note that during microscopic observation, development was often atypical or arrested in unembryonated eggs in samples from treated sows. Eggs commonly had unequal cleavages, satellite and clustering of blastomeres, smaller, more circular shells, a granular and crystalline appearance to the yolk, lack of any apparent development, and abnormal shapes (Figure 2).

**Discussion**

Statistical comparison of sow eggs per gram was not conducted due to the inherent high variability of egg counts when assessing *A. suum* infection. Fecal egg counts are highly variable over time within the same subject, as well as within the same fecal passing. Rather, consistent with the main objectives of the experiments, we assessed the time to cessation of shedding and percent negative sows at the end of respective study periods. In addition, we utilized a calculated environmental burden, which provides a better description of egg shedding, as it uses fecal eggs per gram counts over several time periods to estimate total fecal egg shedding during a period of time. Others have utilized similar calculated estimates of egg contamination. Bernardo et al. used the average eggs per gram counts to calculate a "lifetime burden" in market pigs and modeled the growth impacts of ascariasis. Mejer and Roepstorff calculated a "relative contamination index" from fecal eggs per gram of *Trichuris suis* and *Oesophagostomum dentatum* in pastured pigs in an attempt to compare contamination rates between experimental paddocks.

The environmental burden calculation was utilized to demonstrate the impact of fenbendazole on reduction of total *A. suum* eggs excreted into the environment, which is an important epidemiological aspect of *A. suum* control. As can be noted by the study described in Table 1.

![Figure 1: Survival analysis: Kaplan-Meier survival curves for time-to-negative fecal *Ascaris suum* egg shedding from five experiments (data combined) across three commercial sow farms comparing three fenbendazole dosages and treatment periods to treat *A. suum* in naturally infected gestating sows. Duration of treatment differed among the five experiments; therefore, in-phase censoring of control sows is over-represented. Study described in Table 1.](image)

- The environmental burden values in shedding Experiment 2 differed statistically between control (79.9%) and treatments 1 (60.9%) and 2 (60.8%), but with less numerical difference between the treatments than in the other shedding experiments. This is likely due to several factors, including the shorter duration of the study (14 days), the number of sampling days (4), and the bias of using a “cutoff” in initial fecal eggs per gram values.

- It is important to note that in some fecal samples, eggs per gram was in excess of the 500 eggs per gram cutoff used, and thus introduced bias into the environmental burden calculation. Overall, the use of an eggs per gram cutoff in the shedding experiments resulted in an underestimation of the impact of reduced fecal shedding.

- All but one of the above observations (Experiment 4, Treatment 1, day 8 sample) would have resulted in underestimation of the actual environmental burden calculation, since sample cutoff at day 0 would have resulted in a lower expected environmental burden value, and control samples cutoff post treatment would have underestimated the lower environmental burden compared to treatment groups. This could explain the lower numerical differences and reduction in control environmental burden values observed in shedding experiments 2 and 4 compared to experiments 1, 3, and 5.

- False-positive results due to low fecal eggs per gram counts or coprophagia, when using a highly sensitive test such as the modified Wisconsin sugar flotation technique, are potential sources of misclassification bias. It should be noted that the flotation method used was selected specifically because of its greater sensitivity (approximately 1 egg per gram) relative to other detection methodologies and its common use in North American swine parasitology. In contrast, many European studies utilize a modified McMaster’s technique with a low limit of detection (20 eggs per gram) and recommend a cutoff (200 eggs per gram) to minimize the false-positive effect. For example, if a cutoff of 200 eggs per gram had been used in the previous studies, approximately 50.3% of included sows (93 of 185 sows) would have been excluded for false-positive counts at the time of sow enrollment.
necropsy and daily adult worm recovery from enrolled sows was not practical or performed, it is impossible to classify each positive sample result as true-positive or false-positive; however, a few observations support using these data collectively as valid results. First, all sows were housed individually in partially slatted gestation stalls and had limited access to feces. Second, many enrolled females from the treatment groups passed adult ascarids, which is consistent with observations by Boes et al. Unfortunately this was not recorded. Third, the apparent parasite burden (measured as eggs per gram) for sows in published papers from Europe is much greater when compared to observed eggs per gram values from the present studies and experience in other North American sow herds by the authors (GHM, personal observation, May 2014). The reason for this discrepancy between reports and geography are not fully understood, but may be related to diagnostic methodology (the McMaster’s technique uses a multiplicative calculation and tends to overestimate eggs at the higher concentrations) or inherent management differences (provision of bedding), medication restrictions (anthelmintic use), farm types (multiple ages), and facility designs (group gestation), which result in an overall heavier environmental parasite burden. Another possibility is an inherent geographical variation in egg shedding by adult female parasites, such as has been documented in A lumbricoides. Lastly, a decrease in eggs per gram over time was consistently observed throughout each treatment group within each experiment in the present study, while the majority of control animals continued to shed eggs throughout the sampling period, indicating an effect of the treatment on fecal egg shedding.

