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

by

Jeremy Stuart Pittman, DVM

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Program of Study Committee:
Locke A Karriker, Major Professor
Kenneth J Stalder
Brad J Thacker

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ABSTRACT

Objectives

To determine the time to cessation of *Ascaris suum* egg shedding, the percent of animals that stop shedding, and to estimate the reduction in environmental burden from eggs shed in naturally infected female breeding swine after different treatment levels of fenbendazole to better determine timing of anthelmintic use prior to movement into a farrowing environment. To determine the ovicidal activity of different fenbendazole levels on *Ascaris suum* eggs shed from naturally infected commercial breeding female swine.

Materials and methods

Study 1 – Egg shedding

Five experiments across three commercial sow farms known to be infected with *A suum* were conducted. Breeding gilts and sows were identified with natural *A suum* infections and allocated to one of 4 treatments: CNT = untreated controls, TX1 = 545.5 mg fenbendazole for one day, TX2 = 545.5 mg fenbendazole daily, for 3 consecutive days (1636.5 mg total), and TX3 = 1636.5 mg fenbendazole for one day (TX3 only in experiment 5). Fecal samples were collected on various days and evaluated using the modified Wisconsin sugar flotation technique, with at least 1 EPG considered a positive sample. Time-to-negative was evaluated using Kaplan-Meier survival analysis with Log-Rank test and censoring of animals that reached the end of the study period still shedding.
Percent negative at the end of the experiment was evaluated by Chi-square analysis using Fisher's exact test. Environmental burden (BURD), a calculation of eggs observed versus eggs expected, was evaluated by ANOVA and Tukey's studentized test.

Study 2 - Embryonation

Three experiments were conducted on a commercial breeding farm infected with *A suum*. Breeding gilts and sows were identified with natural *A suum* infections and allocated to one of 4 treatments: CNT = untreated controls, TX1 = 545.5 mg fenbendazole for one day, TX2 = 545.5 mg fenbendazole daily for 3 consecutive days (1636.5 mg total), and TX3 = 1636.5 mg fenbendazole for one day (TX3 only in experiment 3). Eggs were isolated from fecal samples at various days post-treatment (dpt) by experiment and incubated in 0.1 N H$_2$SO$_4$ at room temperature for 60 days. Embryonation rates (ER) for each animal were determined by counting the number of eggs with fully developed larvae out of 100 eggs counted.

Results

Study 1 – Egg shedding

Mean time-to-negative shedding ranged from 9.3 to 13.1 for TX1, 8.9 to 13.1 days and 9.8 for TX3 with 0 to 10 percent censored, while CNT ranged from 13.4 to 28.2 with 70 to 100 percent censored. For all fenbendazole treatment groups, 90 to 100 percent of sows were negative by the end of the study,
compared to 0 to 28.6 percent for CNT. Mean BURD range was 7.0 to 60.9 for TX1, 13.9 to 60.8 for TX2, 29.3 for TX3 and 60.4 to 219.0 for CNT. All fenbendazole treatment values were different from CNT ($P<0.05$) but not from each other for time-to-negative, percent negative and BURD.

Study 2 - Embryonation

ER of *A. suum* eggs shed from CNT animals ranged from 90.3 to 99.3 percent across all experiments and sampling days. ER were significantly ($P<0.0001$, ANOVA) reduced to 29.3 and 30.5 percent for TX1 and TX2 in *A. suum* eggs shed at 8 dpt, and 26.6 percent for TX3 in eggs shed at 6 dpt. Differences in ER between treatments was only seen in eggs shed at 4 dpt; TX1=75.4, TX2=70.9, and TX3=47.0. In addition, many of the *A. suum* eggs shed from treated animals had atypical character, such as abnormal cell division, granular appearance and irregular shapes.

Implications

- Fenbendazole is an effective anthelmintic for the treatment of *Ascaris suum* in naturally infected breeding gilts and sows.

- When using fenbendazole for the control of *A. suum* transmission from dams to offspring, treatment should begin approximately 14 days prior to movement into clean farrowing facilities.
• Fenbendazole was effective at all treatment levels used in decreasing the number of *A suum* eggs shed into the environment.

• Fenbendazole is ovicidal against *A suum* in eggs shed from naturally infected breeding gilts and sows.

• Use of fenbendazole provides additional epidemiological benefits in control of *A suum* through reduced effective environmental contamination due to the reduced number of eggs shed and those that develop to an infectious larvae.
CHAPTER 1. OVERVIEW

1.1 Statement of Problem

The large roundworm of swine, *Ascaris suum* (*A. suum*), is the most common internal parasite of swine and has worldwide distribution (Greve 2012). While the apparent prevalence and severity of infection has decreased in many modern swine production systems, likely due to improved sanitation and effective anthelmintics, the parasite is still prevalent in some farm types and production systems (Roepstorff 1994, Pittman 2010a, Pittman 2010b, Duff 2014). The persistence of *A. suum* is mainly due to the fecundity of the adult female (Kelley 1956, Olsen 1958), the resistance of the *A. suum* egg leading to persistence in the environment (Gaasenbeek 1998) and a direct life cycle with an extra-intestinal migration phase that does not require an intermediate host (Roepstorff 2003). In addition, swine management systems that utilize known epidemiological risk factors will maintain *A. suum* at a prevalence that could produce overt clinical disease and economically important performance losses (Roepstorff 1994, Yaeger 2009, Woods 2012).

The main economic impacts of *A. suum* infection are decreased feed efficiency and daily weight gain in growing pigs (Hale 1985, Stewart 1988), condemnation of “milk spot” livers at slaughter (De Bie 2003, McOrist 2008) and increased medication costs associated with treatment or prevention (Boes 2010, USDA 2007, 2008).

The main goal of a parasite control program is to minimize the economical impacts of disease. Since *A. suum* eggs shed from hosts are not initially
infectious and require a period of time in the environment to develop, the main aspects of a control program are sanitation to remove eggs prior to embryonation to an infectious stage and use of an effective anthelmintic to reduce contamination of the environment. Common control programs in swine breeding herds aim to stop the transmission from dam to piglets and the farrowing environment to piglets. This is accomplished through improved sanitation of the farrowing facilities, washing of the sow prior to movement into those facilities, and treatment of sows with an effective anthelmintic prior to farrowing to eliminate or reduce worm and fecal egg burden (Raffensperger 1927, Behlow 1978, Biehl 1987, Roepstorff 1998). It is important that the anthelmintic utilized results in either significant reductions or complete cessation of egg shedding to minimize contamination of the farrowing environment and subsequent transmission to offspring. Accordingly, it is important to know how long after treatment that cessation of egg shedding occurs to properly time the treatment prior to movement into clean farrowing facilities. If the treatment interval is too close to movement, then contamination of the farrowing environment will occur. This is emphasized by the common misconception that one can simply treat sows at the time of loading farrowing or that treatment can be as soon at 2 days prior to loading (Brad Thacker, personal communication; Jeremy Pittman, personal observation).

The available literature with *A suum* and fenbendazole only report a few time points post-treatment and does not clearly characterize the time required to reach cessation of egg shedding. These reports are usually conducted in
experimental challenge settings with exact dosing of fenbendazole which may not extrapolate to natural infections under commercial management and treatment conditions. Limited data is available for the “decay curve” of fecal egg counts in naturally infected sows that are given treatments in a manner consistent with on-farm practices, which usually either approximates the weight of the animal or are based on a convenience fixed dose (i.e., number of scoops) for all animals regardless of size (Dangolla 1996; Jeremy Pittman, personal observation). In addition, some producers may utilize fenbendazole in a manner inconsistent with the label, such as giving a single day treatment as compared to the labeled 3 day treatment. Much of the experimental fenbendazole literature supports the efficacy of single dose programs with fenbendazole for *A suum*, however there is no reported data available for the evaluation of these lower single doses under commercial conditions and with naturally infected sows.

The purpose of the research contained in this thesis was to characterize the egg shedding pattern and embryonation rates of *A suum* eggs shed in naturally infected sows using common on-farm treatment and dosing methods with fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey).

1.2 Thesis Organization

This thesis is organized into five main chapters and one appendix chapter, including this overview chapter with a statement of the problem that led to the research contained herein.
The second chapter is a literature review of *Ascaris suum* and fenbendazole use in swine. It is imperative that when studying or developing health strategies for a parasite one must understand the parasite (e.g., life cycle, characteristics) and the epidemiological factors associated with prevalence and transmission, in order to develop effective programs to reduce disease and production impacts. There are references to the human roundworm, *Ascaris lumbricoides*, for several reasons. First, for a long time these two closely related but distinct species were considered to be the same parasite, and in fact the swine roundworm has been cited as *Ascaris lumbricoides var suum* in the early literature (Seamster 1950, Rogers 1956) and a search of PubMed returns manuscript titles using this nomenclature up to 1992 (http://www.ncbi.nlm.nih.gov/pubmed/; accessed 07 November 2014). Second, *A suum* and *A lumbricoides* research is complementary and interchangeable in the literature stream responsible for describing life cycles and characteristics. Third, *A suum* infection in pigs and in pig populations has been used consistently as a model to understand *A lumbricoides* worm burdens, disease dynamics, transmission, treatment and control in human populations (Boes 1998, Dold 2011). Lastly, *A suum* and *A lumbricoides* can infect their respective non-host species, with *A suum* frequently infecting humans and *A lumbricoides* having been found in pigs (Anderson 1995, Nejsum 2012, Zhou 2012).

As well as understanding the parasite, an integral part of parasite control in livestock populations is the use of an effective anthelmintic at strategic times based on an understanding of the target parasite’s life cycle and relative to the
host’s production cycle and husbandry management. Therefore a review of fenbendazole has been included in order to provide background on the drug’s efficacy, therapeutic range, safety and mechanisms of action. The literature review focuses on fenbendazole’s documented efficacy against the various life stages of *A suum*, and specifically how it relates to reduction in fecal egg shedding and ovicidal activity which are the basis for the experiments presented in Chapters 3 and 4. In addition, the literature review cites papers that describe other benzimidazole class anthelmintics and their effects on other parasites of several host species, to further support the hypotheses, methodologies and conclusions included in this thesis.

Chapter 3 is a manuscript that was submitted to the *Journal of Swine Health and Production*, the principle journal for practicing swine veterinarians in the United States and Canada, and is formatted according to the guidelines for publication, with the exception of reference citations. The manuscript describes the egg shedding pattern of *A suum* following treatment with various levels of fenbendazole in naturally infected breeding gilts and sows in five studies conducted on three large commercial breeding farms. The goals of this series of five experiments were to characterize the time to cessation of shedding and the percentage of animals that stop shedding *A suum* eggs, and to estimate the relative reduction in environmental contamination, using different levels of fenbendazole. Data presented in this manuscript can be used by veterinarians to make better recommendations to producers on when to apply treatment to sows, and theoretically other ages of swine (e.g., replacement gilts, feeder pigs), prior
to moving to clean facilities in an attempt to control environmental contamination and transmission.

Chapter 4 is a companion manuscript to Chapter 3 and was submitted to the *Journal of Swine Health and Production* and formatted according the guidelines, with the exception of reference citation. The goal of the three experiments included in this manuscript was to evaluate and confirm the ovicidal activity of fenbendazole by determining the embryonation rate of *A suum* eggs excreted in the feces of naturally infected gilts and sows at 0 to 8 days post-treatment, the main shedding period of *A suum* eggs post-treatment with fenbendazole (Chapter 3). The data presented in this manuscript demonstrates the added epidemiological advantage of using fenbendazole as a treatment for *A suum*.

The editors of the *Journal of Swine Health and Production* requested that the two companion manuscripts be combined into a single manuscript, thus publication was pursued with those recommendations and revisions (pending at the writing of this thesis). It was decided however, that the original manuscripts would be kept intact in Chapters 3 and 4 in this thesis to demonstrate extensiveness and completeness of each study and allow for more detailed discussion that was required to be edited out of the combined manuscript.

Components of Chapters 3 and 4 have previously been presented as oral presentations at the 4th European Symposium of Porcine Health Management in
Bruges, Belgium (Pittman 2012) and the 23rd International Pig Veterinary Society Congress in Cancun, Mexico (Pittman 2014a,b).

Chapter 5, a general conclusions chapter, is included in order to tie the results of the two manuscripts in Chapters 3 and 4 together, as one is an extension of the other. Animals in the experiments included in Chapter 4 are a subset of animals from the experiments in Chapter 3. Fenbendazole initially reduces worm burden and fecal egg count, but it also serves to reduce the percentage of those eggs that actually develop to an infectious egg in the environment due to its ovicidal activity, therefore impacting the “next step” of the life cycle and another critical control point.

Finally, Chapter 6 is an appendix detailing the procedures used in the experiments included within this thesis, in the event that others are interested in repeating or continuing this type of work. It was important to include the protocols as they are adaptations and modifications of previously reported methods.

1.3 References


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Raffensperger HB, Connelly JW. The swine sanitation system as developed by the bureau of animal industry in McLean County, ILL. *United States Department of Agriculture, Tech Bull.* 1927;44:1-19.

Roepstorff A. *Ascaris suum* in pigs: population biology and epidemiology. Thesis; The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 2003.


CHAPTER 2. LITERATURE REVIEW

2.1 Ascaris suum

In 1906, in the book Diseases of Swine, author Dr. Robert A. Craig said of the swine roundworm; “Many stockmen have erroneous ideas regarding this parasite.” (Craig 1906) Since then we have learned much about the life cycle and control of Ascaris suum, however one still encounters stockmen and veterinarians with insufficient understanding of the most common and cosmopolitan parasite of swine. In addition, several veterinary colleges within the United States, including those in the main swine producing areas, place little emphasis on swine parasites in current curriculums due to a misconception that parasites are no longer a concern in modern swine production systems (Jeremy Pittman, personal communication).

2.1.1 Life cycle and worm characteristics

The life cycle of A suum is direct and very similar to other Ascarids and has been studied extensively as a model for Ascaris lumbricoides the human Roundworm. Pigs ingest eggs containing an infective L3 larvae from a contaminated environment. If the ingested eggs are unembryonated non-infective they will pass through the intestine and out in feces undamaged and resume embryonation in the environment (Marti 1986, Boes 1997). The eggs begin to hatch in the stomach and small intestine in response to the acidic pH, bile enzymes and peristalsis (Geenen 1999). The liberated L3 larvae penetrate the mucosa of the cecum and proximal spiral colon as early as 3 hours post
ingestion (hpi), but the majority between 6 and 12 hpi (Rhodes 1977, Murrell 1997). Larvae penetrate the mucosa and migrate toward the liver via the hepatic portal system. The majority of larvae can be found in the liver between 8 and 18 hpi, where migration results in mechanical damage leading to a hypersensitivity reaction with eosinophilic inflammation (Murrell 1997). The inflammation results in fibrosis and development of “milk spots”. The larvae leave the liver via the caudal vena cava, travel through the heart and to the pulmonary artery and capillaries (Murrell 1997). Migration through the lungs occurs 4 to 6 days post ingestion (dpi) and results in pulmonary damage, leading to rapid expiratory efforts (“thumps”) and coughing. The larvae penetrate the alveoli and migrate up the bronchial tree to the trachea then the pharynx where the larvae are coughed up and swallowed around 8 to 10 dpi. The L3 larvae then go through two more molts in the small intestine, to an L4 larvae around 10 dpi and an L5 larvae around 23 to 24 dpi, and develop to a mature adult (Roepstorff 2003). When larvae reach the small intestine there is a mass expulsion of many of the larvae, especially with larger infectious doses, which is considered to be related to an immune mechanism or self-population control (Jorgensen 1975, Boes 1998).

Adults live in the small intestinal lumen and feed on ingesta. Adult females begin to produce fertilized unembryonated eggs 6 to 8 weeks post ingestion of embryonated eggs (wpi) if mature males are present. It is interesting to note that migration is not required, as experimental intravenous injection, which bypasses the liver, of in vitro hatched L3 result in patent intestinal infections (Jungersen 1999).
Adult *A suum* are relatively large with females 40 to 45 cm in length and males smaller at 25 cm. The adult female *A suum* has been estimated to produce between 1 and 2 million eggs per day (Kelley 1956, Olsen 1958). By comparison the average daily egg output of *A lumbricoides* has been estimated to be around 240,000 (Brown 1927, Sinniah 1982). The female *A suum* can also produce fertile eggs for 2 weeks after removal of adult males (Jungersen 1997). The majority of adults are expelled by 23 wpi, however some may live for more than a year (Olsen 1958). There is a poor relationship between fecal egg counts and the number of female worms (Bindseil 1974). It is important to note the concept of population overdispersion which has been demonstrated in *A suum* and *A lumbricoides*, in which a small number of hosts harbor the majority of the worm burden in a population while a large percentage of the population may not be infected (Boes 1998).

### 2.1.2 Egg development and resistance

Eggs shed from hosts are not initially infectious, and must go through a period of development in the environment. This is a key aspect to the Ascarid life cycle that is often unrecognized, however is “central in the epidemiology of the parasite” and control programs, allowing time to remove either the eggs (i.e., sanitation) or the susceptible hosts (i.e., early weaning) from the contaminated environment (Roepstorff 2003). Successful embryonation is highly dependent on optimal temperature, humidity and oxygen tension (Seamster 1950, Arene 1986, Gaasenbeek 1998, Brown 1928).
Under ideal conditions the larvae of *A suum* develop from a 1-cell embryo (day 0) to a 2-cell (d2), 3- to 4-cell (d3), early morula (d4), late morula (d6), blastula (d7), gastrula (d8), pre-larvae (d10), L1 (d12) and L2 (d16) (Cruz 2012). A final molt to an L3 occurs in about twice the time it takes to develop to the L2, depending on temperature, at which time they are infectious (Roepstorff 2003). Seamster determined the development and rate of *A suum* eggs in a temperature range from 8.89 to 37.8°C (Seamster 1950). No cleavage was seen at temperatures 11.1°C and lower (Seamster 1950). Motile embryos were seen in ranges between 16.7 and 34.4°C, however there was a decline in the percent of motile embryos at 32.2°C and higher, and at 37.8°C all ova died (Seamster 1950). Seamster determined that optimum development temperature was at 31.1°C (Seamster 1950). Work by Arene (1986) confirmed the increasing rate of development with increased temperatures, and found maximum rate of development at 31°C. However, eggs that were embryonated at 28°C and higher had reduced hatching rate, longevity post hatch and reduced infectivity (Arene 1986). Arene suggested that optimal embryonation occurred at 22°C (Arene 1986) with respect to percent embryonation and percent infectivity.

Egg development in practical environmental settings has been evaluated. Connan described the development of *A suum* eggs under simulated conditions in a pig house in Great Britain in which eggs were placed periodically over 20 months between January 1973 and September 1974 (Connan 1977). Eggs which were started January to May, or after September developed slowly or not at all until June or July when ambient temperature rose above an apparent
development threshold. Eggs started in June and July developed more rapidly and became infectious in August and September. In addition, eggs observed during the months of October and April did not show any additional development during this period, indicating an arrested state (Connan 1977). Stevenson demonstrated similar developmental periods between June 1976 and December 1977 in eggs placed in an unheated swine facility (Stevenson 1979), also in Great Britain. Eggs did not develop between the months of November and May when maximum daily ambient temperatures were below the development threshold of 14.5°C as described by Seamster, however rapid development was seen in the months of June, July and August when maximum temperatures were above 14.5°C (Stevenson 1979, Seamster 1950). A similar study was also conducted in Saskatchewan, Canada by Wagner and Polley, in which eggs were placed at monthly intervals from July 1997 to July 1998 (Wagner 1999). Development was most rapid during the summer months of June, July and August, however in contrast to the work by Connan and Stevenson egg development never ceased, but rather slowed during the spring, fall and winter months (Wagner 1999). Wagner attributed this continued development to a higher ambient temperature within the barns that rarely dropped below the development threshold of 14.5°C, as compared to the studies conducted in Great Britain (Wagner 1999). Stevenson also noted in his work that development of eggs in the environment of pigs could be influenced by supplemental heating, and even consideration of increased ambient temperatures from the body heat generated from pigs in the environment (Stevenson 1979). Interestingly in all
three papers the authors reference the seasonality seen in “milk spots” at the abattoirs as reported by others (Connan 1977, Stevenson 1979, Goodall 1991, Menzies 1994, Lundehein 2010, Sanchez-Vazquez 2012), in which a consistent increase is seen in pigs marketed during the late summer and early fall and subsequent decrease seen in winter and spring. This seasonality is likely related to the timing of marketing growing pigs previously exposed to fully developed larvae in the late spring and summer environments.