Future work could minimize the impact of false-negatives by repeated sampling and evaluation of fecal eggs per gram beyond the initial negative test. False-negatives would affect the survival analysis by overestimating the impact of the treatment on treated sows relative to the control sows. Both types of misclassification, false-positive and false-negative, may occur in subjects of both the treatment and control groups.

Some eggs collected for the embryonation experiments might have been associated with coprophagia. While this type of false-positive diagnosis is important in evaluating infection prevalence and response to treatment, it may not be a significant issue in embryonation studies, since eggs resulting...
from coprophagia still contribute to environmental contamination and are susceptible to the ovidial effects of fenbendazole. Benzimidazoles have significant ovidical activity in vitro, regardless of whether the eggs are extracted from adult worm uteri or collected from feces.\textsuperscript{66,67,70,91} Fenbendazole is maintained at a greater in vitro concentration in the gut lumen during the treatment period due to low bioavailability (27.1%).\textsuperscript{82} 44% to 50%\textsuperscript{60} of the drug remains unchanged and is excreted in the feces, and therefore may have ovidial activity in eggs in the lumen during treatment periods, although this has not been demonstrated directly. Benzimidazole's ovidical activity can affect the embryo even after development is initiated (eg, eggs ingested from the environment after a period of development). Eggs that developed for 9 to 10 days and then were exposed to thiabendazole in vitro ceased further development.\textsuperscript{57}

In the present study, fenbendazole began to have ovidical effects as early as day 4 post treatment at the single-day 1636.5-mg dosage (Treatment 3), and across all treatments by day 6 through 8 post treatment. The lack of a statistical difference in embryonation rate observed in treatments 1 and 2 on day 4 may have been a result of inadequate sample size to show a difference, or may represent an early dose effect. Effect on embryonation beyond day 8 was not evaluated. Other investigators have reported rapid ovidical effects of other helmithic eggs after treatment with various benzimidazoles in sheep (8 hours),\textsuperscript{91} and humans (24 to 72 hours).\textsuperscript{70,81,94-97} The more rapid ovidical activity in these studies, when compared to the present data, may be related to differences in parasite susceptibility, host-parasite interactions, or pharmacokinetics and pharmacodynamics of the anthelmintics for the different host species.

The observations made of atypical egg development, such as unequal cleavages, satellite blastomeres, and crystalline appearance of the yolk, were in agreement with others.\textsuperscript{67,97,98} Microtubules are important for cell structure, proper cleavage, chromosome movement during cell division, and thus embryogenesis.\textsuperscript{72} Affected eggs often have irregular shapes, atypical blastomeres, and unequal divisions, which result in irreversible arrested development.\textsuperscript{34,60,87,98}

It is important to note that A. suum eggs shed from hosts (ie, breeding sows) are not directly infective to the offspring, an important epidemiological aspect often misunderstood by producers and veterinarians.\textsuperscript{45,58,79} Freshly shed A. suum eggs require a developmental period in the environment outside the host, consisting of two molts to an infective L3 larvae.\textsuperscript{99-101} This development usually takes 1 to 3 months or longer, depending on temperature, humidity, and seasonal climate.\textsuperscript{45,79,101-105} It is this external non-infectious developmental period that allows for basic sanitary and husbandry control measures to be effective if applied correctly. In modern swine farms, with early weaning at 3 weeks of age or less, A. suum transmission directly from dam to offspring is unlikely, due to inadequate time for embryo development to an infectious L3. Multivariable risk factor analysis in 413 Scandinavian herds demonstrated that wean age was a significant factor in growing pigs having ascariasis.\textsuperscript{106} Farms that weaned pigs at greater than 6 weeks of age were twice as likely to have finishers positive for A. suum, when compared to farms that weaned between 3 and 5 weeks of age, suggesting that the additional time exposed to farrowing facilities allowed for development of A. suum eggs to an infectious stage. In other studies, age-segregated pork production that results in moving pigs to facilities located at a distance from each other reduced the correlation between sow herd A. suum status and the A. suum status of grow-finish pigs originating from the same sow herd.\textsuperscript{107,108} Transmission to offspring is most likely from older on-site animals (finishing, gilts) or contaminated facilities (finisher barns, gilt development units).\textsuperscript{109} Indirect transmission is still a concern where piglets may be exposed to infectious eggs remaining in the farrowing environment from previous groups.\textsuperscript{109,110} Poorly sanitized farrowing facilities that allow maintenance of “hot spots” as described by Nilsson,\textsuperscript{111} or by mechanical transmission from other farm areas that are contaminated (eg, breeding, gestation, gilt development, finishers), by the sow (eg, fecal matter on skin, feet), stockpersons (hands, clothing, boots), and fomites. Connan\textsuperscript{103} evaluated development of A. suum eggs placed in a commercial swine farm (in England) in order to simulate normal environmental conditions and seasonal influences. Unembryonated eggs placed in June and July became infectious in August and early September, while eggs placed in August and September underwent partial development, then experienced arrested development when conditions were unfavorable (ie, winter), and resumed development the following spring, although with reduced percent embryonation.\textsuperscript{103}