Ascarid eggs have been called “one of the most resistant biological structures” (Wharton 1980). Eggs are highly resistant to chemicals and environmental influences, due to their complex shell which is composed of 4 layers (Foor 1967). The layers include, from the inside-out; an ascaroside lipid layer responsible for the impermeability to many chemicals, a thick chitinous layer which provides structural strength, a thin vitelline layer and an outer “sticky” proteinaceous uterine layer (Roepstorff 2003). Due to the resistance of *A suum* eggs, they are commonly used to test potential disinfection chemicals and as an indicator organism in chemical treatment process protocols for human wastewater treatment facilities (EPA 1994, Pecson 2005). In one study, the eggs of *A suum* were resistant to eleven disinfectants, many of which are commonly used in swine facilities, such as phenol, sodium hydroxide, quaternary ammonium and glutaraldehyde based products (vd Burg 1987). Resistance to povidone-iodine has also been reported (Labare 2013). This resistance to many environmental and chemical influences contributes to the consistent challenges with *A suum* and inability to easily remove the pathogen from the environment.
2.1.3 Environmental epidemiology related to sow to piglet transmission

The main economic impacts of *A suum* are those that occur in the growing pig, therefore much of the focus and control efforts are at reducing the clinical impact of disease in this population. With the development of age-segregated and early weaning production operations, controlling disease transmission between the breeding herd and grow-finish population became easier due to a physical separation of infected and susceptible populations (Harris 2000). While not specifically referenced as a pathogen controlled by these types of operations in Harris’s *Multi-Site Pig Production*, there is a significant advantage for the control *A suum*. The important and common control strategy for *A suum* in modern swine production takes advantage of the fact that eggs shed from hosts are not initially infectious, and therefore allow for a period where targeted measures can significantly minimize or eliminate the transmission risk from sow to piglet. In many modern swine operations, sows are moved from a breeding location to individual farrowing stalls where piglets can be farrowed into a sanitary environment and sows can be managed individually. Piglets are then weaned to an off-site location at a young age (2 to 4 weeks), and prior to the required development period of *A suum* eggs, shed from their dam. In these management styles farrowing rooms or barns are commonly managed with “All-In/All-Out” style of pig flow in which the room is completely emptied and sanitized prior to loading the next group of due-to-farrow sows, which minimizes the transmission of disease from previous groups to subsequent farrowing sows and litters. This management style can minimize or prevent the transmission of *A*
suum from breeding herd to growing herd. Under most standard cleaning protocols (including high pressure washing, use of detergents and disinfectants, and allowing a period for surfaces to dry) the farrowing facility should be a low risk for A suum transmission to piglets from previous contamination. Therefore the main risk of transmission would be from the current birth dam or possibly a surrogate dam shedding eggs. It is important to note that due to the period of development required outside the host, eggs shed into the farrowing environment from sows are not usually directly infectious to her offspring if weaning occurs at an age younger than the period required for the eggs to develop infectious larvae. This is supported by work that shows poor correlation between a sow farm and grow-finish A suum infection status when wean age is less than 5 weeks, however when wean age is 6 weeks or greater, grow-finish animals are twice as likely to be positive if originating from a positive sow farm (Roepstorff 1991, Homgren 1998). While this age segregation has an inherent epidemiological advantage in reducing the transmission from dam to piglet, there are still transmission risks associated with lateral transmission to piglets from unsanitary farrowing environments (“hot spots” from previously housed sows) or fomites from other areas of the farm (Nilsson 1982). Therefore, overall sanitation, management and biosecurity of the entire herd are also important control measures for A suum.

Another common recommendation (referenced in the McLean County and North Carolina Swine Parasite Control programs) on sow farms for control of parasites is to thoroughly wash sows with soap prior to loading into farrowing, in
order to remove contaminated fecal material from the skin and underlining (Raffensperger 1927, Behlow 1978, Biehl 1987). While this recommendation and mode of transmission makes logical sense, it is likely only a significant concern when sows are housed in a highly contaminated areas and are excessively dirty (Thomas 1983). De Deken et al demonstrated a decreased association of finishing ascariasis when sourcing sow farms washed sows and used anthelmintics prior to farrowing (De Deken 1984). Mercy et al identified a lower prevalence in growing pigs from sow herds that routinely washed sows prior to farrowing (Mercy 1989). Johnson et al demonstrated that using anthelmintic alone did not reduce the correlation between sow farm status and finishing status, but that control of gestation contamination in the sow herd was associated with reduced incidence of finishing ascariasis (Johnson 2003). While proper timing and use of effective anthelmintic treatment of breeding females can be an important component of an A suum control program, reduction of the environmental parasite burden, namely through sanitation and proper management of animals and their environment, remain the most fundamental measures in control of A suum in swine herds and farms.

2.1.4 Prevalence

The apparent prevalence and severity of A suum has decreased in many modern swine operations, as has the prevalence of many other swine parasites. Theoretically the main reasons for the reduced prevalence are the improved sanitation of modern facilities, which routinely remove a large amount of the
feces from the environment (e.g., slatted flooring), use of All-In/All-Out
management which helps to break transmission from older pigs to newly placed
pigs and allows better sanitation between groups, separation of age groups to
different off-site facilities, namely off-site weaning of young piglets (<4 weeks of
age) (e.g. two and three-site production, early and isowean systems), and the
strategic use of highly effective anthelmintics. It should be noted here that while
not important for *A suum*, due to a direct life cycle, another cause of reduced
parasite burden in modern swine production has been the removal of
intermediate hosts (e.g. earthworms, beetles) by moving pigs indoors. However,
even with the above advancements in modern swine production, *A suum* can still
be found in modern swine production facilities.

Prevalence in the United States is not well understood as *A suum* is no
longer considered a significant swine disease. In addition, liver condemnations
at slaughter which serves as a metric for ascarid prevalence are no longer
measured at major abattoirs in the US (Meisinger 2004). Current published
estimates of *A suum* prevalence are restricted to the 2006 National Animal
Health Monitoring System (NAHMS) surveys conducted by the United States
Department of Agriculture (USDA), a 2012 survey questionnaire conducted by
Elanco Animal Health (Greenfield, Indiana) and a few production system level
cross-sectional surveys of breeding and grow-finish sites. In the 2006 NAHMS
survey, 26.8 percent of breeding sites indicated that disease problems were
attributed to "roundworms", however only 9.7 percent of these sites had this
confirmed by a veterinarian or a laboratory (USDA 2007b). In nursery age pigs,
15.8 percent of sites indicated “roundworms” as a disease issue with only 13.4 percent of those sites diagnosed by a veterinarian or laboratory (USDA 2007b). In grow-finish sites, 15.5 percent of sites indicated “roundworms” as a disease issue, with only 23.1 percent of those sites having the parasite diagnosed by a veterinarian or laboratory (USDA 2007b). The 2012 USDA NAHMS report for small-enterprise swine operations indicated that 8.2 percent of all breeding operations identified roundworms as a cause of disease in gilts or sows and in 8.6 percent of weaned pigs (USDA 2014). In the Elanco Animal Health’s “Full-Value Pigs” survey, 16.6 percent of those surveyed identified “worms/parasites” as causing disease, however this does not clarify *A suum* from other potential parasites (e.g. *Trichuris suis, Sarcobtes scabiei*, etc.) (Pelger 2012d). In 2002 and 2003, 141 lots across 7 abattoirs were surveyed in the United States and Canada for evidence of mange and roundworms, 25 percent of the lots had at least one animal with “milk spots” (Melancon 2005). In a 2010 survey of a large swine production system located in North Carolina and Virginia, 25 percent (10 of 40 sites) of breeding herds representing 12,000 breeding sows and 38.5 percent (35 of 91 sites) of grow-finish sites representing 370,000 finishing spaces were positive for *A suum* (Pittman 2010 a,b). A 2014 survey of a production system located in eastern North Carolina, looking at 58 sites with breeding age animals, reported a 23 herd percent prevalence of *A suum* (Duff 2014).

Prevalence of *A suum* is better documented in other countries, and is generally considered more of a concern than in the United States. In the same “Full Value Pigs” survey conducted by Elanco Animal Health, parasites were
considered a health challenge in 25.6, 17.2, and 7.8 percent of surveys from Europe, Asia and Latin America respectively (Pelger 2012b,c,d).

A Canadian survey in 2002 indicated that 21.8 percent of 64 farms tested were positive for *A suum*, even though 95.7 percent of the producers used some type of anthelmintic in their sow herds (Young 2002). Previously in two abattoir surveys in Saskatchewan, Canada, 44 percent of 2,500 market animals had some form of liver scarring with 8 percent being severely affected in the first survey, and 17 percent had adult ascarids in the intestine with 50 percent of livers with lesions in the second survey. Out of the total 611 farms surveyed, 477 had pigs with affected livers (Wagner 1997). A similar set of Saskatchewan surveys in 1980 demonstrated 37 percent of animals with adult ascarids and 46 percent with “white spots” in 2,500 market animals observed and in a second study, 44 percent of 2,500 market animals had white spots indicating not much change in prevalence in this region over two decades (Polley 1980).

Prevalence of *A suum* in the European Union (EU) is better understood. In a 2003 review article by De Bie, incidence rates in EU countries were noted as 25 percent of 7,690 livers in France, 47.5 percent of 6,250 livers in Austria, 10.7 percent of 15 million livers in The Netherlands, 50 percent of lots from 9,000 livers in the United Kingdom, 35 percent of pigs in Belgium showed some form of liver pathology and in Germany, 4.3 to 53.6 percent of livers from farms demonstrated white spots (De Bie 2003). Sanchez-Vazquez et al looked at milk spot rates in 12 abattoirs in England between 2005 and 2010 and reported a 4.2 percent rate in milk spots on 34,168 pigs (Sanchez-Vasquez 2012). In Scotland,
5.2 to 5.3 percent of livers evaluated over a two year period from 45,000 carcasses from 150 herds representing 75 percent of Scottish pig production demonstrated milk spots (Strachan 2006). Menzies et al demonstrated an increase in mean annual prevalence of milk spots in Ireland from 4 to 9 percent from 1969 to 1991, demonstrating an increase with modernization of swine production (Menzies 1994). A similar study, conducted in Ireland in 2012 and 2013 evaluating 12,597 finishing pigs at slaughter facilities, demonstrated an average percent of pigs with milk spots within batch to be 11.5 percent (Hidalgo 2014). In addition, the prevalence within batch was greater than 10 percent in 42.8 percent of batches (Hidalgo 2014). In a 2002-2003 survey of Brittany, France abattoirs 10 to 65 percent of pigs within batches demonstrated milk spots (Kanora 2004). In Belgium, 11.1 percent of livers were condemned from 152,364 pigs over a three and a half year period from 56 farms (Vyt 2004). In Sweden, a survey of the six largest abattoirs demonstrated a rate of 2.5 to 6.5 percent milk spots in 729 herds (Lundeheim 2010). In Slovakia, 19,017 animals from 279 herds were inspected from 2000 to 2009 and a decrease in milk spot rates was seen from 39.5 to 6.9 percent (Ondrejková 2012). The authors attributed this decline to an increasing positive attitude toward parasite control within the Slovakian swine industry. In the Czech Republic, between 2003 and 2005, 9,163 livers from 99 farms had a range of 7.1 to 11 percent with milk spots by lot (Žižlavský 2006). A survey in Italy from January to October 2012 evaluated 4,764 batches of slaughter pigs totaling 667,028 pigs and reported an overall
milk spot prevalence of 13.9 percent, a batch prevalence of 17.1 percent and a within batch prevalence range of 8 to 100 percent (Luppi 2014).

In a study between 1986 and 1988, 516 herds located within the Nordic countries (Demark, Sweden, Finland, Norway and including Iceland) were surveyed by fecal analysis. Prevalence rates in sows ranged from 1.4 to 13.4 percent, while gilt prevalence was generally higher at 3.8 to 27.2 percent (Roepstorff 1998). Prevalence rates in finishers ranged from 5 to 34.5 percent (Roepstorff 1998).

*Ascaris suum* prevalence has also been documented in Asian counties. A survey between 1984 and 1986 in Singapore evaluated almost 3 million pigs and reported 4.3 percent had liver lesions, of which 20.4 percent of those were condemned (Tiong 1989). In Japan, between 2005 and 2007, a survey of 150 farms across Japan reported a 2 percent prevalence by fecal analysis, with prevalence being higher in sows than fattening pigs (Kobayashi 2009). In Osaka, Japan, a survey of feces of 129 pigs from an abattoir reported 14.7 percent positive for *A suum* (Matsubayashi 2009). In China, a 2007 to 2009 survey reported *A suum* as the most common parasite in swine, with 20.25 percent of intensive managed sow farms positive and 20.42 percent of extensively managed sow herds positive (Lai 2011).

As the prevalence and severity of *A suum* has generally decreased with modern swine production and facilities, there still exist swine production operations in which particular risk factors are unavoidable, namely organic, niche market or outdoor operations (Roepstorff 2011). In many countries, legislation or
customer demand is forcing change in the management of swine to “improve welfare” that in many ways promotes increased transmission of parasites. In Denmark, such legislation is represented by the requirement to loose house sows in groups, provide access to rooting material (straw, bedding, etc.), solid flooring, sprinklers that increase humidity and restrictions on medication use (Roepstorff 2011). In the US, regulations are very similar for USDA defined “organic” operations (http://www.ams.usda.gov/AMSv1.0/organicinfo). In the EU, Council Directives 2001/88/EC and 2001/93/EC (http://ec.europa.eu/food/animal/welfare/farm/pigs_en.htm) set minimum standards for the provisions of pigs, including several requirements that will potentially increase parasite transmission.

Prevalence of *A. suum* infection is generally higher in the above types (organic, extensive, welfare-friendly) of operations. Pattison *et al* demonstrated a higher prevalence in sows housed on pasture as compared to stalled or tethered sows (Pattison 1980). Interestingly these authors also described a lack of influence of anthelmintics on pasture sows as compared to a positive influence in indoor housed sows (Pattison 1980). Eriksen *et al* described a higher prevalence in outdoor herds and small indoor herds as compared to larger indoor herds, particularly in the growing populations (Eriksen 1996). In a study on Danish organic herds, Cartensen *et al* described a higher prevalence of *A. suum* in these herds as compared to conventional herds, particularly in weaners and fatteners, and indicated the regulations of organic herds (e.g., straw bedding, access to roughage, no prophylactic anthelmintics, extended withdrawal periods and
weaning at 7 weeks of age) as increased risk factors (Cartensen 2002). In this study, *A suum* were found in 14 percent of soil samples from the pastures and in 35 percent of the slaughter pigs (Cartensen 2002). Eijck and Borgsteede surveyed free-range, organic and conventional pig farms in The Netherlands and found a prevalence of 50, 73.7 and 11.1 percent, respectively in each type of operation (Eijck 2005). In a survey of 26 niche market herds in the Midwest US, Yaeger *et al* reported 15.8, 29.4 and 61.9 percent of fecal samples from sows, nursery and finisher were positive for *A suum* which was determined to be a higher prevalence than conventional herds (Yaeger 2009). It was also noted that many of these niche herds commonly used anthelmintics, but appropriately did not achieve successful parasite control.

Prevalence within farm or within a flow of pigs is usually dependent on age of the animals, with older growing pigs having a higher prevalence than younger pigs and breeding sows (Roepstorff 1998). Gilts and younger parity females have a higher prevalence than older sows, likely due to development of immunity over time (Marti 1986, Urban 1989, Roepstorff 1998, Nosal 2008).

### 2.1.5 Production and economic impact

The main production and economic impacts of *A suum* infection are reduced feed conversion (FC), reduced average daily gain (ADG), liver condemnations from milk spots and costs associated with routine anthelmintic treatment and prevention measures (Greve 2012). In general, the level of impact on swine can be related to the level of infectious dose, and thus worm burden of
the individual animal. However when the impact is assessed at a population level, the impact is not generally appreciated, likely due to overdispersion of the parasite burden or the overriding influence of more significant diseases (Hale 1985, Bernardo 1990, Boes 2010). In the case of overdispersion, only a small percentage of the population have the parasite burden, thus the impact on performance of this small percentage could easily be masked by the performance of the majority parasite-free population. However, it is possible that those animals with the higher parasite burden are likely to represent lower value pigs within the population, such as cull or light weight market pigs.

The adult *A suum* lives within the small intestinal tract and consumes nutrients from the lumen of the gut, therefore reducing feed efficiency and conversion. In addition, larval migration through the intestinal mucosa and major organs of the body have a negative impact on feed utilization and growth. Zimmerman *et al*, when studying the effect of the anthelmintic pyrantel on *A suum* in growing pigs, demonstrated an improvement in FC by 11 percent in treated pigs as compared to infected control pigs, but only in groups fed a low protein diet, not in groups fed a high protein diet fed groups (Zimmerman 1971). Nilsson and Martinsson described a significantly reduced growth rate of 37 grams per day in pigs when fecal egg counts were greater than 10,000 (Nilsson 1980). In the commonly cited work done by Hale *et al*, pigs were given three levels of infectious dose of *A suum* eggs (600, 6,000 or 60,000 eggs) to determine the production impact of infection. The authors reported a linear and quadratic trend in reducing final weight and ADG with increasing infectious dose
(Hale 1985). Pigs were 5, 7 and 13 percent less efficient than controls when challenged with 600, 6,000 or 60,000 eggs, respectively (Hale 1985). When evaluating the efficacy of fenbendazole, Stewart et al demonstrated a 15 to 22 percent improved FC in pigs treated 2 to 4 days post-infection (dpi) than control pigs or pigs treated later (6 to 8 dpi), demonstrating that early migration phases have a significant impact on feed conversion (Stewart 1984). In this study, ADG was 23 percent greater than infected controls, regardless of timing of treatment (Stewart 1984). In work by Bernardo et al the number of ascarids at slaughter did not have a significant impact on ADG, but “lifetime burden”, an estimated measure of magnitude and duration of fecal egg counts, was significantly associated with ADG (Bernardo 1990). In the same study the authors indicated that while ascarid infection was detrimental to ADG, only a maximum of 1 percent change in ADG should be expected if parasite control was initiated in heavily infected farms (Bernardo 1990).

In a meta-analysis of endoparasites, Kipper reported pigs with endoparasites had a 5 percent lower daily feed intake, 31 percent lower ADG and 6 percent lower total weight gain than control animals (Kipper 2011). Control animals also demonstrated a 17 percent improved FC ratio (Kipper 2011). Kipper modeled a 4 percent increase in time to a fixed target market weight in pigs with endoparasites, but it must be noted that there was no discrimination as to the type of endoparasite (A suum, T suis, etc.) in this work (Kipper 2011).

The migration of larvae through the liver and resultant “milk spot” pathology is arguably the largest and most appreciated economic impact of A
suum in swine. Livers with severe scarring are condemned or down-graded since they cannot be sold for human consumption. Livers are high in several vitamins and minerals and are either considered delicacies or are a staple of low-income population diets, depending on the country (Marti 2011). In North America, the economic impact is generally not experienced by the producer since payment is based on carcass dressed weight, which does not include the liver and other organs, therefore lost opportunity is experienced by the processor (Hurnik 1995). However, in other countries, a penalty may be imposed on a producer, such as in The Netherlands where a 1 € penalty per pig with a “white spot” liver was applied to a producer to incent better parasite control on the farm (van Wagenberg 2010). McOrist et al calculated the loss of liver condemnations to be 0.30 to 0.50 € kg⁻¹ ($0.18 to 0.31 lb⁻¹) in 2007 in the EU. In a survey of seven packers in the United States, Meisinger reported the lost value of condemned livers to be $0.27 lb⁻¹ to $0.30 per animal (Meisinger 2004). In this same survey 3 of 6 packers reported that domestic and export markets influenced the value of livers. Liver values can vary depending on the domestic demand and packer access to export markets. At the time of this writing (May 2014) the US domestic value of livers was $24.00 cwt⁻¹ for edible livers, $13.50 cwt⁻¹ for inedible livers (used in pet food) and $9.00 cwt⁻¹ for rendered product (personal communication, Charles Allison, Smithfield Packing, Smithfield, Virginia). The export market within the US has seen an increase in edible pork offal, of which liver is a major contributing organ (Marti 2011). In 2012, edible byproduct (variety meats) accounted for 13.7 percent of the total value of US
exports with more than 90 percent of these products exported (Herlihy 2013). The edible byproducts account for 6 percent of the total value of a pig (Marti 2011). To compare the value of livers domestically to an export market, the average value of livers in the US in 2010 was $16.73 cwt$^{-1}$, while the average price in China was $51.80 cwt$^{-1}$ (Marti 2011). As export market demand increases, the value of unblemished livers will increase, and could return focus to *A. suum* control by the producer.

The cost of routine anthelmintics for control or treatment can be significant to swine operations, especially if not required or extensively used (Roepstorff 1997). In the 2006 NAHMS survey in the US, 74.7 to 76.8 percent of sites actively dewormed sows and 64 to 68.2 percent dewormed boars as part of their routine herd health protocols (USDA 2007a, USDA 2008). It is important to note that use of anthelmintic was the most common disease prevention practice used in these herds; even more than antibiotic usage in the feed, water or as an injection. In addition to breeding animals, 30 to 47.2 percent of sites dewormed piglets prior to weaning, 33.4 to 37.1 percent dewormed nursery pigs and 30.6 to 34.0 percent dewormed grow-finish pigs (USDA 2007a, USDA 2008). The average US prices of a single 400 lb sow treatment for the available anthelmintics at the time of this thesis were $2.46 for fenbendazole (Safe-Guard, Merck Animal Health, Summit, NJ), $0.52 for pyrantel tartrate (Banminth, Phibro Animal Health, Teaneck, NJ), $1.55 for ivermectin (Ivomec, Merial LTD, Duluth, GA) and $0.50 for piperazine (Wazine, Fleming Laboratories, Inc, Charlotte, NC) (Prices from QC Supply available at http://www.qcsupply.com/; accessed 01 May
2014, Banmith price provided by Phibro Animal Health). While not available at the time of this writing, the cost to treat a 400 lb sow with dichlorvos was $0.67 (Atgard C, price provided by Boehringer Ingelheim Vetmedica, Inc, St Joseph, MO). Using the above calculations and assuming 10 pigs weaned per sow farrowed, the cost of treating weekly batches of sows can be $0.05-0.25 per weaned pig.

Stewart and Hale utilized the literature available at the time in the US to estimate a production impact (growth and feed conversion only) from *A suum* of $86.7 million to the 1988 US swine industry, representing an average of $4.02 (range $2.36-6.38) per market animal (Stewart 1988). The assumptions made for this calculation were a 91 day feeding period with a start weight of 26.6 kg, a 105.4 kg end weight, daily gains of 0.87 kg, 3.03 feed to gain ratio and a total cost of feed of $42.02 (Stewart 1988). It is important to note that today, the assumptions made by Stewart and Hale are not valid, as the US swine industry is significantly different from 1988 with changes in management, facility design, genetics, nutrition, input costs, market value and *A suum* prevalence; however no recent research has been done to characterize the performance impact of *A suum* on production. Table 2.1 shows current estimated economic impact of *A suum* in grow-finish pigs as $13.36 for each infected animal, based on current feed prices and market value (Iowa State University Extension, Ames, IA), national average pig performance metrics (Agri Stats, Inc, Fort Wayne, IN) and using performance deficiencies as reported by Stewart and Hale (Stewart 1988).
It must be noted that, much of the early work on the economic impact of *A suum* was done with experimental infections and the reduced performance extrapolated to entire populations, which may not be a fair representation of the true economic impact of the disease in swine populations. Urban *et al* could only demonstrate a significant impact in ADG in infected pigs on pasture, but not when groups were housed on concrete (Urban 1989). Boes *et al* failed to demonstrate significant differences in final weight, ADG, FC or lean meat percentage in naturally infected pigs treated with flubendazole as compared to untreated controls (Boes 2010). The authors indicated that other pathogens, such as *Lawsonia intracellularis*, have more of an association with performance metrics than does natural *A suum* infection (Boes 2010). Bernardo *et al* in their modeling work indicated that there was more benefit in controlling respiratory disease in growing pigs than in parasite control (Bernardo 1990). Further work must be done to fully understand the overall production and economic impact on populations and to justify the expense of prevention, control and treatment measures.