Eggs placed from September through May developed synchronously in the subsequent July. This seasonal development may be recognized as seasonal variations in liver condemnation rates at slaughter plants, with the greatest prevalences seen July through December, when growing pigs exposed to infectious eggs are marketed.\textsuperscript{112,113} Seasonal development is seen in pasture-raised pigs, where a “spring rise” and increasing prevalence is observed when pastures are infected the prior fall.\textsuperscript{93,86,114} On the other hand, it is expected that environmentally controlled facilities, such as farrowing rooms and stalls with supplemental heat (eg, heat lamps, heat mats, brooder covers), would promote larval development year round.\textsuperscript{115}

The US label for Safe-Guard EZ Scoop states “For individual 400 lb sow feeding; Mix 1 level scoop (1.07 ounces) of Safe-Guard EZ Scoop premix into 4 to 6 lb of an individual 400 lb sow’s daily ration and feed once daily for 3 consecutive days.” However, extra-label treatment of sows with a single-day, single-scoop dose of Safe-Guard EZ Scoop (ie, Treatment 1), equating to a 3 mg per kg dose for a 181.8-kg animal, is commonly used. In addition, farms usually do not weigh individual sows prior to treatment, but rather estimate an average weight for all sows\textsuperscript{72} (JSP, personal observation, May 2014). Therefore, treatment may be less than indicated by both dose and frequency. The consequence of imprecise dosing and abbreviated treatment regimens on A. suum egg shedding has not been thoroughly evaluated. In early studies of fenbendazole in swine, Baeder et al\textsuperscript{99} used a single oral dose of 5 mg per kg and eliminated 100% of adult A. suum from the intestine, and Batte\textsuperscript{61} demonstrated the efficacy (96.0% adult A. suum removed) of 3 mg per kg as a single dose. Extended treatment (3 days or longer) with fenbendazole, even with a lower daily dose, is usually considered more effective than single doses, namely, for treatment of Trichuris suis.\textsuperscript{61,116} Fenbendazole was used in an off-label manner in this study; however, the authors do not necessarily recommend off-label use under field conditions. It was the authors’ intent to mimic the potential application variation of this product as it might be used in the field by pork producers and veterinarians.
The results of the shedding experiments support using fenbendazole in breeding females prior to farrowing, and indicate that treatment is highly effective and should begin 14 days prior to moving sows into clean farrowing facilities in order to minimize *A. suum* egg shedding into that environment. Treatment of breeding herd animals after this time, or upon entry into the farrowing facility, will likely result in increasing facility contamination and increasing transmission risk to suckling piglets due to carryover of embryonated eggs from previous farrowing groups. The embryonation experiments demonstrated that fenbendazole has ovicidal activity at various treatment levels on *A. suum* eggs shed from naturally infected sows, applied in a manner consistent with practical commercial farm methods. Results from these experiments agree with work by others who have evaluated the ovicidal activity of other benzimidazoles on *A. suum* and *A. lumbricoides* or fenbendazole on other parasites.\(^{33,66,67,70,91}\) To the authors’ knowledge, this is the first description of the ovicidal activity of fenbendazole in *A. suum* eggs collected from the faeces of naturally infected sows.

In addition to sanitation and anthelmintic treatment to reduce adult worm burden and shedding by hosts, using an effective anthelmintic with ovicidal activity, such as fenbendazole, adds an epidemiological advantage by reducing the effective infectious egg load in the environment. Fenbendazole, with its adulticidal, larvical, and ovipical properties, provides added value when implemented in *A. suum* control programs in an infected population.

**Implications**

- Under the conditions of this study, fenbendazole, as a feed top-dress at 545.5 mg for 1 day, 545.5 mg daily for 3 consecutive days, or 1635.5 mg for 1 day, is effective for *A. suum* treatment in naturally infected gestating breeding sows.

- Breeding female swine with naturally occurring *A. suum* should be dewormed with fenbendazole at least 14 days prior to entry into clean farrowing facilities to minimize transmission to offspring and reduce facility contamination.

- Under the conditions of this study, fenbendazole, at 545.5 mg for 1 day, 545.5 mg daily for 3 consecutive days, or 1635.5 mg for 1 day, is ovicidal to *A. suum* eggs shed from naturally infected gestating sows, starting as soon as 4 days post treatment and lasting through at least 8 days post treatment.

- Fenbendazole as a treatment for *A. suum* provides an additional epidemiological advantage through reducing effective environmental contamination resulting from its ovicidal properties.

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**Conflict of interest**

None reported.

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*Non-refereed references.