Other potential impacts of *A suum* infection are an increase in secondary diseases, decrease in vaccine efficacy, reduced nutrient utilization, reduction in carcass quality, decreased sow performance and losses attributed to acute morbidity and mortality with acute infection. These impacts while documented in the literature are generally less appreciated or not fully understood.

The mechanical damage created by the migration of larvae through the intestinal mucosa, liver and lungs allows for the enhancement of secondary
pathogens to cause disease. For example, a decreased rate of clearance of bacteria from the lung of weanling pigs infected with *A suum* has been reported (Curtis 1987). Another aspect of *A suum* resulting in increased secondary bacterial pneumonia is a decreased phagocytic activity of macrophages in pigs infected with *A suum* (Urban 2007).

Co-infection of *A suum* and *Escherichia coli* resulted in a synergistic pneumonia and systemic disease in piglets (Adedeji 1989). Matusyavichus *et al* co-infected pigs with *A suum* and *Erysipelothrix insidiosa [rhusiopathiae]* which resulted in 80 to 100 percent mortality as compared to no mortality in groups infected with either pathogen alone (Matusyavichus 1985). In mice infection models, co-infection with *A suum* and *Pasteurella multocida* resulted in a more severe pneumonia, septicemia and greater mortality than individual infections (Tjørnehøj 1992). Smith *et al* suggested a significant association between *A suum* and *Salmonella* sp. in slaughter animals (Smith 2011). Engle and Bush demonstrated a significant association of porcine circovirus Type 2 associated disease with a history of ascaris and hypothesized that immune stimulation resulting from larval migration could have been the mechanism for pigs to develop to the severe clinical form (Post Weaning Multisystemic Wasting Syndrome) of disease (Engle 2006). In an early study of impact of *A suum* on hog cholera (Classical Swine Fever), Shope demonstrated that co-infection resulted in a more acute, severe and prolonged systemic disease and *A suum* exacerbated latent infections of hog cholera (Shope 1958). Underdahl and Kelley demonstrated ten times greater pulmonary consolidation in pigs exposed to
“virus pneumonia of pigs” [Mycoplasma hyopneumoniae] if exposed after ingestion of A suum eggs (Underdahl 1957). Nayak and Kelley inoculated pigs with swine influenza virus (SIV) 8 days after inoculation with A suum eggs which resulted in 90 percent mortality and a more pronounced dyspnea as compared to 30 and zero percent mortality for SIV and A suum alone exposed groups, respectively (Nayak 1965). The authors suggested the more severe disease and synergism may only be significant during the migratory phase of A suum (approximately 8 to 10 days post-ingestion).

In a case where feeder pigs were placed in highly contaminated outdoor pens, secondary pneumonia with Actinomyces pyogenes and P. multocida occurred three weeks after a spike in larval associated respiratory disease, which resulted in 25 percent morbidity and 10 percent mortality (Perry 1993). Post mortem evaluation revealed severe, diffuse pulmonary edema, multifocal pulmonary hemorrhage and severe parasitic hepatitis (Perry 1993). Microscopically a severe acute, eosinophilic and granulomatous interstitial pneumonia was seen (Perry 1993). In another case, Gjestvang et al described acute respiratory distress and mortality in feeder pigs due to heavily contaminated (320 to 1240 EPG) sawdust bedding (Gjestvang 2004). Pigs began to show signs of lethargy and pneumonia 5 days after arrival, and on day 9 after arrival, 26 of 40 pigs had died from pneumonia due to larval migration (Gjestvang 2004). A case report from Mexico in 2014 described acute and severe respiratory distress and 10 percent mortality in 23 week old finishing pigs (Trujano 2014). At necropsy a significant number of migrating larvae were
visualized throughout the pigs’ airways and intestinal tract (Trujano 2014). These
cases highlight the potential for significant acute mortality when naïve pigs are
exposed to heavily contaminated facilities.

A suum has been loosely associated with gastric ulceration in pigs. Gastric ulceration has been reproduced experimentally in pigs exposed to A
suum (Gaafar 1972), however the clinical significance and prevalence is not well
appreciated. In a case of acute exposure and disease of 3 month old pigs,
gastric ulceration was seen in addition to coughing, dyspnea and fever (Qureshi
1978). The authors suggested the ulceration was due to an immunological
mechanism or simply the off-feed event associated with the disease.

Esophagogastric ulcers were weakly associated with A suum in a study
conducted by Häni et al, but the authors concluded that the association was not
biologically significant in the field (Häni, 1979).

Infection with A suum has been shown to decrease the pig’s response to
vaccine and reduced vaccine efficacy. Steenhard et al demonstrated a reduced
and delayed seroconversion to M hyopneumoniae vaccination when pigs were
consistently exposed to infective A suum eggs before and during the vaccination
period (Steenhard 2009). When pigs were subsequently challenged with M
hyopneumoniae, those exposed to A suum had a higher mean percentage of
lung pathology compared to vaccinated only pigs. The authors suggested that A
suum interferes with vaccine efficacy through an immune modulatory effect,
namely through a Th-2 skewed response (Steenhard 2009).
In addition to synergistic effects related to mechanical damage and immunomodulatory effects, *A suum* infection has a negative impact on nutrient utilization by the pig. In experimentally challenged 15 day old pigs, Stephenson *et al* demonstrated histological changes of the tunica muscularis, intestinal villi, goblet cells and an infiltration of the lamina propria, and hypothesized that this damage could result in decreased nutrient uptake, especially in marginal protein diets (Stephenson 1980). Martin *et al* described marked changes to the mucosal surface and intestinal villi at 58 days post-infection with *A suum* in pigs on low protein diets and suggested an association between the lesions and lactose maldigestion which is commonly seen in human ascariasis (Martin 1984).

Forsum *et al* studied the impact of *A suum* on pigs fed protein deficient diets and demonstrated that reduced growth rates were seen after worms matured to adults and eggs were seen in the feces (Forsum 1981). They described reduced fat digestion, reduced nitrogen retention and a lower lactase activity, resulting in infected pigs using protein less efficiently (Forsum 1981). The impact was more significant in pigs on protein deficient diets, however similar trends were observed in pigs fed normal protein level diets (Forsum 1981). Hale *et al* demonstrated a lower dry matter, crude protein and gross energy digestive coefficient in *A suum* infected pigs between 33 and 37 days post inoculation as compared to control pigs (Hale 1985). Higher nitrogen retention was also seen in control pigs as compared to infected pigs (Hale 1985). In humans, ascariasis can cause vitamin A deficiency if there is a marginal intake of vitamin A and
associated precursors, but this has not been demonstrated in pigs (Mahalanabis 1976).

Reduced nutrient utilization and reduced growth performance can also have an impact on carcass quality in swine. In a study by Knecht et al, the meatiness of swine carcasses were compared between infected and uninfected pigs, and determined to be significantly different with a negative correlation to fecal EPG counts (Knecht 2011). In a follow-up study by Knecht et al, carcasses from infected finishing pigs were statistically more likely to fall into carcass meatiness categories with less than 55 percent lean meat (Knecht 2012). It is important to note that these studies looked at intestinal parasites in general and did not specifically correlate to *A suum* infection alone.

While the impact of *A suum* on growth parameters and feed utilization in growing pigs has been documented, to the author’s knowledge there are very few published assessments of the impact of ascariasis on sow production metrics. Danielson et al compared sow and litter performance in breeding females treated 7 to 10 days prior to farrowing with fenbendazole to untreated controls and demonstrated a significant reduction in average daily feed intake during lactation and an increased number of pigs weaned from treated sows as compared to untreated controls (Danielson 1991). Hagsten et al summarized three studies that looked at sow productivity measures in internal parasite positive sows that either received anthelmintics (dichlorvos or fenbendazole) just prior to farrowing or were left untreated. In each study there were numerical improvements and trends of significance (*P*<0.10) in sow average daily feed
intake, litter size and weight gain of the litters (Hagsten 1994). The paper did not clarify which internal parasites were present in the sows, so the relationship to *A suum* could not be determined.

Pattison *et al* studied the impact of another intestinal parasite of swine, *Oesophagostumum dentatum*, on sows and showed a significant 24.4 kg difference in body weight from service to weaning of infected groups compared to uninfected controls (Pattison 1979). Infected sows weighed 10.5 percent less at weaning, consumed 29 percent more feed during lactation and had lower carcass backfat as compared to uninfected sows (Pattison 1979). Uninfected sows averaged 12.8 total pigs born (TB) and 11.3 pigs born alive (BA) while infected sows averaged 12.4 TB and 9.5 BA (Pattison 1979). While this does not characterize with certainty the potential impact of *A suum* on sows, it does demonstrate the potential negative impact of parasitism on sows and justifies that further work should be considered to better understand the impact of ascariasis on sow performance.

2.1.6 Zoonosis and infection in other species

*Ascaris lumbricoides*, the human roundworm, has an almost identical life cycle and epidemiology to *A suum* (Dold 2011). The two parasites are morphologically indistinguishable from each other and much of the literature and research is used interchangeably (Dold 2011). For a long time these two species were considered to be the same with the only variation being which host it was found in, such that some literature references *A suum* as *Ascaris lumbricoides*
var *suum* (Seamster 1950, Rogers 1956). While the two species are currently considered distinct, they differ genetically by only a small amount (Dold 2011). Zoonotic infection can occur and is relatively common in some areas of the world. It has been suggested that pig derived human ascariasis is more prevalent in developed and developing countries, where human ascariasis is better controlled due to sanitation, while underdeveloped countries where sanitation is poor have a higher endemic prevalence of human derived ascariasis (Nejsum 2005, Nejsum 2012).

Molecular comparison of a ribosomal RNA internal transcribed spacer region (rRNA ITS-1) of nine North American human ascariasis cases indicated that *A suum*, and not *A lumbricoides*, was the causative parasite (Anderson 1995). In Maine, between 2010 and 2013, 14 cases of Ascariasis were documented from people on farms that raised organic produce and livestock (Colby 2013). In a Washington state survey in 1981-1982, 18 of 23 cases of human Ascariasis had exposure to pigs, pig manure or sites where pigs were previously raised (Shoemaker-Nawas 1982). In New Hampshire, five cases of human Ascariasis all reported exposure to pigs or soil contaminated with pig feces (Lord 1982). In the United Kingdom, Bendall *et al* described significant associations between cases of human Ascariasis and living near pigs, with a significant odds ratio of 4.65 for having pigs in the same postcode (Bendall 2011). In Denmark, pigs were identified as the source of 27 cases of human ascariasis, 80 percent of which had known exposure to pig manure (Nejsum 2005). In Japan, comparison of the rRNA ITS-1 region in human ascariasis
cases and ascarids collected from swine slaughterhouses indicated that ascariasis is changing from human to human transmission to pig to human transmission (Arizono 2010). A case of eosinophilic pneumonia, possibly due to *A suum* migrating larvae, was described in a 62 year old man who consumed raw pork liver (Izumikawa 2011). In a survey of human and swine ascariasis in China, 19 of 20 human infections were determined to be direct from pigs (Zhou 2012). The study also described one case of swine ascariasis caused by *A lumbricoides*, demonstrating the potential of complete cross-infection (Zhou 2012). The study estimated that 16.5 percent of all Chinese human ascariasis cases are derived from pigs, and suggested that the main reason for the high rate is due to the common practice of using human and pig manure as fertilizer in underdeveloped endemic areas (Zhou 2012). The use of human and pig manure has been indicated as a risk factor for zoonotic transmission of *Ascaris* sp. (Kutsumi 1969, Arfaa 1984, Ziemer 2010)

*A suum* is periodically diagnosed in other species, namely lambs, calves and cattle as a pneumonia due to larval migration, but does not establish as a patent infection (McCraw 1971, McLennan 1974, Gunn 1980). It is also interesting to note that a sustained infection of pig derived *A suum* has been described in a population of chimpanzees at the Copenhagen Zoo in Denmark (Nejsum 2010).

It is important to note that *A lumbricoides* is a significant parasitic disease in many underdeveloped areas of the world with global prevalence estimated at 800 million people, and is considered one of the top neglected tropical diseases
(Hotez 2009). Therefore continued understanding of the epidemiology and control methods for *Ascaris* sp. and using *A suum* in swine as a model for *A lumbricoides* in humans is valuable.
2.1.7 Tables

Table 2.1 Estimated economic impact of *A suum* on grow-finish pigs using current input costs and production values.

<table>
<thead>
<tr>
<th></th>
<th>121 days feeding&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start Weight&lt;sup&gt;b&lt;/sup&gt; (lbs)</td>
<td>End Weight (lbs)</td>
<td>ADG (lbs)</td>
<td>Feed Gain Ratio</td>
<td>Total Feed Cost&lt;sup&gt;c&lt;/sup&gt; ($)</td>
<td>Feed Cost Difference ($)</td>
<td>Market Value&lt;sup&gt;d&lt;/sup&gt; ($/cwt, live weight)</td>
<td>Market Value difference ($/hd)</td>
</tr>
<tr>
<td>Infected</td>
<td>58.5</td>
<td>264&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.92&lt;sup&gt;e&lt;/sup&gt;</td>
<td>190.44</td>
<td>+$3.44</td>
<td>163.68</td>
<td>-$9.92</td>
</tr>
<tr>
<td>Control</td>
<td>58.5</td>
<td>280&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.66&lt;sup&gt;f&lt;/sup&gt;</td>
<td>187.00</td>
<td>---</td>
<td>173.60</td>
<td>---</td>
</tr>
</tbody>
</table>

<sup>a</sup> Model assumes a fixed-time system using the performance of the control group

<sup>b</sup> Used same initial start weight reported by Stewart and Hale, 1988

<sup>c</sup> Feed cost estimated at $0.32/lb, Iowa State University Extension and Outreach (available at: http://www.econ.iastate.edu/estimated-returns, accessed 05 May 2014)

<sup>d</sup> Market value estimated at $62.00/cwt live weight, Iowa State University Extension and Outreach (available at: http://www.econ.iastate.edu/estimated-returns, accessed 05 May 2014)

<sup>e</sup> Calculated using decreased performance metrics (as a percentage) of “all infected groups” from Stewart and Hale, 1988

<sup>f</sup> Agri Stats, Inc, Fort Wayne, IN, accessed 05 May 2014.
2.2 Fenbendazole

2.2.1 Benzimidazole class, fenbendazole and current formulations

Chemical structure

The first benzimidazole (BZ), thiabendazole (TBZ), was discovered in 1961 and began an exploration into other variations (Brown 1961). The basic chemical structure of the BZ class is a methyl 5-substituted benzimidazol-2-yl-carbamate. Fenbendazole [methyl 5-(phenyl-thio)-2-benzimidazole-carbamate] has a substitution of a sulfide structure at the C5 carbon of the benzene ring (Baeder 1974) (Table 2.2). The reversible metabolite of fenbendazole is its sulphoxide derivative, oxfendazole (also an effective anthelmintic) which then undergoes further and irreversible metabolism to fenbendazole sulphone (not effective as an anthelmintic) (McKellar 1990, Corwin 1977). (Table 2.2)

Safety

The benzimidazole class is ideal because at low doses it provides a broad spectrum of activity against helminths of many host species, has a high level of efficacy against many helminth species, and has a high margin of safety for mammals. The LD$_{50}$ in mice is very high at 10,000 mg·kg$^{-1}$, and single oral doses greater than 5,000 mg·kg$^{-1}$ in swine were well tolerated (Düwel 1977). Fenbendazole does produce a transient leukopenia and increased sorbitol dehydrogenase levels in swine after 2,000 mg·kg$^{-1}$ daily for 14 days or with 75 and 125 mg·kg$^{-1}$ for 5 days, compared to the labeled 9 mg·kg$^{-1}$ dose (Hayes 1979). Morgan studied the effects of oxfendazole, one of the metabolites of
fenbendazole, on pregnant sows and found no observed toxic or teratogenic
effects in pregnant sows dosed at 4.5 and 13.5 mg·kg\(^{-1}\) at repeated times during
early pregnancy (Morgan 1982).

Formulations

Benzimidazoles are insoluble in water and thus all formulations are enteral
in the form of a feed additive, paste or suspension (McKellar 1990). In the United
States, fenbendazole is currently available for swine in the form of a 20 percent
Type A Medicated Article (Safe-Guard Dewormer 10 percent), a 1.8 percent
Type B feed medication that is also labeled for feed top-dress (Safe-Guard EZ
Scoop), and a 0.5 percent pellet feed top-dress (Safe-Guard Medicated
Dewormer) (North American Compendium available at:
http://swinemeds.naccvp.com; accessed 14 May 2014). All products are
currently manufactured and sold by Merck Animal Health (Summit, New Jersey).
Fenbendazole is also currently available for swine in the European Union as a
200 mg·mL\(^{-1}\) oral suspension for use in water delivery systems (Panacur
Aquasol, MSD Animal Health, division of Merck Animal Health, Summit, New
Jersey).

Other relevant benzimidazoles

Examples of other common BZ class anthelmintics used in human and
veterinary medicine, and referenced within this thesis are; thiabendazole,

2.2.2 Pharmacokinetics and pharmacodynamics

The benzimidazoles are considered to have the same efficacy and mode of action, but differ in their effectiveness due to solubility, absorption and distribution in the host and parasite (McKellar 1990). The more soluble metabolite oxfendazole has a higher plasma concentration, than the less soluble fenbendazole. In pigs, 44-50 percent of oral fenbendazole is excreted unchanged in the feces and 0.1 percent in the urine (Düwel 1977). Both metabolites can be found in urine and feces (Düwel 1977). Petersen and Friis demonstrated a bioavailability of 27.1 percent in orally administered fenbendazole in pigs (Petersen 2000). The pharmacokinetics of fenbendazole was determined after a single oral administration of 5 mg·kg⁻¹ (Petersen 2000).

Parameter means (±SD) for fenbendazole and its metabolites, oxfendazole and fenbendazole sulfone, were evaluated and are summarized in Table 2.3. Metabolism and excretion of fenbendazole in pigs is rapid and occurs by microsomal oxidation within the liver (Szprenger-Jusztkiewicz 2002). The rapid metabolism and excretion of fenbendazole is supported by its small percent of the total AUC, low Cmax and low Tmax, compared to the metabolites. Half-life of fenbendazole is approximately 8.3 hours (Petersen 2000). In a study where 12 pigs were fed 5 mg·kg⁻¹ fenbendazole in feed for five days, the metabolite fenbendazole sulfone was evaluated at 24, 48 and 72 hours post completion of
treatment. Liver, muscle, kidney and fat tissue residue levels were demonstrated to be below established maximum residue levels (MRLs) by 48 hours post treatment in muscle, kidney and fat and by 72 hours in liver, and thus the authors suggested that a 3 day withdrawal after completion of treatment would be sufficient (Capece 1999). Further plasma (n=8) and tissue (n=12) pharmacokinetic studies in pigs with a single 5 mg·kg⁻¹ oral dose found fenbendazole only in plasma at 2 hours with a mean concentration of 0.015 ug·mL⁻¹. Metabolite levels reached a mean maximum concentration of 0.116 ± 0.029 ug·mL⁻¹ in plasma at 8 hours and were below the limit of detection by 60 hours post treatment (Szprengier-Juszkiewicz 2002). Fenbendazole could not be detected in kidney, liver or muscle tissue and only low concentrations of metabolites, below the currently established MRLs (500 ug·kg⁻¹ for liver and 50 ug·kg⁻¹ for muscle, kidney and fat), could be found in liver tissue at 4 days after treatment. The authors supported a 3 day withdrawal period (Szprengier-Juszkiewicz 2002). Plasma pharmacokinetics of the sum of fenbendazole metabolites from this study can also be found in Table 2.2.

The rapid metabolism and excretion of fenbendazole permits a labeled zero day pre-slaughter withdrawal period in the United States. However, the MRL’s for many foreign markets to the US require a 21 day pre-slaughter withdrawal from swine (US Pork Board MRL Database, available at: http://www.pork.org/Resources/214/MaximumResidueLimits.aspx, accessed 14 May 2014), which according to the work done by Capece and Szprengier-Juszkiewicz et al is excessive (Capece 1999, Szprengier-Juszkiewicz 2002).
2.2.3 Mechanism of action

The mechanism of action of benzimidazole anthelmintics is not completely understood, however the main aspect involves the disruption of tubulin formation in both adult helminths and developing embryos (Friedman 1980, Lacey 1990, McKellar 1990). Specifically, fenbendazole interacts with embryonic tubulin at a colchicine binding domain on the leading edge of tubulin polymerization, and prevents the formation of microtubules (Friedman 1980, Lacey 1987, Martin 1997). Microtubules are important for cell structure, proper cleavage, chromosome movement during cell division and embryogenesis (Martin 1997). Due to the interference with microtubules, eggs exposed to benzimidazole anthelmintics, both in vivo and in vitro, often have irregular shapes, atypical blastomeres and unequal divisions which result in arrested and irreversible development (Boes 1998, Düwel 1977, Egerton 1969, Kirsh 1982). Tubulin is a relatively conserved protein across species, and while there is affinity of benzimidazoles to mammalian tubulin, studies have shown that with the low doses used therapeutically, the affinity of benizmidazoles to helminth tubulin is 250-500 times higher than that of mammalian tubulin, allowing for the high range of safety of the benzimidazoles (Friedman 1990).

Other effects of benzimidazoles, specifically on A suum, have been reported, but are considered to be secondary mechanisms subsequent to microtubule disruption (Friedman 1990). Mebendazole was shown to irreversibly inhibit glucose uptake resulting in a depletion of energy reserves (Friedman 1990). Mebendazole interferes with cytoplasmic microtubules in adult A suum...
intestinal cells, resulting in disruption in the transport of secretory granules, cell lysis and death of the parasite (Friedman 1990). In the same study, a higher concentration of the drug was found in the intestinal cells than in other tissues of *A suum*. In one study, *A suum* intestinal tissue showed a reduced uptake of sodium after treatment with benzimidazoles, but could have resulted from reduced glucose uptake (McKellar 1990). Several benzimidazoles also inhibited cytoplasmic and mitochondrial malate dehydrogenase and fumarate reductase in *A suum* (Friedman 1990, McKellar 1990).

2.2.4 Spectrum and efficacy against *Ascaris suum* adults and larvae

Fenbendazole is effective against a wide range of parasites and many larval stages, and is specifically labeled in swine for: *Ascaris suum*, *Trichuris suis* (swine whipworm), *Hyostrongylus rubidus* (small stomach worm), *Oesophagostomum dentatum* and *O. quadrupulatum* (nodular worms), *Metastrongylus apri* and *M. pudendotectus* (lungworms) and *Stephanurus dentatus* (kidney worm) (Kirsch 1975, Marti 1978, Stewart 1981, Becker 1981).

For *A suum*, fenbendazole has been shown to be effective against the migrating L3 larvae within the liver and lungs, the pre-adult L4 larvae and the L5 adults (Stewart 1981, Stewart 1984, Stewart 1986). This effectiveness is beneficial in that treatment of all larval stages present within a host or population at a single time, extends the period to re-shedding of infective eggs post treatment. Anthelmintics that are only effective against adult stages, allow for
continued development of pre-adult larval stages and a shorter time to re-shedding of eggs.

In the early description of the molecule, Baeder noted that a single 5 mg·kg⁻¹ oral dose eliminated 100 percent of adult A suum from pigs, but did not provide details on the material and methods of the study, number treated or comparison to untreated animals (Baeder 1974).

Fenbendazole was evaluated as an anthelmintic for swine in its initial commercial form of Panacur (Merck Animal Health, Summit, New Jersey), for effectiveness against Hyostrongylus rubidus and Oesophagostomum spp (Kirsch 1975). Kirsch and Dülwel used single doses of 3.5 and 5 mg·kg⁻¹ at 5, 16 and 42 days post infection, and demonstrated a relatively lower efficacy in 5 day old worms (migrating larvae) than in 16 and 42 day old worms, with the exception of 16 day Oesophagostomum spp. which had only 44 percent efficacy (Kirsch 1975).

Batte demonstrated efficacy of fenbendazole against A suum with single treatments of varying levels. Single dose treatments of 3, 5, 7.5, 10, 15 and 25 mg·kg⁻¹ fenbendazole were 96.0, 76.9, 100, 96.8, 98.8 and 98.4 percent effective in expelling adult intestinal A suum (Batte 1978). In addition, Batte showed 98.9 and 100 percent efficacy of 3 or 5 mg·kg⁻¹ daily doses for 3 days, respectively (Batte 1978).

In another study where the efficacy of 3 and 5 mg·kg⁻¹ fenbendazole daily for 3 days was studied against A suum, both treatments were 100 percent effective at removing adults (Stewart 1981). Marti et al compared the efficacy of
different anthelmintics against *A suum*, with fenbendazole given at 3 mg·kg⁻¹ for 3 days (Marti 1978). Feces was collected on day 0 and 5 and evaluated for worms and EPG, and all pigs were necropsied on day 7 and assessed for number of intestinal worms. Fenbendazole was 92.4 percent effective at removing *A suum* from the intestine and reduced mean EPG by 76 percent at day 5 post-treatment (Marti 1978). Düwel *et al* demonstrated that fenbendazole was 100 percent effective at removing adult *A suum* from experimentally challenged pigs when given at 0.33 mg·kg⁻¹ daily for 15 days (Düwel 1980).

Evaluation of fecal EPG in 35 naturally infected pigs treated with single doses of 5 (n=15) and 10 mg·kg⁻¹ (n=15) fenbendazole or left as untreated controls (n=5), showed reduction in pre-treatment EPG levels (1,200-3,200) to zero by day 3, and remained negative at days 7, 14 and 21 post treatment, while controls had “no reduction” (Bali 1980).

Becker and Bradley evaluated fenbendazole for the treatment of *Stephanurus dentatus* in naturally infected captured feral swine, but also reported on *A suum* fecal EPG and intestinal worms. Pigs were treated with 3 mg·kg⁻¹ for 3 days and necropsied at 17 to 20 days post completion of treatment. Fecal EPG was evaluated prior to treatment and again at necropsy, while number of intestinal worms were counted at necropsy. In treated pigs, mean fecal EPG fell from 9,180 to zero and no intestinal worms were found at necropsy, while mean fecal EPG increased from 17,500 to 20,900 with 3.6 mean intestinal worms in control pigs (Becker 1981).
Kennedy et al evaluated the efficacy of fenbendazole against migrating *A suum* in pigs fed 3 mg·kg\(^{-1}\) daily for 3 days (days 1, 2 and 3 of challenge period) to pigs experimentally infected with 500 *A suum* eggs daily over a 14 day period. Fenbendazole did not prevent migration of all larvae through the liver and lungs, but did show a reduced number of intestinal worms at 28 days post inoculation as compared to controls (Kennedy 1987). It should be noted that the 14 day exposure period would be longer than the anticipated treatment effect of fenbendazole given on days 1 through 3 of the experiment, and eggs ingested by pigs on days 5 through 14 would not be exposed to the effects of fenbendazole, based on the pharmacokinetics detailed above.

Twelve pigs treated with 3 mg·kg\(^{-1}\) fenbendazole for 3 days, 50 days after inoculation with *A suum* eggs, had no intestinal worms 7 days after completion of treatment, as compared to a mean intestinal worm count of 64 in controls (Marchiondo 1987). No fecal eggs were seen in treated pigs, while 5 of 9, 3 of 9 and 7 of 9 control pigs were positive by fecal EPG at 21, 35 and 49 days (Marchiondo 1987).

Kulkarni and Rao experimentally infected 3 pigs with 20,000 *A suum* eggs and treated (time post infection not reported) with 5 mg·kg\(^{-1}\) daily for 3 days and evaluated efficacy with pre- and post-treatment fecal EPG, expulsion of worms and number of worms at necropsy on day 4 post-treatment as compared to 3 infected control pigs (Kulkarni 1990). Fecal EPG were zero by day 3 post treatment in all treated pigs while fecal EPG of controls was 2,066 on the same sample day (Kulkarni 1990). A mean of 6 worms were expelled during the
treatment period and no worms were found on necropsy in the treated pigs, while no worms were expelled and a mean of 9.3 intestinal worms were found at necropsy in control pigs (Kulkarni 1990).

An evaluation of low dose fenbendazole against *A suum* was reported by Bjørn et al in which a dose of 0.35 mg·kg\(^{-1}\) was used to treat experimentally infected pigs, however there was no effect on excretion of *A suum* egg in the feces (Bjørn 1997). It is unclear in this report as to the duration of treatment or the number of pigs evaluated.

Maiti et al evaluated the response to fenbendazole by measuring fecal EPG in pigs treated with 7.5 mg·kg\(^{-1}\) for 2 days compared to untreated controls. A 68 percent reduction in mean EPG counts from 2,422 to 766 was seen 3 days after treatment and all animals were negative at days 7 and 14 post treatment, while control mean EPG remained above 2,000 (Maiti 2011).

In recently reported work using a similar benzimidazole, flubendazole, Jourquin et al demonstrated the daily reduction in fecal EPG count and percent of subjects positive for *A suum* after treatment. Sows were treated with either 1 mg·kg\(^{-1}\) flubendazole for 5 consecutive days (n=11) or left as untreated controls (n=9) (Jourquin 2014). Fecal EPG showed numeric, but not statistical differences starting 3 days after treatment and negative fecal EPG by 8 days in treated sows (Jourquin 2014).

Fenbendazole has also been evaluated for its efficacy against the larval and migrating stages of *A suum* and found to be highly effective. Stewart et al evaluated the efficacy of fenbendazole on migrating larvae while in the liver and
the lung by treating experimentally infected pigs with 3 mg·kg⁻¹ for 3 days starting 2 (liver) or 6 days (lung) after inoculation (Stewart 1984). Pigs were euthanized 24 days after inoculation and the mean number of *A suum* recovered in each treatment and control group were reported. The mean number of worms recovered in the treatment groups was 0.62 and 0.25, for treatment start day 2 and 6 respectively while mean number of worms was 133.57 for control pigs (Stewart 1984).

Stewart *et al* inoculated pigs with embryonated *A suum* eggs on three occasions 11 days apart, each followed by a treatment of 3 days of 3 mg·kg⁻¹ fenbendazole, either on days 2 through 4 or 6 through 8 post-infection, in an attempt to elicit an immunological response. Treated animals had a decreased number of pigs positive for *A suum*, a reduced mean number of worms per pig and the length and weight of the recovered worms was less than that of worms recovered from control animals (Stewart 1984/85).

In a study targeting L4 larvae after liver and lung migration, Stewart *et al* treated pigs with 3 mg·kg⁻¹ fenbendazole on days 8 through 10 post inoculation of L4 larvae and found no worms in any treated pigs 5 days post completion of treatment, while a mean number of 13.6 L4 and L5 worms were recovered from control pigs (Stewart 1986).

It is recognized that extended treatment is more effective than single day treatment and may be necessary for complete treatment of some parasites (e.g. *T suis*) (McKellar 1990). Corwin *et al* showed that there was no difference in feeding 9 mg·kg⁻¹ total over periods of 3, 6 and 12 days on the removal of adult *A*
suum (Corwin 1984). This was important, due to the fact that in many swine operations the application of a 3 day diet may be restrictive to feed milling and feed delivery efficiency and management at the farm level.

A possible treatment regimen in swine breeding farms is to give only a single day of treatment of 3 mg·kg\(^{-1}\) to pregnant sows prior to farrowing (Jeremy Pittman, personal observation). Batte showed a single treatment of 3 mg·kg\(^{-1}\) of fenbendazole was 96.0 percent effective in expelling adult A suum (Batte 1978). Praslička evaluated 0.05, 0.10, 0.25, 1.0 and 2.5 mg·kg\(^{-1}\) of fenbendazole against A suum in pigs. Rates of 0.25, 1.0, and 2.5 mg·kg\(^{-1}\) had high efficacy against adult A suum, at 98.6, 99.9 and 100, respectively (Praslička 1997). Fenbendazole treatment rates of 1.0 and 2.5 mg·kg\(^{-1}\) had efficacy against immature worms, while other rates showed no efficacy (Praslička 1997). While the single 3 mg·kg\(^{-1}\) and reduced treatment levels of fenbendazole appear to be effective, the limited amount of work may not be enough to support reduced doses in the field and should be studied further. A summary of reported literature, found herein, on the effectiveness of fenbendazole on Ascaris suum can be found in Table 2.4.

2.2.5 Ovicidal activity

Members of the benzimidazole class of anthelmintics, namely thiabendazole, mebendazole and fenbendazole, have been shown to have ovicidal activity against helminths in humans (A lumbricoides), swine (A suum) and other species (Friedman 1980, Egerton 1969, Kirsch 1978, Carvalho 1992,
Embryos exposed to benzimidazoles either *in vivo* or *in vitro* develop abnormally or fail to cleave during embryogenesis. The ovicidal activity of benzimidazoles is related to its mode of action on tubulin. Work by Friedman and Platzer demonstrated an almost complete noncompetitive binding of fenbendazole and mebendazole to *A suum* embryonic tubulin with colchicine (Friedman 1980). They proposed that the ovicidal activity was due to prevention of microtubule polymerization due to the benzimidazole molecule binding to the tubules.

Egerton demonstrated complete inhibition of *A suum* embryo development with the parent benzimidazole, thiabendazole, at levels less than 1 ppm (Egerton 1969). Düwel reported ovicidal activity of fenbendazole against trichostrongylids at levels of 0.1 mg·kg⁻¹ after 3 doses (Düwel 1977). This effect was also seen in eggs *in utero* in adult female trichostrongylids. Kirsch studied the ovicidal effects of fenbendazole *in vivo* and *in vitro* on three helminthes (*Ostertagia ostertagi*, *Haemonchus contortus* and *Trichostrongylus colubriformis*) of sheep. With both *in vivo* and *in vitro* testing, Kirsch showed reduced embryonation of all three helminthes, and estimated that the 0.5 ppm *in vitro* concentration used was optimal for ovicidal activity (Kirsch 1978). Additional work by Kirsch and Schleich demonstrated this ovicidal effect at an early stage in egg development, and even *in utero*, related to the formation of atypical blastomeres (Kirsch 1982).

Wagner and Chavarria demonstrated rapid changes to *Trichuris trichiura* eggs from human patients as soon as 1 day after treatment with mebendazole. Changes included increases and decreases in size from normal, distorted
elongation and shapes, changes in the size and number of polar plugs and abnormal appearing yolk material. In addition, many of these eggs (>92 percent) failed to develop to the larval stage (Wagner 1974b). In another study by the same authors, they showed this same impact on *T. trichiura* and also demonstrated complete ovicidal effect on human hookworm eggs after 1 day of treatment with mebendazole (Wagner 1974a). Carvalho *et al* demonstrated reduced embryonation of *A. lumbricoides* eggs extracted from the uteri of expelled adult females in patients treated with thiabendazole (Carvalho 1992). Ovicidal effects have also been shown with albendazole against *A. lumbricoides* (Maisonneuve 1985). Massara *et al* demonstrated a complete ovicidal effect of thiabendazole in *A. lumbricoides* eggs eliminated in patients by 48 hours after treatment. In addition, eliminated eggs had a reduced infectivity as measured by retrieval of larvae from lungs and hearts of challenged mice 48 and 72 hours after infection (Massara 1991). When thiabendazole was used *in vitro* on eggs from expelled *A. lumbricoides* of human patients at different concentrations and for different times, a dose and time dependent effect was observed on embryonation. Dilutions of thiabendazole at 1 and 2.5 ppm did not have an effect on embryonation. Concentration of 5 ppm had a 0 to 31 percent reduction in embryonation at 72 hours post-treatment, while 10 ppm resulted in 0 to 72 percent reduction at 24 hours and 100 percent at 48 and 72 hours post-treatment (Massara 2001).

Lacey *et al* studied the effects of different benzimidazoles and their metabolites on egg hatch *in vivo* of *Haemonchus contortus* from sheep. The egg
hatch rate was reduced when the metabolites of fenbendazole were used, compared to fenbendazole. Sulfur-oxidation to fenbendazole sulphone reduced ovicidal activity 40-fold and was not active with fenbendazole sulphone (Lacey 1987). In addition, the hydroxylation, oxidation and decarbamoylation of fenbendazole also reduced or eliminated the ovicidal effects of fenbendazole. The authors hypothesized this reduced efficacy could have been due to an inability of the polar metabolites to penetrate the egg's shell, rather than inability to inhibit the tubule structures (Lacey 1987). Therefore it is likely for A suum that the ovicidal activity observed with fenbendazole is due in large part to the parent molecule fenbendazole, and not to its metabolites.

*Ascaris suum* egg embryonation and infectivity was evaluated in eggs from expelled worms from pigs treated with albendazole, pyrantel, ivermectin and piperazine. In the albendazole group, only 7 percent of the eggs developed into larval stages, while most were arrested at the one-cell stage (Boes 1998). The other anthelmintics had no significant impact on embryonation rates. When remaining embryonated eggs were inoculated into mice, there was no significant difference in larvae recovered from albendazole treated eggs compared to control eggs, suggesting fenbendazole did not affect hatchability or infectivity of those eggs that fully developed to L3 larvae (Boes 1998).
2.2.6 Tables

Table 2.2 Chemical structure of fenbendazole and its sulphone (oxfendazole) and suphone metabolites.

<table>
<thead>
<tr>
<th>Benzimidazole</th>
<th>Chemical Structure*</th>
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<tr>
<td>Fenbendazole</td>
<td><img src="image" alt="Fenbendazole" /></td>
</tr>
<tr>
<td>Oxfendazole (FBZ sulphoxide)</td>
<td><img src="image" alt="Oxfendazole" /></td>
</tr>
<tr>
<td>Fenbendazole Sulphone</td>
<td><img src="image" alt="Fenbendazole Sulphone" /></td>
</tr>
</tbody>
</table>

Table 2.3 Pharmacokinetic parameters (means ± SD) of fenbendazole and metabolites in pigs given one 5 mg·kg\(^{-1}\) dose orally.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Petersen and Friis, 2000 (n=4)</th>
<th>Szprengier-Juszkiewicz \textit{et al}, 2002 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fenbendazole</td>
<td>Oxfendazole</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>3.75 ± 1.50</td>
<td>12.50 ± 8.06</td>
</tr>
<tr>
<td>(C_{\text{max}}) (ug/mL)</td>
<td>0.07 ± 0.04</td>
<td>0.66 ± 0.22</td>
</tr>
<tr>
<td>(T_{\text{half}}) (h)</td>
<td>8.38 ± 4.28</td>
<td>NR</td>
</tr>
<tr>
<td>PCT Total AUC</td>
<td>4.5 ± 1.6</td>
<td>65.9 ± 10.0</td>
</tr>
<tr>
<td>AUC (ug*h/mL)</td>
<td>1.00 ± 0.34</td>
<td>15.61 ± 5.24</td>
</tr>
</tbody>
</table>

From Petersen and Friis, 2000 and Szprengier-Juszkiewicz \textit{et al}, 2002
AUC = area under the curve. PCT Total = percent of total AUC. \(T_{\text{half}}\) = Half life. \(C_{\text{max}}\) = Maximum plasma concentration. \(T_{\text{max}}\) = Time to \(C_{\text{max}}\). NR = Not reported.
Table 2.4 Review of published literature for fenbendazole efficacy against *Ascaris suum*.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose</th>
<th>Treatment Duration</th>
<th>n</th>
<th>Determinant of efficacy</th>
<th>Result</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baeder et al, 1974</td>
<td>5 mg·kg⁻¹</td>
<td>1 day</td>
<td>NR</td>
<td>Percent elimination of intestinal adults</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Batte, 1978</td>
<td>3 mg·kg⁻¹</td>
<td>1 day</td>
<td>12</td>
<td></td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 mg·kg⁻¹</td>
<td></td>
<td>12</td>
<td></td>
<td>76.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5 mg·kg⁻¹</td>
<td></td>
<td>10</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg·kg⁻¹</td>
<td></td>
<td>20</td>
<td></td>
<td>96.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 mg·kg⁻¹</td>
<td></td>
<td>20</td>
<td></td>
<td>98.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg·kg⁻¹</td>
<td></td>
<td>10</td>
<td></td>
<td>98.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 mg·kg⁻¹</td>
<td>3 days</td>
<td>12</td>
<td>Percent worms expelled at 7 dpt</td>
<td>98.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 mg·kg⁻¹</td>
<td></td>
<td>12</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Marti et al, 1978</td>
<td>3 mg·kg⁻¹</td>
<td>3 days</td>
<td>11</td>
<td>Fecal EPG reduction at 5 dpt</td>
<td>92.4</td>
<td>Avg EPG reduced from 1,158 to 276</td>
</tr>
<tr>
<td>Bali and Singh, 1980</td>
<td>3 mg·kg⁻¹</td>
<td>1 day</td>
<td>15</td>
<td>Mean fecal EPG at 3, 7, 14 and 21 dpt</td>
<td>0</td>
<td>Pre-treatment EPG range was 1,200-3,200</td>
</tr>
<tr>
<td></td>
<td>10 mg·kg⁻¹</td>
<td>1 day</td>
<td>15</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>5</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Becker and Bradley, 1981</td>
<td>3 mg·kg⁻¹</td>
<td>3 days</td>
<td>10</td>
<td>a) Mean fecal EPG pre and 7-10 dpt b) Mean worms at necropsy at 7-10 dpt</td>
<td>9,180 to 0</td>
<td>Naturally infected feral swine</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>10</td>
<td></td>
<td>17,500 to 20,900</td>
<td></td>
</tr>
<tr>
<td>Stewart et al, 1981</td>
<td>3 mg·kg⁻¹</td>
<td>3 days</td>
<td>11</td>
<td>Mean worms in intestine at 4-5 dpt</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 mg·kg⁻¹</td>
<td>3 days</td>
<td>11</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>11</td>
<td></td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Corwin et al, 1984</td>
<td>3 mg·kg⁻¹</td>
<td>3 days</td>
<td>10</td>
<td>Mean intestinal worm recovery at 24 dpi</td>
<td>0.62</td>
<td>Targeted migrating larvae</td>
</tr>
<tr>
<td></td>
<td>1.5 mg·kg⁻¹</td>
<td>6 days</td>
<td>10</td>
<td></td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.75 mg·kg⁻¹</td>
<td>12 days</td>
<td>10</td>
<td></td>
<td>13.6</td>
<td>Targeted 4th stage larvae</td>
</tr>
<tr>
<td>Stewart et al, 1984</td>
<td>3 mg·kg⁻¹</td>
<td>3 days (2-4 dpi)</td>
<td>8</td>
<td>Mean intestinal worm recovery at 24 dpi</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 mg·kg⁻¹</td>
<td>3 days (6-8 dpi)</td>
<td>8</td>
<td></td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td></td>
<td>8</td>
<td></td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Stewart and Rowell, 1986</td>
<td>3 mg·kg⁻¹</td>
<td>3 days (8-10 dpi)</td>
<td>8</td>
<td>Mean intestinal worm recovery at 15 dpi</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td></td>
<td>8</td>
<td></td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Kennedy et al, 1987</td>
<td>3 mg·kg⁻¹</td>
<td>3 days (1-3 dpi)</td>
<td>10</td>
<td>Number of larvae in intestine 14 and 24 dpi</td>
<td>80</td>
<td>Pigs exposed to 500 eggs daily for 14 days</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td></td>
<td>10</td>
<td></td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Marchiondo and Szanto, 1987</td>
<td>3 mg·kg⁻¹</td>
<td>3 days</td>
<td>10</td>
<td>a) Positive EPG 25, 35 and 49 dpi b) Mean worms 7 and 81 dpi</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>10</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Kulkarni and Rao, 1990</td>
<td>5 mg·kg⁻¹</td>
<td>3 days</td>
<td>3</td>
<td>a) EPG reduction pre and 7-10 dpt b) Mean worms voided 24-72 hpt c) Mean intestinal worms at 4 dpi</td>
<td>3,822 to 356</td>
<td>Pigs infected with 20,000 eggs</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>3</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Maiti et al, 2008</td>
<td>7.5 mg·kg⁻¹</td>
<td>2 days</td>
<td>10</td>
<td>Fecal EPG 0, 3, 7 and 14 dpi</td>
<td>2.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>10</td>
<td></td>
<td>2.27</td>
<td></td>
</tr>
</tbody>
</table>

NR=not reported, dpi=days post inoculation, dpt=days post treatment, EPG=eggs per gram
2.3 References


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CHAPTER 3. EFFECT OF FENBENDAZOLE ON ASCARIS SUUM INFECTIONS OF NATURALLY INFECTED SOWS: I. EGG SHEDDING

A paper to be submitted to Journal of Swine Health and Production

Jeremy S Pittman, DVM, Dipl ABVP, Gil Myers, PhD, Kenneth J Stalder, PhD, Locke A Karriker, DVM, MS, Dipl ACVPM

JSP: Murphy-Brown, LLC – North Division, Waverly, Virginia.
GM: Myers Parasitology Services, Magnolia, Kentucky
KJS: Department of Animal Science, College of Agriculture, Iowa State University, Ames, Iowa
LAK: Swine Medicine Education Center, College of Veterinary Medicine, Iowa State University, Ames, Iowa

3.1 Summary

Objective: To determine the time to cessation of *Ascaris suum* egg shedding, the percent of animals that stop shedding, and to estimate the reduction in environmental burden from eggs shed in naturally infected female breeding swine after different treatment levels of fenbendazole to better determine timing of anthelmintic use prior to movement into a farrowing environment.

Materials and methods: Five experiments across three commercial sow farms, known to be infected with *A suum* were conducted. Breeding gilts and sows were identified with natural *A suum* infections and allocated to one of 4 treatments;
CNT = untreated controls, TX1 = 545.5 mg fenbendazole for one day, TX2 = 545.5 mg fenbendazole daily, for 3 consecutive days (1636.5 mg total), TX3 = 1636.5 mg fenbendazole for one day. Fecal samples were collected on various days and evaluated using the modified Wisconsin sugar flotation technique. Time-to-negative was evaluated using Kaplan-Meier survival analysis, percent negative was evaluated by Chi-square analysis and environmental burden (BURD) (eggs observed versus eggs expected) was evaluated by analysis of variance.

**Results:** Mean time-to-negative ranged from 9.3 to 13.1 for TX1, 8.9 to 13.1 days for TX2 and 9.8 for TX3, while CNT ranged from 13.4 to 28.2 with 70 to 100 percent censored. For all TX, 90 to 100 percent of sows were negative by the end of the study, compared to 0 to 28.6 percent for CNT. Mean BURD range was 7.0 to 60.9 for TX1, 13.9 to 60.8 for TX2, 29.3 for TX3 and 60.4 to 219.0 for CNT. All TX were different from CNT ($P<0.05$) but not from each other.

**Implications:** Fenbendazole was effective at all treatment levels in decreasing the number of *A. suum* eggs shed into the environment. When using fenbendazole for the control of *A. suum* transmission from dams to offspring, treatment should begin approximately 14 days prior to movement into clean farrowing facilities.

**Key words:** swine, *Ascaris suum*, fenbendazole, eggs, shedding
3.2 Introduction

The Large Roundworm of swine, *Ascaris suum* (*A. suum*), has worldwide distribution and can be found in all types of swine operations. (Roepstorff 1994, De Bie 2003, Greve 2012, Pelger 2012a-d) Changes in modern swine production systems that decrease contacts with intermediate hosts and environments, increased sanitation, separation of production stages, and use of effective anthelmintics, have created the perception that the incidence and severity of swine parasites in general has declined. Some producers and veterinarians have developed a false assumption that raising pigs indoors completely prevents parasitism, and hence parasites are often not considered to be of major importance in modern swine production. However, due to several key characteristics of *A. suum*, the parasite persists in modern production systems (Roepstorff 1994, Johnson 2003, Melancon 2005, Pittman 2010a,b). Those characteristics are a highly fecund adult female roundworm (estimated to produce 1 to 2 million eggs per day) (Kelley 1956, Olsen 1958), eggs that are highly resistant to environmental influences and disinfectants (Wharton 1979, Gaasenbeek 1998, Roepstorff 1998), a direct life cycle involving extra-intestinal migration (Roepstorff 2003), and a relatively short patency period (6-8 weeks) (Roepstorff 2003). These characteristics combined with common oversight, allow infected carriers to enter into negative sites or continue contaminating already positive sites (Connor 2012). The capacity for rapid environmental contamination and inherent resistance of the eggs makes it nearly impossible to
eradicate the parasite from contaminated facilities, thus continued monitoring, treatment and control measures are required (Myers 1988, Roepstorff 1998).

Current trends in the swine industry will permit an increase in swine parasite prevalence as a consequence of exposure to epidemiological risk factors (Dangolla 1996b, Sundrum 2010, Roepstorff 2011). Such changes including housing pregnant sows in groups, requiring provision of nesting or bedding materials and restrictions of drug use will inevitably result in systems that permit A suum transmission and increased prevalence within farms (Haugegaard 2010, Roespstorff 2011). The increase in niche production systems (eg. organic, differentiated markets, welfare-friendly, pasture raised) and swine associated with regional and national exhibition show circuits provide environments that maintain parasites, especially A suum (Eriksen 1996, Cartensen 2002, Eijck 2005, Yaeger 2009, Woods 2012).

The main economic impacts of A suum in swine are reduced feed efficiency (Hale 1985, Stewart 1988), decreased average daily gain (Nilsson 1980, Bernardo 1990a, Kipper 2011), increased liver condemnations at slaughter due to larval migration and organ pathology ("milk spots" on livers) (Bernardo 1990b, Hurnik 1995, Harr 2001, Kanora 2004, Meisinger 2004, Melancon 2005, McOrist 2008) and increased medication costs related to treatment (Boes 2010, USDA 2007). Additional A suum impacts include reduced vaccine efficacy from migrating larvae (Urban 2007, Steenhard 2009), organ damage from larval migrans (i.e, lung, liver, small intestine, stomach) predisposing the host to secondary diseases (Shope 1958, Underdahl 1957, Nayak 1965, Qureshi 1978,

The goal for most parasite control programs is to minimize the production impact through preventing transmission and reducing environmental contamination (Raffensperger 1927, Behlow 1978, Biehl 1987, Myers 1988, Stewart 1993). This is accomplished by increased sanitation, management of pig flow and use of anthelmintics at key times in the parasite’s life cycle and the host’s production cycle. Common practice in the swine industry is to provide an anthelmintic to sows prior to farrowing before they enter clean farrowing facilities (Myers 1988, Roepstorff 1998a). This reduces transmission to the farrowing environment (eg. stall, pasture, etc) and from the sow to piglets. Treatment timing prior to farrowing can vary considerably and if insufficient time elapses before farrowing to allow cessation of fecal shedding into the farrowing environment, offspring exposure is not prevented.
Fenbendazole is a broad spectrum benzimidazole (Class I anthelmintic) approved for use in swine by the FDA. Fenbendazole has a wide safety margin, is highly effective against both the adult and larval stages of *A suum*, and has ovicidal activity against the eggs of many helminths (Batte 1978, Friedman 1980, Corwin 1984, Stewart 1984, Stewart 1986). It 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 EZ Scoop, Merck Animal Health, Summit, New Jersey). The drug is also available for other species in North America and for swine in the other countries under the trade name Panacur (MSD Animal Health, Summit, New Jersey). The individual top-dress can be considered as therapeutic and enables judicious treatment of individual animals or subpopulations without requiring simultaneously medicating the entire population served by the feed system. It also eliminates the need for on-farm feed mixing, which may not be available.

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 lbs of an individual 400 lb sow’s daily ration and feed once daily for 3 consecutive days”. However, off-label treatment of sows with a single day, single scoop dose of Safe-Guard EZ Scoop, equating to a 3 mg·kg⁻¹ dose for a 181.8 kg animal is commonly used (Jeremy Pittman, personal observations). In addition, farms usually do not weigh individual animals prior to applying fenbendazole using the Safe-Guard EZ Scoop (Dangolla 1996a, Jeremy Pittman, personal observations). Therefore, treatment may be less than indicated by both
dose and duration. The consequence of imprecise dosing and abbreviated treatment regimen on *A suum* egg shedding has not been thoroughly evaluated. In early studies of fenbendazole in swine, Baeder *et al* used a single oral dose of 5 mg·kg⁻¹ and eliminated 100 percent of adult *A suum* from the intestine and Batte demonstrated the efficacy (96.0 percent adult *A suum* removed) of 3 mg·kg⁻¹ as a single dose (Baeder 1974, Batte 1978). 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* (Batte 1978, McKellar 1990).

While fenbendazole use in swine has been demonstrated in small group infections, useful information about the drug’s impact on population dynamics of *A suum* in dynamic, commercial pig populations is not available. 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. The experiments presented herein were conducted to characterize the impact of fenbendazole on *A suum* egg shedding in naturally infected gestating sows under commercial conditions with commonly used protocols.

### 3.3 Materials and Methods

#### 3.3.1 Farms

The herds in which the experiments were conducted were sow farms in a commercial swine production company, using Large White-Landrace cross
maternal genetics, between 2010 and 2013. 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/Ag_Guide_3rd_ed.pdf) and Pork Quality Assurance Plus (PQA Plus) guidelines (http://www.pork.org/Certification/2341/pqaPlusMaterials.aspx). 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 (Pittman 2010a). Farm A was a 1,000-sow one-site farrow-to-finish operation located in Southeastern Virginia. Farm B and C were each 2,000 farrow-to-feeder pig (10 wks of age) operations, both located in Northeastern North Carolina. All farms were managed with weekly batches of gestating, multiparous sows which were housed in individual gestation and farrowing stalls, weaning pigs at approximately 3 weeks of age. Replacement gilts were housed in pens in groups of 4 to 5 until bred, at which time they were moved into individual gestation stalls. All farms utilized fenbendazole as a feed top-dress (Safe-Guard EZ scoop), weekly to gestating groups of sows 2 weeks prior to farrowing, but all routine fenbendazole use was suspended prior to the start of the experiment and for the duration. Animals were fed individually through automated drop boxes one time per day and individual water nipples were available for each animal at all times.
3.3.2 Experiments

Five separate experiments were conducted; one at Farm A and B each and three at Farm C. Each experimental design differed slightly with regard to collection dates, treatments and duration. All subject enrollment, sample collection, sample processing and application of treatments was consistent between experiments. No animals were used in more than one experiment at Farm C.

3.3.3 Sow inclusion and exclusion criteria

Based on reported literature and previous observations in the herd, younger parity animals (gilts through second parity sows) were initially screened for A. suum infection status, due to a higher expected prevalence of infection (Marti 1986, Roepstorff 1998b, Nosal 2008). 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 nitrile glove was used for each animal to prevent sample cross-contamination. Fecal samples were placed in a 15 mL centrifuge tube (VWR, Radnor, Pennsylvania) with a screw top lid, pre-filled with 5 mL of a concentrated sugar solution (Sheather’s solution; 454 g sugar in 355 mL water with specific gravity of 1.27) (David 1982). Samples were processed immediately on site. Fecal samples and sugar solution were homogenized within the 15 mL tube manually by vigorously shaking the tube.
Supplemental homogenization, if required, was accomplished using a disposable wooden stirrer that was discarded after a single use. Tubes were placed in test tube racks and a volume of sugar solution added sufficient to create a meniscus at the top of the tube. A 2 cm by 2 cm glass coverslip was placed on top of each tube and allowed to sit for a minimum of 10 minutes. Coverslips were removed, placed on a glass microscope slide and examined under 40X magnification (4X objective and 10X eyepiece) for presence of *A suum* eggs. Each coverslip was examined until confirmation of at least 1 egg was observed or the entire coverslip determined to be negative. Animals with at least 1 *A suum* egg were considered positive and enrolled into the study. Animals where no eggs were observed from their fecal sample were considered negative and excluded from the experiments.

### 3.3.4 Treatments

Treatments were applied in a manner consistent with existing on-farm processes. Gilts and sows were not weighed at any point in the experiments. Safe-Guard EZ Scoop was used for all fenbendazole treatments, and subject dose allotments were established using the manufacturer’s scoop provided in the package. The scoop, when level full, provides approximately 545.5 mg of fenbendazole, sufficient to provide 3 mg·kg⁻¹ bodyweight to a 181.8 kg animal. In practice, a single full scoop is provided to each sow, regardless of bodyweight, and treatments were based on this methodology.
Five experiments were conducted to compare egg shedding patterns over time between four treatments. Treatments were: control with no treatment (CNT), a single 545.5 mg scoop of FBZ for one day (TX1), a single 545.5 mg scoop of FBZ daily for three consecutive days (total 1636.5 mg) (TX2), or three 545.5 mg scoops of FBZ on a single day (total 1636.5 mg) (TX3). In experiments 1 through 4, subjects were randomly assigned into CNT, TX1 or TX2. In experiment 5, sows were randomly assigned to CNT, TX1, TX2 or TX3 groups. Anthelmintic intake was monitored by ensuring each sow consumed their entire feed allotment prior to the next feeding. Sows that did not completely consume medicated feed were dropped from the study and not included in the analysis. A summary of the experimental design, treatments and number of sows in each treatment can be found in Table 1.

3.3.5 Sample collection and processing

At each sample day, individual fecal samples were collected directly per rectum from each enrolled sow. Fecal collection days varied by experiment and a summary of sampling days by experiment can be found in Table 1. A new clean nitrile glove was used for each fecal collection. Approximately 5 grams of feces was extracted from the rectum of each sow. If feces were not readily available, collection was attempted later the same day. If a fecal collection from a sow failed in two attempts they were excluded from the day’s collection. After collection, fecal samples were placed into 50 mL screw-top centrifuge tubes (VWR, Radnor, Pennsylvania) and labeled appropriately. Samples were kept
Samples were either processed in-house by the primary author or submitted to the secondary author’s laboratory (Myers Parasitology Services, Magnolia, Kentucky) using the same methodology with consistent qualitative results between laboratories as determined by a priori comparisons (kappa = 0.96).

Samples were processed using the modified Wisconsin sugar flotation technique (Bliss 1997). Sheather’s solution was evaluated for a specific gravity of 1.27 with a hygrometer (David 1982). Sample homogenization occurred within the 50 mL collection tube prior to collecting a 2 gram sub-sample. The 2 gram sub-sample was homogenized with 15 mL of Sheather’s solution in a 147.8 mL wax paper cup using a disposable wooden stick. The fecal-sugar solution homogenate was strained into a second wax paper cup through a tea strainer to remove large organic particles. The strained homogenate was then transferred to 15 mL centrifuge tube and a volume of Sheather’s solution added to restore a total volume to 15 mL. Tubes were centrifuged at 145.5 $\times g$ for 10 minutes. Centrifuged tubes were placed in a test tube rack and a sufficient volume of Sheather’s solution was added to each tube until a meniscus was formed. A 2 cm by 2 cm coverslip was placed on the meniscus and remained for a minimum of 10 minutes. The coverslip was removed and placed on a microscope slide for evaluation. Slides were evaluated using 40X magnification and the $A$ suum eggs number on the entire slide was counted. To calculate the egg per gram (EPG) of feces, the total number of eggs was divided by 2 (initial grams of feces used).
Samples with greater than 500 EPG were recorded as 500 eggs. Samples were considered positive if at least 1 egg per coverslip was identified.

3.3.6 Statistical analysis

All statistical analyses were completed 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 study was the duration of fecal *A suum* egg shedding measured as the time to first negative fecal exam from experimental sows. Survival analysis (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. Effects determined non-significant based on analysis were dropped from further analysis. Comparisons between treatment groups were made using Kaplan-Meier methods as an estimation for survival function from life time data. Mean survival times (±SE) 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 (BURD) value for each treatment group were evaluated using analysis of variance 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 BURD calculation was an attempt to compare observed eggs excreted [EPG_{obs}] for the duration of the
study period compared to an expected eggs excreted \( [EPG_{exp}] \) for each sow and between treatments as a measure of the potential environmental contamination provided by each group. An individual’s \( EP_{exp} \) value was that individual’s initial (day 0) EPG multiplied by the total duration days of the study. The \( EP_{obs} \) value was calculated as the cumulative sum of EPG at one sampling day \( (D_i) \) times the number of days until the next sampling day \( (D_{i+x}) \) through the duration of the study \( \sum (EPG_{D_i} \times (D_{i+x} - D_i)) \). For example, an animal has sample day EPG values of 100, 80 and 20 on days 0, 4 and 8 respectively. The \( EP_{exp} \) for this particular animals is 900 \( [100 \text{ EPG}_{Day0} \times 9 \text{ total days}] \), the \( EP_{obs} \) is 740 \( [(100 \text{ EPG}_{Day0} \times 4 \text{ days}) + (80 \text{ EPG}_{Day4} \times 4 \text{ days}) + (20 \text{ EPG}_{Day8} \times 1 \text{ day})] \), and the BURD is 0.822 \( [740 \text{ EPG}_{obs} / 900 \text{ EPG}_{exp}] \). This BURD estimate represents that the individual shed 82.2 percent of the eggs over the study duration as was anticipated for that individual.

### 3.4 Results

In the survival analyses there were significant farm (experiment) effects \( (P<0.0001) \), and evidence of a trend in farm-treatment interaction \( (P=0.0540) \) in the full model, therefore data was analyzed and reported independently by experiment in a reduced model. There was no parity effect and was therefore excluded from the reduced models. In each experiment there was a high censoring rate in the CNT groups (range 70.0 to 100.0 percent), as many subjects remained positive throughout the period of testing. In each experiment, Kaplan-Meier survival analysis demonstrated a significant difference in time-to-
negative egg shedding between CNT and each treatment group. In all experiments, TX1, TX2 and TX3 were not different between one another. Mean time to negative egg shedding for CNT groups were 18.0 (SE 0.0), 14.0 (0.0), 22.3 (1.6), 28.2 (1.5) and 13.4 (0.8) for experiments 1 through 5 respectively. Mean time to negative for TX1 groups were 13.1 (0.9), 9.3 (1.0), 11.7 (0.4), 11.5 (0.7) and 9.5 (0.6) in experiments 1 through 5 respectively. Mean time to negative for TX2 groups were 11.0 (1.2), 10.6 (0.8), 11.3 (0.5), 13.1 (1.0) and 8.9 (0.8) in experiments 1 through 5 respectively. In experiment 5, TX3 survival time was 9.8 (0.7). Complete mean time-to-negative, standard error, range and percent censored for each experiment can be found in TABLE 2. A graph of the survival analysis has been included (Figure 1) for all of the data from experiments 1 through 5 combined. Note that due to the different durations of the 5 experiments, the CNT group contains a large percentage of censored data points, while the most number of sows censored in any fenbendazole treated group or experiment was one. Therefore this graph significantly underestimates the impact of fenbendazole treatments and is a conservative assessment.

When combining all experiments, only 9 of 40 (22.5%) CNT animals were negative for *A suum* eggs throughout the respective study periods. Of the treatment groups, 66 of 67 (98.5%) for TX1, 65 of 67 (97.0%) for TX2 and all of 11 (100.0%) TX3 were negative for *A suum* eggs at respective experiment completion. The percent negative at end of study differences between CNT and each treatment was significant by Fisher’s exact test ($P<0.0001$). There was no
difference in percent negative at end of study between TX1, TX2 or TX3 in any of the five experiments. The results for each experiment can be found in TABLE 3.

For BURD analysis, a significant effect of treatment ($P<0.0001$) and farm ($P=0.0003$) and a trend towards significance in the treatment-farm ($P=0.0540$) interaction was observed, therefore data was analyzed and reported by experiment. The BURD analysis demonstrated a significant difference ($P<0.05$) between CNT and all fenbendazole treatments in each experiment by Tukey’s studentized range test. There was no difference between TX1, TX2 or TX3 in any of the five experiments. The BURD estimate for individuals in some experiments were greater than 1.00 as a result of increased EPG counts on subsequent samplings from their day 0 EPG value. It should be noted that while significant, the BURD values in experiment 2 between CNT (79.9) and TX1 (60.9) and TX2 (60.8) are numerically not very different as compared to the other experiments. This is likely due to several factors, including the shorter duration of study (14 days), the number of sampling days (4) and the bias of “capping” initial fecal EPG values described below. Average BURD and 95 percent confidence intervals are listed for each experiment in TABLE 4.

It is important to note that in some fecal samples, EPG was in excess of the 500 EPG cut-off used, and thus could introduce bias into the BURD calculation. Overall, the use of an EPG “cap” in these experiments resulted in an underestimation of the impact of reduced fecal shedding. In Experiment 1 there were no recorded samples above 500 EPG. In Experiment 2, two TX1 and three TX2 at day 0 were above 500. In Experiment 3, a CNT sample at day 8 and 10
and one TX2 day 0 sample were above 500. In Experiment 4, eleven CNT samples were greater than 500, with 10 of the eleven coming between days 8 and 22 of sampling, while ten TX1 and six TX2 day 0 samples were above 500 EPG. There was one TX1 day 8 sample greater than 500 in experiment 4. In Experiment 5, one TX2 and TX3 day 0 sample each had above 500 EPG, while one CNT sample on days 8, 10 and 21 had a fecal EPG above 500. All but one of the above observations (Experiment 4 TX1 day 8 sample) would have resulted in underestimation of the actual BURD calculation, since sample “capped” at day 0 would have resulted in a lower expected BURD value and CNT samples “capped” post-treatment would have underestimated the reduced BURD compared to treatment groups. This could explain the more minimal numerical differences and reduction in CNT BURD values observed in Experiments 2 and 4, as compares to Experiments 1, 3 and 5. It is not well understood how this influences transmission or clinical disease as fecal EPG values are not well correlated with disease burden and are inherently highly variable in swine and are not well understood (Roepstorff 1998a).

3.5 Discussion

The results from the experiments presented here, suggest using fenbendazole in breeding females prior to farrowing, and treatment should begin 14 days prior to moving sows into clean farrowing facilities in order to minimize *A suum* eggs shedding into that environment. Treatment of breeding herd animals
after this time or upon entry into the farrowing facility will likely result in some facility contamination and increase transmission risk to suckling piglets.

The number of sows in each experiment and between treatment groups varies considerably, as compared to other reported literature. Since the experiments were completed on naturally infected sows on commercial farms, and due to the method of enrollment described above, in which a rapid survey of a large number of animals was conducted, there was significant exclusion of subjects after enrollment. The initial screening of sows was conducted on a large number of animals (e.g. 100 to 250), on-farm and initially positive animals were randomly allotted to treatment groups. Initial allocations attempted to place an equal number of sows per treatment group in experiments 1, 2, 3 and 5, while in experiment 4 twice as many sows were enrolled in TX1 and TX2 as in the CNT group. After allotment, the day 0 fecal sample was collected and treatment was initiated. Initial screening, enrollment, day 0 fecal collection and treatment were all conducted on-farm on the same day within experiments in order to be more efficient. Once the day 0 fecal sample was processed and “false positives” from the initial screening were determined, those sows were excluded from the study, resulting in uneven treatment groups for data collection and analysis.

While egg per gram was recorded, comparison of EPG between treatments was not considered due to the inherent high variability of egg counts when assessing *A suum* infection. Egg counts are highly variable over time within the same subject as well as within the same fecal passing (Roepstorff 1998a, personal observations), therefore use of absolute counts or snapshots
can be very misleading. Interpretation of fecal EPG counts in swine are difficult because egg counts are not well correlated with worm burden or clinical impact (Roepstorff 1998a). In addition, the main goal of these experiments where to assess the time to cessation of shedding, and not necessarily a reduction in average EPG. Therefore use of a calculated burden (BURD) was utilized, which may provide a better description of egg shedding as it represents an average of fecal egg counts over several time periods, rather than use of a single time point. The estimation of BURD in this paper is an attempt to describe the level of environmental contamination by the different treatment groups, which is an important epidemiological aspect of A suum control. Others have utilized similar calculated estimates of egg contamination. Bernardo et al used the average egg per gram count between consecutive visits multiplied by the time elapsed between those visits to calculate a raw “lifetime burden” in market pigs (Bernardo 1990a). Those values were then standardized to an average lifetime burden to generate a value relative to 1 (average lifetime burden) and used in models to determine growth impacts of ascariasis. Mejer et al calculated a “relative contamination index” from fecal egg counts of Trichuris suis and Oesophagostomum dentatum in pastured pigs as an attempt to compare contamination rates between experimental paddocks (Mejer 2006). The calculation utilized in this paper was an attempt to demonstrate the impact of fenbendazole treatment on reduction of total eggs excreted into the environment, while accounting for the theoretical contamination if those animals were not treated. As can be noted by the increased BURD values in CNT in experiments
1, 3 and 5, non-treated animals have the potential to perpetuate contamination and even increase contamination of the environment.

It is important to note, that *A suum* eggs shed from breeding sows are not directly infective to the offspring (Arene 1986, Stewart 1993, Roepstorff 2003). Freshly shed *A suum* eggs require a developmental period in the environment outside the host, consisting of 2 moltings to an infective L3 larvae (Stoll 1933, Fagerholm 2000, Kirchgässner 2008). This development usually takes 1 to 3 months or longer, which is largely influenced by temperature, humidity and seasonal climate (Seamster 1950, Connan 1977, Arene 1986, Geenen 1999, Roepstorff 2003, Kirchgässner 2008, Kim 2012). In modern, early weaning (3 weeks of age or less) swine farms, *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 always a significant factor in growing pigs having ascariasis (Roepstorff 1999). Farms which weaned pigs greater than 6 weeks of age were twice as likely to have downstream finishing pigs 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 separating pigs in facilities that are located some 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 (Roepstorff 1991, Homgren 1998), supporting the idea that
transmission to offspring is much more likely from older animals (grow-finish, gilts) or contaminated facilities (finisher barns, gilt development units) (Thomas 1983). However, indirect transmission is still a concern where piglets may be exposed to infectious eggs remaining in the farrowing environment from previous groups (Thomas 1983, Roepstorff 1997), poorly sanitized farrowing facilities that allow maintenance of “hot spots” as described by Nilsson (Nilsson 1982), or by mechanical transmission from other farm areas that are contaminated (e.g., breeding, gestation, gilt development, finishers), by the sow (e.g., fecal matter on skin, feet), employees (hands, clothing, boots) and fomites (hearding boards, equipment).

It should be noted that the estimated FBZ treatment ranges of 3 and 9 mg·kg⁻¹ from the present studies may vary from published scientific literature recommendations for FBZ treatment ranges, however the different efficacy results from various FBZ treatment levels and duration is likely not biologically significant because FBZ has been found to be highly efficacious under multiple treatment regimens (Batte 1978, Stewart 1981, Corwin 1984, Stewart 1986). Previously published scientific literature reports are in agreement with the presented findings which demonstrate that positive EPG counts can be seen beyond 3 days post-treatment, and while overall environmental contamination is reduced even low shedding levels have the potential to transmit infection to offspring (Bindseil 1974, Marti 1986, Nosal 2008). Piglet exposure and ingestion of A suum eggs, even at low levels, has been described by Marti and Hale (Marti 1986). This was theorized to have been from ingesting “pen debris” in the
farrowing unit. In contrast, Roepstorff dismissed this as a major transmission aspect in intensive production farms where wean age and sanitation are likely more significant control factors (Roepstorff 1994). Regardless, this demonstrates an unappreciated aspect in vertical parasite control regardless of wean age or sow anthelmintic control programs; 1) piglets can ingest infective embryonated eggs from a contaminated farrowing environment at a young age and 2) piglets may ingest unembryonated eggs and act as carriers to destination facilities, where the ingested eggs can be shed, resume embryonation and contaminate the facilities where they are housed.

The fenbendazole levels used in treatments TX1 and TX3 from the present studies are an “off-label” use of the product, as well as the potential “off-label” dosing of TX2 in the event that the sow treated was greater than 181.8 kg. It was the authors’ intent to mimic the potential application variation of this product as it is used in the field by pork producers and veterinarians.

False positive results due to coprophagia when using a highly sensitive test, such as the modified Wisconsin sugar flotation technique used in the present studies (Boes 1997), is a potential source of misclassification bias. It should be noted that the flotation method used was selected specifically because of its greater sensitivity (≈1 EPG) relative to other detection methodologies (Egwang 1981, Bliss 1997, Dryden 2005) and its common use in North American swine parasitology. In contrast, many *A suum* European studies, especially those from Scandinavia, utilize McMaster’s technique with increased sensitivity (detect 20 EPG) and have a recommended cut-off (200 EPG) to minimize the
false positive effect (Boes 1997, Roepstorff 1998). Since necropsy and daily adult worm recovery from enrolled sows was not practical and thus not performed in any of the present studies, it is impossible to classify each sample result as true or false positive, however a few aspects support using these data collectively as valid results. First, all sows were housed individually in partially slatted gestation stalls for the entire experimental period and thus had limited access to fecal material when compared to group housed sows. Second, many enrolled females from the treatment group passed adult ascarids as visually observed on the floor behind the sow’s stall or by rectal extraction during sample collection days, notably on days 6 and 8 post treatment, which is consistent with observations by Boes et al (Boes 1998). Unfortunately this was not recorded for every enrolled animal as complete daily individual sow feces observation could not be completed. Third, the apparent parasite burden (measured as EPG) for sows in published papers from Europe is much greater when compared to observed EPG values from the present studies and experience in other North American sow herds by the authors (Johnson 2003, Pittman 2010a, Myers G personal communication). For example, if a 200 EPG cut-off was used in the present studies as is suggested, approximately 56.7 percent (93 of 215 sows) of enrolled sows would have been excluded for false positive counts when sow enrollment occurred. The reason for this discrepancy between studies and geography are not fully understood, but it may be related to diagnostic methodology (the McMaster’s technique uses a multiplicative calculation and tends to overestimate eggs at the higher concentrations (Egwang 1981)) or
inherent management differences (provision of bedding), regulatory restrictions (anthelmintic use), farm type (multiple ages), and facility design (group housing) which results in an overall heavier environmental parasite burden. Another possibility is an inherent geographical variation in egg shedding by adult females, such as has been documented in *A lumbricoides* (Hall 2000). Lastly, EPG reduction was consistently observed in each fenbendazole treatment group within each experiment in the present study, while the majority of control animals continued to shed eggs throughout the sampling duration, indicating an effect of the treatment on fecal egg shedding at these lower “false positive” EPG counts.

Similarly, false negative results could have occurred from extremely low fecal egg per gram counts, below the detection sensitivity of the fecal flotation method used, and as a result of using the first negative fecal test as the time to negative. Repeated work could minimize the impact of false negatives by repeated sampling and evaluation of fecal EPG beyond the initial negative test. False negatives would impact 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, can occur in subjects of both the treatment and control groups.

### 3.6 Implications

- The Large Roundworm of swine, *Ascaris suum*, is still present in modern swine production and should be considered an important pathogen.
A *suum* in gestating breeding animals is sensitive to treatment with fenbendazole as a feed top-dress at different dose and duration levels.

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.

3.7 Acknowledgements

The primary author would like to thank the farm management and staff at the sites in which these studies were conducted. The primary author would like to thank the numerous veterinary students that assisted with sample collection during the research project time period. Funding for the sample collection supplies and diagnostic testing was provided by Murphy-Brown, LLC.

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### 3.9 Tables and Figures

Table 3.1 Treatment regimens, number of sows (N) and fecal sample collection days in five experiments across three commercial sow farms comparing different fenbendazole treatment† levels and duration for the treatment of *Ascaris suum* in naturally infected gestating sows*.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Farm</th>
<th>Treatment Group</th>
<th>Anthelmintic</th>
<th>Dose (mg)</th>
<th>Duration (days)</th>
<th>Total Dose (mg)</th>
<th>N</th>
<th>Fecal Sample Collection Days</th>
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†CNT = untreated control sows; TX1 = 545.5 mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day; TX2 = 545.5 mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total); TX3 = 1636.5 mg fenbendazole given on a single day.

*Sow; F1 1/2 Large White x 1/2 Landrace, multiparous
Table 3.2 Survival analysis. Number of sows (N), number failed (stopped shedding), censored (remained shedding or loss to follow-up), percent censored, mean time, standard error (±SE) and range to stop shedding in five experiments across three commercial sow farms comparing different fenbendazole treatment† levels and duration for the treatment of *Ascaris suum* in naturally infected gestating sows.

Experiment 1

<table>
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<th>% Censored</th>
<th>Time to stop shedding</th>
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</thead>
<tbody>
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<td></td>
<td></td>
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<td></td>
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<td>0</td>
<td>0.0</td>
<td>11.0^b</td>
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</tbody>
</table>

*Duration of study: 20 days

ab Values with different superscripts are statistically significant (P=0.0024; Log-rank Test)

Experiment 2

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<th>% Censored</th>
<th>Time to stop shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>Mean</td>
</tr>
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Duration of study: 14 days

ab Values with different superscripts are statistically significant (P=0.0019; Log-rank Test)

Experiment 3

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<td>11</td>
<td>1</td>
<td>8.3</td>
<td>11.3^b</td>
</tr>
</tbody>
</table>

Duration of study: 31 days

ab Values with different superscripts are statistically significant (P=0.0002; Log-rank Test)

Experiment 4

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Failed</th>
<th>Censored</th>
<th>% Censored</th>
<th>Time to stop shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>CNT</td>
<td>14</td>
<td>3</td>
<td>11</td>
<td>78.6</td>
<td>28.2^a</td>
</tr>
<tr>
<td>TX1</td>
<td>29</td>
<td>28</td>
<td>1</td>
<td>3.5</td>
<td>11.5^b</td>
</tr>
<tr>
<td>TX2</td>
<td>26</td>
<td>26</td>
<td>0</td>
<td>0.0</td>
<td>13.1^b</td>
</tr>
</tbody>
</table>
Duration of study: 37 days

\(^{ab}\) Values with different superscripts are statistically significant (\(P<0.0001; \) Log-rank Test)

Experiment 5

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Failed</th>
<th>Censored</th>
<th>% Censored</th>
<th>Mean</th>
<th>SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>71.4</td>
<td>13.4(^a)</td>
<td>0.8</td>
<td>10-21</td>
</tr>
<tr>
<td>TX1</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0.0</td>
<td>9.5(^b)</td>
<td>0.6</td>
<td>6-14</td>
</tr>
<tr>
<td>TX2</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0.0</td>
<td>8.9(^b)</td>
<td>0.8</td>
<td>6-14</td>
</tr>
<tr>
<td>TX3</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>0.0</td>
<td>9.8(^b)</td>
<td>0.7</td>
<td>8-14</td>
</tr>
</tbody>
</table>

Duration of study: 21 days

\(^{ab}\) Values with different superscripts are statistically significant (\(P=0.0004; \) Log-rank Test)

\(^{\dagger}\) CNT = untreated control sows; TX1 = 545.5 mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day; TX2 = 545.5 mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total); TX3 = 1636.5 mg fenbendazole given on a single day.
Table 3.3 Percent of sows negative or positive for *A suum* by fecal egg shedding at completion of experimental sample period by treatment, for each of five experiments across three commercial sow farms comparing different fenbendazole treatment† levels and duration for the treatment of *Ascaris suum* in naturally infected gestating sows.

**Experiment 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>4</td>
<td>1 (25.0)</td>
<td>3 (75.0)​^a</td>
</tr>
<tr>
<td>TX1</td>
<td>7</td>
<td>7 (100.0)</td>
<td>0 (0.0)​^b</td>
</tr>
<tr>
<td>TX2</td>
<td>10</td>
<td>10 (100.0)</td>
<td>0 (0.0)​^b</td>
</tr>
</tbody>
</table>

Duration of study: 20 days

\(^{ab}\) Values with different superscripts are statistically significant (\(P=0.0030;\) Fisher’s Exact)

**Experiment 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>5</td>
<td>0 (0.0)</td>
<td>5 (100.0)​^a</td>
</tr>
<tr>
<td>TX1</td>
<td>6</td>
<td>6 (100.0)</td>
<td>0 (0.0)​^b</td>
</tr>
<tr>
<td>TX2</td>
<td>10</td>
<td>9 (90.0)</td>
<td>1 (10.0)​^b</td>
</tr>
</tbody>
</table>

Duration of study: 14 days

\(^{ab}\) Values with different superscripts are statistically significant (\(P=0.0003;\) Fisher’s Exact)

**Experiment 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>10</td>
<td>3 (30.0)</td>
<td>7 (70.0)​^a</td>
</tr>
<tr>
<td>TX1</td>
<td>13</td>
<td>13 (100.0)</td>
<td>0 (0.0)​^b</td>
</tr>
<tr>
<td>TX2</td>
<td>12</td>
<td>11 (91.7)</td>
<td>1 (8.33)​^b</td>
</tr>
</tbody>
</table>

Duration of study: 31 days

\(^{ab}\) Values with different superscripts are statistically significant (\(P=0.0001;\) Fisher’s Exact)

**Experiment 4**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>14</td>
<td>3 (21.4)</td>
<td>11 (78.6)​^a</td>
</tr>
<tr>
<td>TX1</td>
<td>29</td>
<td>28 (96.6)</td>
<td>1 (3.5)​^b</td>
</tr>
<tr>
<td>TX2</td>
<td>26</td>
<td>26 (100.0)</td>
<td>0 (0.0)​^b</td>
</tr>
</tbody>
</table>

Duration of study: 37 days

\(^{ab}\) Values with different superscripts are statistically significant (\(P<0.0001;\) Fisher’s Exact)
Experiment 5

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>7</td>
<td>2 (28.6)</td>
<td>5 (71.4) a</td>
</tr>
<tr>
<td>TX1</td>
<td>12</td>
<td>12 (100.0)</td>
<td>0 (0.0) b</td>
</tr>
<tr>
<td>TX2</td>
<td>9</td>
<td>9 (100.0)</td>
<td>0 (0.0) b</td>
</tr>
<tr>
<td>TX3</td>
<td>11</td>
<td>11 (100.0)</td>
<td>0 (0.0) b</td>
</tr>
</tbody>
</table>

Duration of study: 21 days

ab Values with different superscripts are statistically significant (P<0.0001; Fisher’s Exact)

¹CNT = untreated control sows; TX1 = 545.5 mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day; TX2 = 545.5 mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total); TX3 = 1636.5 mg fenbendazole given on a single day.
Table 3.4 Mean environmental burden (BURD)* and 95 percent confidence intervals in five experiments across three commercial sow farms comparing different fenbendazole treatment† levels and duration for the treatment of *Ascaris suum* in naturally infected gestating sows.

### Experiment 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean BURD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>136.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-49.4, 322.6</td>
</tr>
<tr>
<td>TX1</td>
<td>46.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.2, 65.5</td>
</tr>
<tr>
<td>TX2</td>
<td>36.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.9, 48.2</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Values with different superscripts are statistically significant (*P*=0.0105; ANOVA)

### Experiment 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean BURD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>79.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.8, 86.0</td>
</tr>
<tr>
<td>TX1</td>
<td>60.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.5, 67.3</td>
</tr>
<tr>
<td>TX2</td>
<td>60.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.8, 65.9</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Values with different superscripts are statistically significant (*P*<0.0001; ANOVA)

### Experiment 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean BURD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>219.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.7, 416.2</td>
</tr>
<tr>
<td>TX1</td>
<td>44.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.6, 63.2</td>
</tr>
<tr>
<td>TX2</td>
<td>33.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.2, 41.0</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Values with different superscripts are statistically significant (*P*=0.0083; ANOVA)

### Experiment 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean BURD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>60.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.6, 88.2</td>
</tr>
<tr>
<td>TX1</td>
<td>7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6, 12.4</td>
</tr>
<tr>
<td>TX2</td>
<td>13.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5, 27.3</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Values with different superscripts are statistically significant (*P*<0.0001; ANOVA)
Experiment 5

<table>
<thead>
<tr>
<th>Group</th>
<th>BURD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>118.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.5, 191.7</td>
</tr>
<tr>
<td>TX1</td>
<td>32.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.8, 40.9</td>
</tr>
<tr>
<td>TX2</td>
<td>20.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.0, 26.2</td>
</tr>
<tr>
<td>TX3</td>
<td>29.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.7, 50.0</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Values with different superscripts are statistically significant (P<0.0001; ANOVA)

*Environmental burden (BURD) is the cumulative sum (fecal EPG x number of days between sample days) of observed fecal EPG compared to the expected fecal EPG (initial day 0 EPG X days in study), expressed as a percentage.

†CNT = untreated control sows; TX1 = 545.5 mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day; TX2 = 545.5 mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total); TX3 = 1636.5 mg fenbendazole given on a single day.
Figure 3.1 Survival analysis. Kaplan-Meier survival curves for time-to-negative fecal *A suum* egg shedding from five experiments (data combined for chart) across three commercial sow farms comparing different fenbendazole treatment† levels and duration for the treatment of *Ascaris suum* in naturally infected gestating sows. Note that each of five experiments had different days of duration, therefore in-phase censoring of CNT is overrepresented.

†CNT (black) = untreated control sows; TX1 (red) = 545.5 mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day; TX2 (green) = 545.5 mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total); TX3 (blue) = 1636.5 mg fenbendazole given on a single day. Open circles represent censored observations.
CHAPTER 4. EFFECT OF FENBENDAZOLE ON ASCARIS SUUM INFECTIONS OF NATURALLY INFECTED SOWS: II. OVICIDAL ACTIVITY AND EMBRYONATION RATES

A paper to be submitted to Journal of Swine Health and Production

Jeremy S Pittman, DVM, Dipl ABVP, Gil Myers, PhD, Kenneth J Stalder, PhD, Locke A Karriker, DVM, MS, Dipl ACVPM

JSP: Murphy-Brown, LLC – North Division, Waverly, Virginia.
GM: Myers Parasitology Services, Magnolia, Kentucky
KJS: Department of Animal Science, College of Agriculture, Iowa State University, Ames, Iowa
LAK: Swine Medicine Education Center, College of Veterinary Medicine, Iowa State University, Ames, Iowa

4.1 Summary

Objective: To determine the ovicidal activity of different fenbendazole levels on Ascaris suum eggs shed from naturally infected commercial breeding female swine.

Materials and methods: Three experiments were conducted on a commercial breeding farm infected with A suum. Breeding gilts and sows were identified with natural A suum infections and allocated to one of 4 treatments; CNT = untreated controls, TX1 = 545.5 mg fenbendazole for one day, TX2 = 545.5 mg fenbendazole daily, for 3 consecutive days (1636.5 mg total), TX3 = 1636.5 mg
fenbendazole for one day. Eggs were isolated from fecal samples at various days post-treatment (dpt) by experiment and incubated for 60 days. Embryonation rates (ER) for each animal were determined by counting the number of eggs with fully developed larvae out of 100 eggs counted.

**Results:** Embryonation rates were significantly ($P<0.0001$, ANOVA) reduced to 29.3 and 30.5 percent for TX1 and TX2 in *A suum* eggs shed 8 dpt, and 26.6 percent for TX3 in eggs shed 6 dpt. *A suum* eggs shed from CNT animals ranged from 90.3 to 99.3 across all experiments and sampling days. Difference in ER between treatments was only seen in eggs shed 4 dpt; TX1=75.4, TX2=70.9, TX3=47.0. In addition, many of the *A suum* eggs shed from treated animals had atypical character, such as unequal cell division, granular appearance and irregular shapes.

**Implications:** Fenbendazole is ovicidal for *A suum*. Use of fenbendazole provides additional epidemiological benefits in control of *A suum* through reduced effective environmental contamination due to the reduced number of eggs that develop to an infectious larvae.

**Key words:** swine, *Ascaris suum*, fenbendazole, eggs, ovicidal activity
4.2 Introduction


Significant changes in the way most modern swine are housed and managed, such as housing pigs indoors, increased sanitation and effective anthelmintic use have either eliminated or significantly reduced parasite incidence and severity. Due to reduced prevalence and overdispersion within populations (Boes 1998b), parasites are mainly a subclinical disease and not often considered to be of major importance in modern swine production (Myers 1988, Boes 2010). However, due to several characteristics of *A suum*, the parasite still persists in modern production systems (Roepstorff 1994, Johnson 2003, Pittman 2010a, b, Duff 2014). Those characteristics are a highly fecund adult female (estimated to produce 1 to 2 million eggs per day for up to 55 weeks) (Kelley 1956, Olsen 1958), eggs that are highly resistant to environmental conditions and disinfectants (Wharton 1979, Gaasenbeek 1998, Roepstorff 1998a), a direct life cycle involving extra-intestinal migration
(Roepstorff 2003), and a relatively short patency period (6-8 weeks) (Roepstorff 2003). The high environmental contamination level and inherent egg resistance makes it nearly impossible to eradicate the parasite from contaminated facilities, thus continuous control measure implementation is required (Myers 1988, Roepstorff 1998a).

Recent housing requirements driven by concerns other than parasite control, such as group sow housing, bedding material provision and drug use restrictions are re-introducing known risk factors, and are creating exposures that promote *A suum* transmission resulting in increased prevalence and clinical severity within those farm types (Dangolla 1996b, Sundrum 2010, Roepstorff 2011). In addition, niche production systems (eg. organic, differentiated markets, pasture raised) and swine associated with regional and national exhibition show circuits provide environments for the parasite’s maintenance, especially *A suum* (Eriksen 1996, Cartensen 2002, Eijck 2005, Yaeger 2009, Woods 2012).

The two fundamental aspects for successful parasite control programs are reducing environmental contamination and minimizing transmission to susceptible animals. The ultimate goal in these programs is to minimize clinical disease and production impacts associated with parasitism. Environmental contamination reduction can be accomplished through sanitation programs that remove contaminated fecal material from the animals’ environment and often require moving animals from infected to un-infected facilities. 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 (Raffensperger 1927, Behlow 1978, Biehl 1987, Roepstorff 1998a). Reducing host shedding can be accomplished through treatment with effective targeted anthelmintics at key times in production cycles (e.g., prior to farrowing, entry into breeding herd, etc). To prevent egg transmission from sow to piglets or farm environment, a common practice involves using anthelmintics on sows prior to farrowing (Myers 1988, Roepstorff 1998a).

Fenbendazole is a broad spectrum benzimidazole (Class I) anthelmintic used in swine. Fenbendazole is widely used because of its efficacy against the most common internal parasites in swine including both the adult and larval parasitic stages (Baeder 1974, Düwel 1977, Batte 1978, Stewart 1981, Corwin 1984, Stewart 1986). Additionally, fenbendazole has a high safety margin (Baeder 1974, Düwel 1977). 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 EZ Scoop, Merck Animal Health, Summit, New Jersey) (Jacela 2009). The drug is available for other species in North America and for swine in the other countries under the trade name Panacur (MSD Animal Health, Summit, New Jersey).

The benzimidazole class of anthelmintics have ovicidal activity against parasites from a number of species (Egerton 1961, Egerton 1969, Friedman 1980, Lacey 1987, Boes 1998a, Massara 2001). This activity results from fenbendazole binding with embryonic tubulin at the leading edge of polymerization which prevents microtubule formation (Friedman 1980, Lacey...
Microtubules are important for cell structure, proper cleavage, chromosome movement during cell division, and thus embryogenesis (Martin 1997). Affected eggs often have irregular shapes, atypical blastomeres and unequal divisions which result in irreversible arrested development (Egerton 1969, Düwel 1977, Kirsh 1982, Boes 1998). The ovicidal activity resulting from benzimidazole administration provides additional benefits for reducing environmental contamination and parasite control strategies over other anthelmintic classes currently available in the US market place.

The use of fenbendazole in swine has been studied extensively and its use in sow herds is common (Dangolla 1996a). However, little information is available regarding fenbendazole’s impact on embryonation of shed *A suum* eggs post treatment under common commercial conditions. Understanding the dynamic of reduced environmental contamination is of benefit in developing effective control measures for *A suum* in commercial swine operations worldwide. Further knowledge for controlling *A suum* in swine can be used to model *A lumbricoides* control in humans (Boes 1998b, Dold 2011). The study series presented herein were conducted to characterize the embryonation rates for *A suum* eggs that were shed from naturally infected gestating sows after treatment with various fenbendazole levels under commercial conditions.
4.3 Materials and Methods

4.3.1 Farm

All three experiments were conducted on a commercial 2000-sow breed-to-feeder pig sow farm in Northeastern North Carolina. 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/Ag_Guide_3rd_ed.pdf) and Pork Quality Assurance Plus (PQA Plus) guidelines (http://www.pork.org/Certification/2341/pqaPlusMaterials.aspx). The farm was selected because it had been previously diagnosed with A suum infection in breeding sows as part of a system surveillance study (Pittman 2010a). The farm was managed with weekly batches of gestating, multiparous sows housed in individual gestation and farrowing stalls. Replacement gilts were housed in pens in groups of 4 to 5 until bred, at which time they were moved into individual gestation stalls. The farm utilized fenbendazole as an individual feed top-dress (Safe-Guard EZ Scoop) applied each week to gestating groups 2 weeks prior to farrowing, however all routine fenbendazole use was suspended for the duration of these experiments. Animals were fed individually through automated drop boxes and individual water nipples were available for each animal at all times.

4.3.2 Experiments

Three separate experiments were conducted between January 2012 and December 2013 to evaluate embryonation rates of A suum eggs shed from
naturally infected sows after treatment with three different fenbendazole levels. Each experimental design differed slightly in number of subjects, sample collection dates and treatments. (Table 1) All subject enrollment, treatment applications, sample collection, sample processing and embryonation evaluation were consistent between experiments. No animals were used in more than one experiment.

4.3.3 Sow inclusion and exclusion criteria

Based on reported literature and previous experience, younger parity animals (gilts through second parity sows) were initially screened for *A suum* infection status, since a greater infection prevalence was expected (Marti 1986, Roepstorff 1998b, Nosal 2008). In order to screen a large number of animals a modified fecal flotation method was utilized. Approximately 1 gram of feces was collected directly from the rectum of selected gilts and sows using a new clean nitrile glove for each animal. Fecal samples were placed in 15 mL centrifuge tubes (VWR, Radnor, Pennsylvania) with a screw top lid, pre-filled with concentrated sugar solution (5 mL) (Sheather’s solution; 454 g sugar in 355 mL water with specific gravity of 1.27) (David 1982). Samples were processed immediately on site. Fecal samples and sugar solution were homogenized within the 15 mL tube by vigorously shaking by hand. More complete homogenization, if required, was accomplished by using a clean disposable wooden stirrer. Tubes were placed in test tube racks and a volume of sugar solution added in order to create a meniscus at the top of the tube. A 2 cm x 2 cm glass coverslip was
placed on top of each tube and allowed to sit a minimum of 10 minutes. Coverslips were removed and placed on a glass microscope slide and examined under 40X magnification (4X objective and 10X eyepiece) for presence of *A suum* eggs. Each coverslip was examined until at least 1 egg was seen or the entire coverslip was examined and confirmed to be negative. Animals providing positive test samples were enrolled into the study and randomly allocated to treatment and control groups, while animals with negative test samples were excluded from enrollment. All enrolled animals had a day 0 fecal sample collection and egg per gram (EPG) quantification using the modified Wisconsin sugar flotation technique (Bliss 1997). 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.

4.3.4 Treatments

Safe-Guard EZ Scoop was used for all fenbendazole treatments, and subject dose allotments were with the manufacturer scoop provided in the package. The scoop when level full provides approximately 545.5 mg of fenbendazole, sufficient to provide 3 mg·kg\(^{-1}\) bodyweight to a 181.8 kg animal. In practice, a level full scoop is provided to each sow, regardless of bodyweight, and treatments were based on this methodology. Therefore, a single scoop has been loosely estimated to be 545.5 mg for each animal.

Treatments were as follows: control with no treatment (CNT), a single 545.5 mg scoop of fenbendazole for one day (TX1), a single 545.5 mg scoop of
fenbendazole daily, for three consecutive days (total 1636.5 mg) (TX2) or three 545.5 mg scoops of fenbendazole on a single day (total 1636.5 mg) (TX3). In experiments 1 and 2, subjects were randomly allocated to CNT, TX1 or TX 2. In experiment 3, subjects were randomly allocated to CNT, TX1, TX2 or TX3.

Treatment was applied to sows’ individual feed drop box 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. Animals that did not completely consume medicated feed were not included in the studies.

4.3.5 Sample collection and processing

All samples in all experiments were collected and processed in the same manner. 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 prior to the start of treatment (day 0) and at days 2, 4 and 6 after the start of treatment. At each collection day, using a new nitrile glove, the animal was stimulated to defecate by gently rubbing the dorsal surface of the rectum. Feces was collected (~100 to 500 g) in a clean plastic sample bag. If rectal stimulation did not result in defecation, fecal collection from that animal was attempted a second time later that day. If an animal had two failed attempts they were excluded from sample collection for that time point.

Samples were held at 4ºC when not being processed. Samples were processed in order to obtain a large number of A suum eggs, not for fecal
quantification. Processing and sample embryonation was developed based on several reported methodologies (Arene 1986, Okasen 1990, Massara 1991, Boes 1998a, Massara 2001) and equipment availability. A *suum* eggs were isolated using a method similar to the modified Wisconsin sugar flotation technique but adjusted for a large sample volume. Samples were homogenized by hand within the collection bags and a 100 g sub-sample was weighed out and placed into a one liter plastic container. Feces was then mixed with 200 mL of tap water and homogenized in the bucket using a kitchen potato masher. The fecal-water homogenate was then strained through a large tea strainer into a second one liter plastic container to remove large organic material. Up to 200 mL of the strained contents was then poured into 200 mL dilution bottles. The bottles were centrifuged at 145 g for 10 min in a large bucket centrifuge. The supernatant was discarded and the pellet was re-suspended in a volume quantity sufficient to 200 mL of Sheather’s sugar solution. The solution was then centrifuged at 145 g for 10 min. The solution was allowed to stand for a minimum of 10 minutes. The top 10 to 15 mL of solution was poured off into 50 mL conical centrifuge tubes. Tap water was added to a quantity sufficient 45 mL and the tubes were homogenized manually by vigorously shaking the tube. The tube was centrifuged at 145 g for 10 min and the supernatant discarded. The pellet was re-suspended in 30 mL of 0.1 N H₂SO₄ and transferred into a 50 mL filtered top culture flask (VWR, Radnor, Pennsylvania). Egg concentration was evaluated by counting the egg number in a 10 µL sub-sample at 40X magnification. Samples with greater than 25 eggs per µL were diluted with 0.1 N
H$_2$SO$_4$ to achieve this maximum concentration, as it has been reported that egg density influences development (Eriksen 1990). Culture flasks were held at 4ºC until embryonation incubation was initiated for all samples within an experiment.

4.3.6 Embryonation

Completion of embryonation occurred independently for each experiment once all samples from all collection days were processed. Once all samples within an experiment were processed, culture flasks were simultaneously incubated at room temperature (approximately 25ºC) in the dark for 60 days to ensure complete larval development (Arene 1986). Flasks were agitated by hand three to four times per week for aeration. At the end of 60 days, flasks were held at 4ºC 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 145 $g$ for 10 min. The supernatant was poured off and the pellet re-suspended in 5 mL of concentrated sugar solution. Tubes were placed in test tube racks and a volume of sugar solution added in order to create a meniscus at the tube top. A 2 cm by 2 cm glass coverslip was placed on top of each tube and allowed to sit a minimum of 10 minutes. Coverslips were removed and placed on a glass microscope slide then examined under 100X magnification (10X objective and 10X eyepiece) for $A$ suum eggs presence and embryonation stage. The first one-hundred $A$ 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 (Cruz 2012)). If less 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 subject from the experiment. Embryonation rate (ER) was recorded as the number of eggs containing fully embryonated larvae out of 100 eggs.

4.3.7 Statistical analysis

All statistical analyses were completed using Enterprise Guide 5.1 software (SAS Institute, INC, Cary, North Carolina). Analysis of variance 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 experiment due to difference in sampling protocol followed. In each case sow was considered the experimental unit. The dependent variable in each study was ER. Independent variables considered were treatment, sampling day and a treatment-sampling day interaction.

4.4 Results

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 experiment 1, ER for CNT was 95.4 percent compared to 29.3
and 30.5 percent for TX1 and TX2, respectively ($P<0.05$). In experiment 2, day 0 ER between CNT and treatment groups were not different. At sampling day 8, CNT ER was different from both treatment groups ($P<0.05$), but there was no difference between TX1 and TX2, with CNT, TX1 and TX2 having ER of 95.6, 29.4 and 38.2 percent respectively. In experiment 3, ER rates on day 0 and 2 ranged between 93.1 and 99.1 percent for all treatments, and no statistical difference was observed between treatment groups. Ovicidal activity was realized on day 4 post-treatment. Embryonation rates on day 4 were 99.3, 74.5, 70.9 and 47.0 percent for CNT, TX1, TX2 and TX3 respectively. While there was numerical reduction in ER for TX1 and TX2, only TX3 was different from CNT ($P<0.05$). By day 6, all FBZ treatments differed from CNT with ER being 90.3, 48.6, 28.6 and 26.6 percent for CNT, TX1, TX2 and TX3 respectively ($P<0.05$). Complete embryonation rates and standard deviations for all experiments are shown in Table 2.

The only ER differences between fenbendazole levels used in the present study, were observed on day 4 post-treatment in experiment 3. A numerical reduction in ER was observed in TX1 and TX2, although not statistically significant when compared to CNT. This could be due to insignificant power due to sample size or represent a dose effect. By day 6, there was no longer a difference observed between the fenbendazole treatment groups, and all were different from CNT.
4.5 Discussion

The present experiments demonstrated the fenbendazole ovicidal activity at various treatment levels, applied in a manner consistent with practical commercial farm methods, on *A suum* eggs shed from naturally infected sows. Results from these experiments agree with work by others on the benzimidazole ovicidal activity, and specifically fenbendazole on *A suum*. While others have evaluated the ovicidal activity from other benzimidazoles on *A suum* or fenbendazole on other parasites, to the authors knowledge this paper is the first to describe in detail the ovicidal activity for fenbendazole in *A suum* eggs collected from feces that were from naturally infected sows.

When *A suum* eggs are shed in the feces they are not initially infectious and require a developmental period in the environment; an important epidemiological aspect that may not be fully understood by producers and veterinarians. Since *A suum* eggs require an embryonation period outside the host, it is this transmission epidemiology aspect for *A suum* that allows effective control measures. Ascarid embryo development has been studied extensively (Alicata 1935, Cruz 2012), but specific detailed aspects are still being fully understood, namely those related to preventing embryonation or death (Fagerholm 2000, Kirchgässner 2008, Kim 2012, Katakam 2013). Embryo development in the environment is dependent on temperature, oxygen tension and humidity (Brown 1928, Connan 1977, Arene 1986, Boisvenue 1990). Eggs undergo development at temperature ranges between 16 and 38°C, with an increasing embryonation rate as temperature increases (Arene 1986). Larval
development occurs between 16 and 34°C, with non-larval arrested stages developing between 34 and 38°C. An important aspect to development is that optimal temperature for larval development is not the same as optimal temperature for larval viability (Arene 1986, Boisvenue 1990), as rapidly developing larvae have reduced viability and infectivity post hatch.

Connan evaluated *A suum* egg development when placed in a commercial swine farm [assumed in England] over time 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 (i.e., winter), and resumed development the following Spring, however with a reduced embryonation rate (Connan 1977). Eggs placed from September through May developed synchronously in the subsequent July. It is logical that environmentally controlled facilities, such as farrowing rooms and stalls with supplemental heat (e.g., heat lamps, heat mats, covered creep areas) would promote development year round (Murrell 1986). This seasonal development can be seen as seasonal variations in liver condemnation rates at slaughter plants, with the greatest prevalence’s seen July through December when growing pigs exposed to infectious eggs are marketed (Goodall 1991, Menzies 1994). Seasonal development is seen in pasture raised pigs, where a “spring rise” and increasing prevalence is observed when pastures are infected the prior fall (Larsen 1999, Mejer 2006, Roepstorff 2011).
Many unembryonated eggs visualized in the samples from treated subjects had atypical larval development and arrested development. Eggs commonly had unequal cleavages, satellite blastomeres, clustering of abnormal blastomeres, smaller and more circular shells, a granular or crystalline appearance to the yolk, lack of any apparent development and abnormal shapes, indicating arrested development. Fully embryonated larvae could be seen moving within the egg during evaluation. Atypical helminth eggs after treatment with benzimidazoles have been noted by others. Egerton reported on benzimidazole ovicidal activity and demonstrated abnormal and unequal cell divisions in *A suum* eggs after treatment with thiabendazole (Egerton 1969). Wagner and Chavarria noted granular appearing yolk material and changes and distortion in the shape and size of *T trichiura* eggs shed after treatment with mebendazole (Wagner 1974). Kirsch and Schleich reported different sized blastomeres, with an abnormal granular appearance, knot-like and crater-like, as well as undeveloped blastomere clusters next to apparently developing embryos in eggs of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* after host animals were treated treatment with fenbendazole (Kirsch 1982).

In the present study, it is important to note that the true egg origin when collected from feces and used in this study are not known with regards to their status at the time of interaction with fenbendazole. Eggs could have originated from an adult ascarid female’s uterus after treatment or liberated from dead *A suum*, free eggs in the intestinal lumen expelled from resident adult female *A suum* prior to treatment, or from unembryonated or partially embryonated eggs
from the environment ingested through coprophagia (Boes 1997). In addition, egg fertilization status at the time of collection is not known, however the vast majority of eggs collected from CNT animals developed into full larvae, suggesting ample and extensive fertilization. Egg origin effects should not have an influence on normal embryonation rates, as Okasen observed no development differences between eggs isolated from feces or from the uterus of expelled adult females (Okasen 1990). It is also important to note that adult female Ascaris can continue to shed fertilized eggs for long periods after the removal of adult males from the intestinal tract (Olsen 1958).

There is evidence that benzimidazole ovicidal activity would be effective during the treatment phase against eggs having different origin. The ovicidal activity of benzimidazoles occurs in vitro, in vivo and in utero. Boes et al demonstrated ovicidal activity in utero of female A suum expelled after treatment with albendazole (Boes 1998). In utero fenbendazole ovicidal effects on Ostertagia sp. has been reported for sheep (Kirsch 1982). Similar effects have been observed with thiabendazole on A lumbricoides eggs expelled from humans (Carvalho 1992). Wagner and Chavarria described an average of 34.6 and 66.7 percent abnormal T trichiura eggs expelled from mebendazole treated human patients (Wagner 1974b).

Some eggs excreted in the present experiments could have been due to coprophagia (Boes 1997). While this “false positive” diagnosis aspect is important in evaluating infection prevalence and response to treatment, it may not be a significant issue in embryonation studies since eggs from coprophagia
still represent environmental risk and at treatment should be susceptible to fenbendazole’s ovicidal effects in the intestinal lumen. Fenbendazole and other benzimidazoles have been shown to have significant ovicidal activity in vitro to several helminths, regardless if they were extracted from adult worm uteri (expelled therapeutically or mechanically) or collected from feces (Egerton 1961, Egerton 1969, Kirsch 1978, Massara 2001). Fenbendazole is maintained at a greater “in vitro” level in gut lumen during the treatment period due to low bioavailability (27.1 percent) and 44-50 percent of the drug remaining unchanged and excreted in the feces (Duwel 1977, Peterson 2000). Thiabendazole, a related benzimidazole, has been used successfully as an “in vitro” ovicidal treatment for *A lumbricoides* and *T trichiura* eggs in “night soil” (human waste used for fertilizer) to reduce environmental contamination and decrease infection prevalence in local populations (Kutsumi 1969). Therefore, there is still potential benefit from reducing *A suum* egg embryonation from coprophagic origin. In addition, any eggs expelled from adult females into the intestinal lumen would be subjected to the effects of fenbendazole. Benzimidazole’s ovicidal activity can affect the embryo after development is initiated. Eggs that developed for 9-10 days and then were exposed to thiabendazole in vitro, ceased further development (Egerton 1969). In theory, partially embryonated *A suum* eggs from a contaminated environment ingested by coprophagic animals would be subjected to the ovicidal effects in the intestinal lumen during fenbendazole treatment.
In the present paper, fenbendazole began to have ovicidal effects as early as day 4 post-treatment at the single day 1636.5 mg level (TX3), and across all treatments by day 6 through 8 post-treatment. Effect on embryonation beyond day 8 was not evaluated.

Other scientific literature has reported ovicidal effects rapidly after treatment with benzimidazoles. Ovicidal activity was seen in eggs in utero from adult A lumbricoides expelled 72 hours after treatment with thiabendazole (Carvalho 1992). Kirsch reported ovicidal activity in expelled Trichostrongylus colubriformis eggs as early as 8 hours post-treatment from infected sheep with 5 mg·kg⁻¹ fenbendazole (Kirsch 1978). It has been reported that using low fenbendazole levels (0.05-0.01 mg·kg⁻¹ for 7 or 18 days) resulted in atypical Trichostrongylus eggs that were identified 24 hours after the second treatment day and prior to reductions in egg counts in sheep (Kirsch 1982). Southcott reported that thiabendazole had ovicidal activity in vivo against several parasites infecting sheep as early as 8 hours post-treatment (Southcott 1963). Wagner and Chavarria evaluated mebendazole’s ovicidal effects on T trichiura eggs and the human hookworm (Necator sp) shed in feces and found reduced development in both types as early as 1 day post-treatment (Wagner 1974a,b). Maisonneuve and Rossignol reported ovicidal activity in A lumbricoides, T trichiura, Ancylostoma duodenale and Necator americanus eggs shed from humans 1 day after treatment with albendazole (Maisonneuve 1985). A lumbricoides eggs were observed to have decreased embryonation and subsequent reduced infectivity to mice as soon as 24 hours after the treatment.
start with thiabendazole or mebendazole in humans (Massara 1991). In addition, Massara et al reported thiabendazole’s effects in vitro on *A lumbricoides* egg embryonation when extracted from adult worms’ uteri as early as 48 hours post incubation (Massara 2001). The more rapid ovicidal activity in these studies, when compared to the present data, may be related to differences in anthelmintic properties, parasite characteristics, the specific anthelmintic-parasite interactions, or anthelmintic bioavailability, pharmacokinetics and pharmacodynamics for the different host species.

The overall effect is that shed *A suum* eggs, regardless of origin, have reduced embryonation rates after treatment with fenbendazole, and thus do not contribute to environmental contamination. In addition to sanitation and anthelmintic treatment to reduce adult worm burden and shedding by hosts, using an anthelmintic having ovicidal activity adds an additional control level by reducing the effective infectious egg load in the environment. Parasite control goals are to reduce environmental contamination, minimize transmission, reduce egg shedding and worm burden in hosts, in order to minimize clinical disease and production impact from parasitism, therefore fenbendazole with its adulticidal, larvical and ovicidal effects provides additional value when implemented in the overall parasite control programs instituted in populations.
4.6 Implications

- Fenbendazole, at various treatment levels, is ovicidal to *Ascaris 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.

- *Ascaris suum* eggs shed during 4 to 8 days post sow treatment with fenbendazole have reduced embryonation rates, and therefore do not contribute to the environmental contamination.

- Fenbendazole as a treatment for *Ascaris suum* provides an additional epidemiological advantage through reducing effective environmental contamination resulting from its ovicidal properties, when compared to other anthelmintic classes.

4.7 Acknowledgements

The primary author would like to thank the farm management and farm staff at the sites in which these studies were conducted. The primary author would like to thank the numerous veterinary students who assisted with sample collection over the course of these studies.

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4.9 Tables and Figures

Table 4.1 Summary of sample collection days for egg embryonation post treatment (DPT) and number of sows (N) in three experiments comparing different fenbendazole treatment† levels and duration on ovicidal activity of *Ascaris suum* eggs shed from naturally infected crossbred* gestating sows.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DPT</th>
<th>Treatment (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CNT</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

†CNT = untreated control sows; TX1 = 545.5 mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day; TX2 = 545.5 mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total); TX3 = 1636.5 mg fenbendazole given on a single day.

*Sow; F1 1/2 Large White x 1/2 Landrace, multiparous
Table 4.2 Results. Mean *A suum* embryonation rate (±SD) by days post-treatment (DPT) in three experiments comparing different fenbendazole treatment\(^\dagger\) levels and duration on ovicidal activity of *Ascaris suum* eggs shed from naturally infected crossbred* gestating sows.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DPT</th>
<th>Treatment Group</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CNT</td>
<td>TX1</td>
<td>TX2</td>
<td>TX3</td>
<td>(P)</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>95.4(^a) (8.6)</td>
<td>29.3(^b) (24.2)</td>
<td>30.5(^b) (18.2)</td>
<td>ND</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>92.8(^a) (13.3)</td>
<td>92.5(^a) (17.4)</td>
<td>85.0(^a) (17.9)</td>
<td>ND</td>
<td>0.3949</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>95.6(^a) (8.6)</td>
<td>29.4(^b) (24.0)</td>
<td>38.2(^b) (21.2)</td>
<td>ND</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>98.6(^a) (2.9)</td>
<td>97.8(^a) (2.9)</td>
<td>94.0(^a) (5.6)</td>
<td>97.1(^a) (4.2)</td>
<td>0.1131</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>99.1(^a) (1.1)</td>
<td>99.0(^a) (1.5)</td>
<td>93.1(^a) (10.9)</td>
<td>95.9(^a) (8.7)</td>
<td>0.2687</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>99.3(^a) (0.8)</td>
<td>75.4(^a) (31.0)</td>
<td>70.9(^a) (20.1)</td>
<td>47.0(^b) (34.1)</td>
<td>0.0064</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>90.3(^a) (21.3)</td>
<td>48.6(^b) (20.9)</td>
<td>28.6(^b) (26.1)</td>
<td>26.6(^b) (28.9)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^{ab}\) Values within row with different superscripts are different (reported \(P\); ANOVA Tukey’s studentized test).

\(^\dagger\)CNT = untreated control sows; TX1 = 545.5 mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day; TX2 = 545.5 mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total); TX3 = 1636.5 mg fenbendazole given on a single day.

*F1 Large White x Landrace multiparous sows
Figure 4.1. Micrographs of embryonated and unembryonated *Ascaris suum* eggs after expulsion in three experiments comparing different fenbendazole treatment† levels and duration on ovicidal activity of *A suum* eggs shed from naturally infected crossbred* gestating sows.

Normally developed larvae isolated from sows on day 0, prior to treatment with fenbendazole. (ER = 99 percent)
A: abnormal crystalline-like yolk; B: unequal cell division; C: satellite blastomeres. Sample collected on day 4 after 545.5 mg fenbendazole for 3 consecutive days (TX2). (ER = 33 percent)

A: Unequal cell division; B: clustering of blastomeres around a single cell. Sample collected day 8 after 545.5 mg fenbendazole for a single day (TX1). (ER = 21 percent)
Mix of A: Normally developed larvae; B: undeveloped eggs; C: crystalline-like yolk. Sample collected day 8 after 545.5 mg fenbendazole for a single day (TX1). (ER = 21 percent)

A: arrested 3-cell division; B: small circular egg; C: crystalline-like yolk; D: abnormal divided cell structure. Sample collected on day 4 after 545.5 mg fenbendazole for 3 consecutive days (TX2). (ER = 33 percent)
Clustering of smaller blastomeres around an undivided cell. Sample collected on day 4 after 545.5 mg fenbendazole for 3 consecutive days (TX2). (ER = 33 percent)

Satellite blastomere next to an undivided cell. Sample collected on day 6 after 1636.5 mg fenbendazole for a single day (TX3). (ER = percent)

Abnormally shaped cell. Sample collected on day 6 after 1636.5 mg fenbendazole for a single day (TX3). (ER = 69 percent)

\(^1\text{CNT} = \text{untreated control sows}; \text{TX1} = 545.5 \text{ mg fenbendazole (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey) given on a single day}; \text{TX2} = 545.5 \text{ mg fenbendazole given on three consecutive days (1636.5 mg fenbendazole total)}; \text{TX3} = 1636.5 \text{ mg fenbendazole given on a single day}; \text{ER} = \text{embryonation rate, percent of 100 eggs with visible developed larvae after 60 days of incubation at room temperature}.

\(^*\text{Sow; F1 1/2 Large White x 1/2 Landrace, multiparous}
CHAPTER 5. GENERAL CONCLUSIONS

5.1 Effective Environmental Contamination

The impact of fenbendazole on the eggs of *A suum* is described in Chapter 3 as a reduction in fecal shedding and in Chapter 4 as reduced embryonation rates of shed eggs relative to non-treated controls during the study periods defined. However, these two aspects do not happen independently and both are important aspects of reducing the overall effective environmental contamination in an infected population. In theory, this should have an impact on clinical severity, population infection, re-infection and transmission dynamics of *A suum* in swine. This has been shown in human population studies with *A lumbricoides* and *T trichiura* (Kutsumi 1969), but has not been studied in swine populations to the author’s knowledge.

To demonstrate this concept, data from studies in Chapter 3 and 4 were combined and modeled in the example below. Reduction in environmental burden (BURD) and embryonation rates (ER) were combined to estimate an “effective burden” (EFF BURD). Due to the variation in sample collection days and an inability to match BURD and ER data for all subjects the below data is generated from several selected data sets and is therefore an approximation of the overall effect. In order to more clearly describe the theory and minimize across treatment effects, only data from the control group and TX2, the labeled treatment of fenbendazole, have been used for description of the model. Therefore the data should not be used as a valid mathematical model, however should be considered as a basis for directing further research efforts. The
following adjustments and assumptions were made in the following data set; 1) individual subject data was combined from multiple studies, 2) fecal EPG counts were averaged for each sample day-treatment group, 3) average fecal EPG for sample day 2 has been calculated as the average of day 0 and 4 EPG, since no fecal EPG counts were conducted for day 2 post-treatment in any of the data sets, 4) BURD was calculated from the group average EPG, not as an average of the individual BURD values for each subject. No data was generated beyond 8 days post treatment since embryonation rates were not conducted beyond this period, thus extrapolation would not be appropriate, however control subjects would continue to shed eggs capable of full embryonation into the environment beyond this period, while treated animals would contribute very few eggs to the environment during this period (Chapter 3). When using the above methods it can be theorized that the treated group sheds 54.3 percent less total eggs into the environment over the 8 day period than untreated controls. When embryonation rates are included to estimate the EFF BURD, the treated group sheds 62.5 percent less eggs that have the potential to become infectious as compared to the controls. Data is presented in tabular (Table 5.1) and graphic (Figure 5.1) form below.

While not directly evaluated in this thesis, the larvacidal effect of fenbendazole also has additional impact on the epidemiology of *A suum* as treatment of migrating larvae will effectively lengthen the period it will take for sows to shed eggs from reinfection.
5.2 Recommendations for Future Research and Study Limitations

Further work that would be complementary to the data presented in this thesis would be to evaluate the larval hatch rates and infectivity of those eggs that undergo full development in the presence of fenbendazole. There have been several studies that have looked at larval hatch rates or infectivity after treatment with various chemicals, including benzimidazole anthelmintics, however none have looked at the combination of fenbendazole in A suum using a pig infectivity model. Boes et al evaluated the infectivity of developed A suum eggs expelled after treatment with albendazole in a mouse model but showed no difference in the number of larvae recovered from lung and liver tissue between treated and positive control groups (Boes 1998). This could be accomplished by repeating the work reported in Chapter 4 and using the recovered developed eggs in a hatch model similar to that described by Han et al as well as an adaption of infectivity and larval recovery models as described by Boes et al and Slotved et al (Slotved 1996, Boes 1998, Han 2000). This would determine if there is additional benefit to the use of fenbendazole beyond reduced shedding and reduced embryonation as it relates to environmental contamination and transmission.

One limitation to the work presented in this thesis is the use of fecal egg shedding as an indicator of treatment effectiveness. Fecal egg counts of A suum can be highly variable in pigs (Roepstorff 1998) and can also represent false positive results from coprophagia (Boes 1997). Confirmation of effectiveness of anthelmintics are done with worm recovery, normally at necropsy, however this is
prohibitive in a commercial setting and costly in a research setting, therefore
fecal egg counts in these experiments were used as a proxy for infection. The
concern is that not all subjects’ true infection status was known, and therefore
false positive and false negative sampling points are a strong possibility as noted
in Chapter 3. Therefore, the work contained within this thesis could be repeated
in an experimental setting where coprophagia can be controlled and infection
status confirmed with other methods, such as necropsy, collection of feces and
worm identification during the study period or worm elimination (by using a
different non-ovicidal anthelmintic such as piperazine) at the completion of the
study period. In addition, more frequent serial fecal collections or longer
collection periods could help confirm the infection status (eliminate false
negatives) and possibly minimize the variability in fecal EPG counts.

Another advantage of using an experimental infection model would be the
ability to establish a homogenous infection status of all the subjects, which is not
the case in the subjects enrolled in studies included in this thesis. Subjects
enrolled in these studies most likely represented egg shedding at variable days
post infection or even post reinfection, which could have had an impact on
intestinal adult worm burden, migrating larval burden, magnitude and variation of
fecal egg counts, the dynamics of fecal egg shedding post treatment and
response to treatment. It should be noted that the work contained herein was
purposely conducted in commercial farm settings to account for variability
normally experienced in the field, which should provide a more practical example.
The last aspect that could be controlled in future studies are the treatment doses provided to the subjects. In the included studies, a practical application to the treatment doses (i.e., the manufacture’s provided scoop) was used as that is how the product is commonly applied on-farm. While the labeled daily dose states $3 \text{ mg} \cdot \text{kg}^{-1}$, and the Safe-Guard label states “1 level scoop per 400 lbs of sow” to achieve this dose, it should be noted that individual sow weights are at best estimated on commercial sow farms and the vast majority of commercial multiparous sows are greater than 400 lbs. Therefore, practical application of fenbendazole in increments different than the scoop provided (e.g., $1 \frac{3}{4}$ scoops for a 500 lbs sow) are not likely feasible in a commercial setting. However, given the data provided in Chapter 3 and published literature reviewed in Chapter 2 concerning fenbendazole effectiveness at different dose levels, this aspect is not likely a major biological influence.

5.3 Summary

*Ascaris suum* is still present in modern swine production systems worldwide, and will continue to be present with the current methods of control that are available. In addition, *A suum* has increased prevalence in alternative swine production systems (niche production, outdoor operations, hobby farms, etc.) that support epidemiological risk factors associated with the parasite (access to pasture or dirt, reduced ability to sanitize facilities and remove manure, one-site multi-stage production, limitations on anthelmintic use). While overt clinical disease is not commonly reported in the literature today, cases of
acute mortality and severe disease are still encountered and subclinical infection has the potential to greatly decrease the efficiency and profitability of swine. It should be noted that in non-industrialized swine production, for example "small village swine herds", clinical ascariasis is still a significant health concern for both the pigs and the humans. In addition the closely related human ascarid, *A lumbricoides*, continues to be a significant disease in human populations throughout the world, while *A suum* is a significant zoonotic contributor to human cases of ascariasis. Continued understanding of the life cycle, epidemiology, transmission and methods of control are important for better application of current control strategies and development of future interventions. The data generated from the studies included in this thesis fill an area of practical ascarid research in swine. Those areas specifically being the time to negative after treatment of naturally infected breeding sows with fenbendazole and characterization of the ovicidal effects of fenbendazole on the eggs of *A suum*. The current literature available does not specifically provide this information to parasitologists, veterinarians and pork producers. Prior to this work, recommendations on when to initiate treatment to sows prior to movement into the farrowing environment was supported only by a few studies with single sampling points or extrapolated from other drugs or species. This work presented herein should provide a better set of guidelines for use of fenbendazole in breeding sows. The current recommendation by the author is to utilize fenbendazole at least 14 days prior to movement into a clean farrowing facility. This treatment will minimize the number of infectious eggs shed into the
farrowing environment by the dam for the entire lactation period (assuming 4 week or less lactation), reduce the risk of transmission to her offspring and mitigate or eliminate downstream production impacts in the grow-finish herd derived from vertical transmission.

5.4 References


### 5.5 Tables and Figures

Table 5.1 Average fecal EPG, BURD, embryonation rates and effective burden estimated from multiple studies.

<table>
<thead>
<tr>
<th>Day</th>
<th>CNT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TX2&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>EMB&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>184.3</td>
<td>0.949</td>
</tr>
<tr>
<td>2&lt;sup&gt;g&lt;/sup&gt;</td>
<td>188.2</td>
<td>0.991</td>
</tr>
<tr>
<td>4</td>
<td>192.1</td>
<td>0.993</td>
</tr>
<tr>
<td>6</td>
<td>206.3</td>
<td>0.903</td>
</tr>
<tr>
<td>8</td>
<td>234.6</td>
<td>0.955</td>
</tr>
<tr>
<td>Average</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Overall Difference&lt;sup&gt;h&lt;/sup&gt;</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

<sup>a</sup> CNT = untreated control group  
<sup>b</sup> TX2 = treated group; treated with 545.5 mg fenbendazole daily for 3 consecutive days  
<sup>c</sup> EPG = eggs per gram, average fecal EPG for all subjects within treatment-day  
<sup>d</sup> EMB = embryonation rate, average EMB for all subject within treatment-day  
<sup>e</sup> BURD = burden, calculated sample day EPG as percent initial (day 0) EPG.  
<sup>f</sup> EFF BURD = effective burden, calculated sample day EPG*sample day EMB as percent of initial (day 0) EPG.  
<sup>g</sup> EPG values for day 2 are an average of day 0 and 4 values within treatment as fecal EPG were not collected  
<sup>h</sup> Difference = overall percent difference in embryonated eggs between CNT and TX2 groups over the 8 day evaluation period.
Figure 5.1 Graph depicting the theoretical difference in total *A. suum* eggs and potentially infectious *A. suum* eggs shed from sows left untreated (CNT) or treated with 545.5 mg fenbendazole daily for 3 consecutive days (TX2).
APPENDICES. PROTOCOLS

6.1 Modified Wisconsin Sugar Flotation Technique

1. Weigh 2-5 grams of feces and place into a 147.8 mL (5 oz) wax paper cup.
2. Add 15 mL of Sheather’s solution into the cup.
3. Mix until homogenized using a disposable wooden stirrer.
4. Pour solution through a tea strainer and collect in a new wax paper cup.
5. Using a wooden tongue depressor, squeeze the liquid out of the feces that is left in the strainer.
6. Pour strained material into a 15 mL conical centrifuge tube.
7. Centrifuge the tube for 5 to 10 minutes at 1000 rpm (145 g).
8. Place the tube in a test tube rack and fill with Sheather’s solution until a meniscus is formed.
9. Place a 2 cm by 2 cm coverslip on top of the meniscus.
10. Let coverslip sit for 10 minutes, then remove the cover slip and place on a microscope slide.
11. Examine the entire cover slip at 40X magnification (10X eyepiece and 4X objective) and count the number of eggs observed. Slides can be viewed at

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1Adapted from:

2Sheather’s solution is made by dissolving 454 g sugar in 355 mL water. Solution should have a final specific gravity of 1.25-1.27; check with a hygrometer
100X magnification to better distinguish between eggs and debris, if necessary.

12. The number of eggs counted is for the total amount of feces used in Step 1, therefore the egg per gram (EPG) is the total number of eggs counted divided by the number of grams of feces used in Step 1. [example: 225 total eggs counted ÷ 3 grams feces used = 75 EPG]
6.2 *Ascaris suum* Egg Isolation and Embryonation

**Isolation of eggs from feces**

1. Collect a large volume (100 grams or more) of fresh feces from known positive animal.

2. Homogenize sample or take multiple sub samples.

3. Homogenize 100 grams of feces with 200 mL water in a large 1 L plastic container. A kitchen potato masher works well for homogenization.

4. Strain fecal-water solution through a large tea strainer into second 1 L plastic container.

5. Pour 200 mL of strained material into 200 mL dilution bottle.

6. Centrifuge for 10 minutes at 1000 rpm (145 g) in a large bucket centrifuge.

7. Discard supernatant.

8. Add a small volume of Sheather’s sugar solution and re-suspend pellet.

9. Fill bottle to 200 mL with Sheather’s sugar solution.

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Adapted from:


10. Mix well and centrifuge for 10 minutes at 1000 rpm (145 g) in a large bucket centrifuge.

11. Allow bottle to sit upright for 10 minutes.

12. Transfer (pour, pipette, etc.) the top 10-15 mL of solution into a 50 mL centrifuge tube.

13. Fill 50 mL centrifuge tube with water, mix and centrifuge for 10 minutes at 1000 rpm (145 g).

14. Pour off supernatant.

15. Re-suspend pellet with 30 mL of 0.1 N H₂SO₄.

16. Pour contents into a filter top 50 mL tissue culture flask.

**Enumeration of eggs**

17. Mix contents of culture flask and pipette 10 uL of solution and place on microscope slide. Cover with a 2 cm X 2 cm coverslip.

18. Using 40X magnification (10X eyepiece and 4 X objective) count total number of eggs per slide.

19. Dilute with 0.1 N H₂SO₄ to achieve an egg concentration less than 25 eggs·uL⁻¹.

**Incubation of eggs**

20. Hold at 4°C until ready to initiate incubation.

21. Place flasks in dark area at room temperature (approximately 23-25°C).

22. Flasks should be gently shaken to aerate every 2 to 3 days.

23. Incubate for 28 days or longer.
Calculating embryonation rate

24. After incubation, hold samples at 4°C until ready to evaluation embryonation rate.

25. Remove 10 to 15 mL of incubated solution and place in a 15 mL conical centrifuge tube.

26. Centrifuge the tube for 10 minutes at 1000 rpm (145 g).

27. Discard supernatant.

28. Add 5 mL of Sheather’s solution and re-suspend the pellet.

29. Add 10 mL of Sheather’s solution.

30. Centrifuge the tube for 10 minutes at 1000 rpm (145 g).

31. Place the tube in a test tube rack and fill with Sheather’s solution until a meniscus is formed.

32. Place a 2 cm by 2 cm coverslip on top of the meniscus.

33. Let coverslip sit for 10 minutes, then remove the cover slip and place on a microscope slide.

34. Examine the cover slip at 100X magnification (10X eyepiece and 10X objective) and determine the embryonation status of a number of eggs desired (usually 100 or greater).

35. The embryonation rate (ER) is defined as the percent of embryos with a fully developed larvae within the egg over the total number of eggs observed. These final steps can be repeated any number of times and an average taken of the subsets evaluated.
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I want to thank the management and employees of the farms where the research for this thesis was conducted. My frequent presence on the farm, the treatment protocols and periodic assistance from the farm staff is outside normal operating requirements. I appreciate their continued patience and assistance
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NAME OF AUTHOR: Jeremy Stuart Pittman

DATE AND PLACE OF BIRTH: April 1, 1978, Hackensack, NJ

DEGREES AWARDED:

B.S. in Animal Science, North Carolina State University, 2000
B.S. in Zoology, North Carolina State University, 2000
Minor in Genetics, North Carolina State University, 2000
Minor in Nutrition, North Carolina State University, 2000
D.V.M., North Carolina State University, 2004

HONORS AND AWARDS:

Diplomate American Board Veterinary Practitioners – Swine Health Management, 2012
Pfizer Top 10 swine veterinarians under 40 Award, 2011
Ben Harrington Award for Student Mentorship, North Carolina State University, 2012

PROFESSIONAL EXPERIENCE:

Staff Veterinarian, Murphy-Brown, LLC, Waverly, Virginia, 2004-present
Adjunct Clinical Faculty, North Carolina State University, 2010-present

PROFESSIONAL PUBLICATIONS:


