Title

Investigation following a putative adverse reaction to thiophanate anthelminthic in a herd of breeding sows

Applicant identification number

xxxxxxx

Role in case study

ABVP candidate xxxxxxx was responsible for follow-up investigation and resolution of this case (at the request of the farmer) after initial consultation and treatment was conducted by a non-specialist local veterinarian.

Introduction

One-hundred forty-eight gestating and lactating sows at a commercial pork farm were orally dosed over a two-day period with thiophanate anthelmintic mixed into their feed. After initial refusal, sows consumed the diet over the next 12 hours then began to present with signs of an adverse reaction including malaise, weakness, muscle tremors, hypersalivation, recumbency, and four deaths. The clinical signs were treated, and an investigation was undertaken to confirm the cause of the adverse reaction.

The significance of internal parasites on the health and productivity of pigs has been recognized for many years.[1] Though commercial pork industries in most countries have evolved to raising pigs indoors where exposure to soil contaminated with eggs and larvae of the major swine nematodes has largely been eliminated, internal parasitism remains a chronic problem. While as

21 many as 20 species of internal parasites have been reported in feral pigs,[2] only three major
22 helminth species predominate amongst indoor-housed pigs in temperate areas of the world
23 including *Ascaris suum* (the large round worm), *Trichuris suis* (whipworm), and
24 *Oesophagostomum* spp. (nodular worm).[3,4] In addition to these three species, modern pig farms
25 choosing to rear some or all of their animals outdoors are more likely to also become infected
26 with *Metastrongylus* spp. (which requires the earthworm as an intermediate host) and
27 *Hyostrongylus rubidus* as compared to their indoor-housed counterparts.[5,6]

28 The prevalence of these nematodes is not only influenced by host environment (indoor or
29 outdoor production, or feral), they are also influenced by stage of production, pig age, and
30 geography.[7] As an example, the prevalence of *A. suum* in indoor pigs in some Nordic countries
31 has been estimated to be 21.5% in slaughter pigs but only 11.3% in sows,[8] and *T. suis* has been
32 found only sporadically.[9] This contrasts with the situation in pigs reared in extensive systems in
33 the same region in which nearly all farms surveyed in Denmark were infected with *T. suis* and
34 37.5% were positive in the Netherlands.[10,11] Studies on the prevalence of *Oesophagostomum* spp.
35 are less frequent but data that are available suggests that its prevalence is also impacted by farm
36 factors with prevalence nearly twice as high amongst sows on outdoor herds (38%) as compared
37 to indoor herds (22%).[10] A study of indoor herds in Denmark found that 13% of sows in 20 herds
38 that were surveyed were infected with the parasite[12]. A study from Germany documented the
39 persistence of infection with nodular worms.[13] In this study, 11% of pooled fecal samples from
40 finishing pigs were positive early in their feeding period, decreased to 0% in the weeks after a
41 single dose of anthelminthic, but had increased to 6% by the end of the growing period.

42 Even in countries with large, integrated pork industries based on total-confinement housing
43 (generally over perforated flooring) such as the United States, internal parasites appear to be

common. In a statistically valid survey of the United States pork industry, farmers reported roundworms being present on around 8 to 10% of sites housing growing pigs and nearly 40% of breeding herds reported roundworms being present.[14]

The relationship between age and occurrence of each of these nematodes is not constant nor does it appear to be consistent between farms and farm type. For example, one study found that the occurrence of *A. suum* and *Oesophagostomum* spp. was strongly related to the age of the animals with *A. suum* present nearly three times as frequently in growing pigs than in adult stock.[9] However, on the same farms the prevalence of *Oesophagostomum* spp. tended to increase with the age of the pig. Another study looked more broadly at herd-level risk factors related to infection with *A. suum* and *Oesophagostomum* spp. using fecal sample and survey data collected from 83 Danish pig herds.[12] Amongst variables that were evaluated, use of bedding for sows proved to be the most significant factor for a herd being infected with *A. suum* or nodular worms; a herd was 5.4 times more likely to be infected with *A. suum* and more than 6 times more likely to be infected with nodular worms than those that did not use bedding. Data from this study and others suggest that indoor herds, particular those that use bedding, have generally poor hygiene, or raise pigs in a 'continuous flow' pattern are unlikely to remain free of *A. suum* and nodular worms while *T. suis* and *Hyostrongylus rubidus* can be effectively controlled or eliminated by removing pigs from outdoor, free-range systems.[15-17] Other authors have emphasized however, the importance of individual farm-specific risk factors that may not be included in a standardized questionnaire such as farm history, parity distribution, biosecurity measure (and compliance with those measures), and previous or ongoing use of anthelminthic programs.[18]

The cost of internal parasitism in terms of clinical signs of disease and performance measures such as reduced average daily gain and reduced feed efficiency has been studied. In pigs

experimentally infected with *T. suis*, young growing pigs in four treatment groups were dosed with 0, 550, 1,100, or 1,650 eggs per kg of bodyweight, then followed over an 11-week period.[19] Pigs receiving the lowest dose did not grow at a rate significantly different than uninfected controls but the two high dose groups grew 16% and 26% more slowly, respectively than the control pigs. The raw data on feed efficiency rate showed an identical pattern but the authors noted the differences were not significantly different due to extremely wide variability in the infected groups. The same authors used similar experimental approaches for estimating the influence of nodular worms or *A. suum* on growth rate and feed efficiency. Despite inducing infection with nodular worms over six dose levels, no significant differences were observed in either average daily gain or feed efficiency over the 11-week period, though significant differences were observed over the first 21-days post-inoculation.[20] When measuring the effect of *A. suum* on gain and efficiency, the authors reported a strong negative linear effect of infective dose on average daily gain ($p < 0.07$, no statistical measure reported) with a 10% decrease in daily gain observed in those pigs administered the highest dose (60,000 eggs). Dose of *A. suum* had a significant linear effect ($p < 0.01$) on feed efficiency, with increasing doses having negative effects in the range of 5% to 15% on feed efficiency.[21] A number of additional studies have been published reporting estimates of the effects on performance of internal parasitism on pigs and the studies cited above are only indicative of the magnitude of the consequences that might be experienced on any particular farm. The influence of farm management, pig age, presence of concurrent diseases, the infectious dose that was received by the pig (and whether the dose was received continually in small amounts or as an acute single point-in-time), the presence of non-infectious stressors, nutrition, and other co-factors mentioned in the preceding discussion can have dramatic effects on the true cost of internal parasitism to a

farmer. The reader is encouraged to refer to one of several excellent reviews published in textbooks or the scientific literature for a more complete discussion of the topic.[1,22-25]

Three internal nematodes including *A. suum* (the large round worm), *T. suis* (whipworm), and *Oesophagostomum* spp. (nodular worms) are consistently found in indoor-housed commercial pigs. Large roundworms are by far the most important and prevalent of these three intestinal worms[26] and the life cycle of the parasite has been reviewed by others and is briefly described here. The female adult worm reaches 25 to 40cm in length and resides unattached to the wall in the small intestine. The female adult lays thick-shelled, ovoid-shaped eggs that are passed in the feces and are coated with a sticky, brownish layer. A female can lay many hundreds of thousands of eggs per day over a life span of about 6 months. The eggs are resistant to environmental challenges and can remain infective for years, so any environment in which infected pigs have resided is likely to be heavily contaminated.

Roundworms have a direct life cycle and do not require an intermediate host. Once shed in the feces, eggs (65 × 50 µm) become infective in two to four weeks depending on humidity and temperature of the environment. During this time, the eggs larvate into first stage larvae. After ingestion, the infective larvae hatch and penetrate the jejunal wall where they enter the portal circulation and are deposited in the liver one or two days after ingestion. In the liver, the larvae burrow through the liver parenchyma to enter the venous circulation where they are transported to the lung; this larval migration through the liver causes an inflammatory reaction that produces characteristic white spots or ‘milk spots’ on the surface of the liver, a hallmark of the disease. The larvae are then carried by the circulation to the lungs where after spending a few days, they leave the pulmonary capillaries, enter the bronchioles, are coughed up, and swallowed. The

larvae reach the small intestine approximately 10 to 15 days after the initial infection where they mature to adults. Egg-laying begins at about 43 days after infection.

The sticky outer coating of the *A. suum* egg facilitates mechanical transport by farm equipment, boots, insects, and transport vehicles. On endemically infected farms, young piglets are exposed to moderate numbers of eggs over time and by the time they are 5 to 6 months old, they are relatively resistant to further migrating larvae due to acquired resistance; clinical signs of ascariasis in this situation may be absent apart from suboptimal growth and feed efficiency. Some of the most dramatic disease associated with ascariasis is seen in young adults acutely exposed to large numbers of eggs over a short period of time such as might be the case when introducing high-health naive replacement gilts into a heavily contaminated isolation facility prior to entry into a breeding herd. In acute, massive exposures there can be substantial evidence of inflammation in both the liver and lungs related to larval migration; larvae can occasionally be seen on histopathological examination.

Clinical signs of 'verminous' pneumonia can occur from the simultaneous migration of numerous larvae through the lung.[24] Mild coughing is associated with migration of smaller numbers of larvae through the lung (coughing likely facilitates the swallowing of larvae necessary for completion of the parasite's life cycle) though little gross or microscopic pathology is noticeable. However, in the case of verminous pneumonia due to migration of large numbers of larvae, petechial hemorrhages in the lungs are apparent along with areas of interstitial pneumonia, bronchiolitis, and alveolar edema. The interstitial pneumonia is characterized by the presence of large numbers of eosinophils and histiocytes, with the occasional presence of nematode larvae.[27] Coughing and dyspnea can be severe, potentially leading to death in severe cases. The condition can exacerbate or mimic other concurrent bacterial or viral pneumonias that

may be present including those caused by *Mycoplasma hyopneumoniae*, influenza virus, and *Actinobacillus pleuropneumonia*.

In endemic situations, gross and microscopic evidence of infection with *A. suum* may be absent apart from 'white spots' on the liver. These spots will resolve by around 35 days post-inoculation.[28] Repeated or on-going exposure to round worms generates a robust immune response at the level of the small intestine, effectively limiting most further migration events.[29]

The biology and epidemiology of *Oesophagostomum* spp. have been thoroughly studied by authors; a brief review of the topic follows.[24] In contrast to *A. suum, Oesophagostomum* spp. are nematodes that reside in the cecum and colon, living in the mucosal surface rather than swimming freely in the lumen. The adults are substantially smaller than ascarids, ranging from 8 to 15 mm in length. There are several species of *Oesophagostomum* but all are quite similar and it is not typical for veterinarians or diagnostic laboratories to attempt to speciate them. Eggs are ovate (70 × 40 µm) and thin-shelled. They have a direct life cycle and therefore do not require an intermediate host. Eggs are passed in the feces where in contrast to *A. suum*, they develop into infective first stage larvae within the fecal matter in about a week. Like other strongyles, the larvae crawl away from the feces and move onto vegetation where they are eventually ingested by swine. *Oesophagostomum* larvae are not nearly as environmentally hardy as *A. suum* eggs but are still able to survive for up to one-year under ideal conditions. When ingested by a pig, the larvae enter the mucosal glands of the cecum and colon, penetrate the lamina propria, molt, then return as adults to the colonic lumen after about two weeks. The prepatent period is substantially shorter than for *A. suum* with eggs appearing in the feces three to six weeks after the initial infection.

Adult stages of the nematode appear to cause minimal damage to the mucosa and as they do not undergo any systemic migration as do ascarids, there is often little in the way of clinical signs. However, their brief migration into the lamina propria does often cause formation of one to two mm microabscesses or 'nodules' which gives the parasite its common name.

Members of the *Trichuris* genus exist in most mammalian species. Biology, pathology, and epidemiology of the infection of pigs has been comprehensively reviewed by others and a summary of that work follows. *Trichuris suis*, similar to *Oesophagostomum* spp., resides in the large bowel, primarily in the cecum. Adult females of the species rarely exceed 60 mm in length and have a characteristic 'whip-like' shape. About two-thirds of their length is the filamentous anterior or esophageal portion of the body (attached to the cecal mucosa) and one-third comprises the thicker posterior portion of the body. Typically, only the thicker posterior part of the worm is visible on gross inspection as most of the head portion is buried within the cecal mucosa. Eggs (55 × 25 µm) laid by *T. suis* have a very characteristic appearance; they are thick-shelled, barrel-shaped, and have a unique clear plug filling an opening at each pole of the egg. The eggs may only be shed intermittently but when seen on a fecal float examination, they are readily identifiable.

The life cycle of *T. suis* is direct and does not require an intermediate host. Once passed in the feces, first stage larvae begin to form inside the shell and after three to four weeks under ideal conditions, they will have become infective; eggs are reasonably resistant to environmental degradation and can remain viable for several years. After an egg is ingested by a susceptible pig, the larvae are released into the lower small intestine or cecum where they penetrate the lamina propria of the lower small intestine and cecum for approximately two weeks and undergo several molts after which time the posterior end of the adult whipworm's body begins to emerge

and extends out into the lumen of the cecum. Eggs begins to appear in the feces around six to seven weeks after infection, similar to that of the roundworm.

Relative to ascarids and nodular worms, *T. suis* typically creates more severe gross and histopathological lesions. Small populations of adult *T. suis* may be associated with only minimal lesions in the cecum. However, heavy infections are associated with ulceration of the mucosa, mucosal edema, hemorrhage/dysentery, and in chronic cases can produce a fibrinonecrotic membrane over the cecal mucosa. Much of this tissue damage is caused by the larval stages and therefore the absence of adult worms should not rule-out acute whipworm infection.

Control of internal parasites in pigs is achieved through a combination of management procedures, biosecurity, and anthelminthic treatment. As described above, several risk factors have been described that put farms and animals at risk for infection. Control of parasitism therefore involves management of these risk factors.

The first step in establishing a control program is focused on determining what parasites are present on the farm, and in what age group, stage of production, or physical location they exist on the farm. This step is most commonly done through collection of fecal samples from representative animal populations and examining them for the presence of nematode eggs using one of many published procedures for fecal egg floatation and enumeration.[30] Opinions vary as to what interpretations should be made around the quantity of eggs present in a sample, as opposed to simply describing an animal (or population) as positive or negative. At a farm level, quantifying the number of eggs in a fecal sample is probably not that critical as the key pieces of information that are required for establishing a control program are ‘What parasites are present in

the population' and 'What populations on the farm are infected?' Answers to these questions will provide a farmer or veterinarian with the information needed to make rational choices about what anthelminthic drug class(es) should be used, when and how often they need to be used, and in what populations they should be used. By having information about the presence of parasites in different animal classes and locations, the epidemiology of the infections including the most likely and important transmission pathways and/or risk factor for infection can be managed and therefore improve the expected response to treatment. Quantification of 'eggs per gram of feces' or determining the prevalence of infection (number of infected animals in a class divided by total number of animals in the class) may have more utility after a control program has been initiated as it can serve to provide information about changes in anthelminthic efficacy over time (i.e. resistance) and allow cost optimization of the program through more targeted use of therapy. Aside from fecal egg counts, periodic examination (and diagnostic workup) of deceased pigs can also be quite helpful in monitoring the success of a parasite control program. Some veterinarians advocate the use of 'slaughter checks' as a rapid and inexpensive means of assessing large numbers of pigs for the presence of worms though practically this may be limited to only observation for the presence of ascarid-induced milk spots on the liver; the condition is monitored at a national level in some countries such as New Zealand.[31]

Once internal parasitism has been confirmed on a farm, a control program can be created based around a combination of periodic and strategic deworming, and management of risk factors. Nematode eggs can persistent in the environment for extended periods, years in the case of *A. suum, Oesophagostomum* spp., and *T. suis*. Therefore, it stands to reason that once a farm has been populated with pigs that are infected with these parasites, animals born or moved onto that farm will remain at risk of becoming infected well into the future. This is particularly true for

225 farms that rear pigs outdoors as there are few practical ways to eliminate nematode eggs and
226 larvae from soil. Rearing pigs in a completely confined, indoor environment can make control
227 much more feasible especially in housing that allows pigs to minimize contact with their feces
228 through use of perforated flooring and avoids the use of bedding.

229 One potential transmission pathway for parasites on pig farms is from a dam to her offspring. On
230 modern commercial farms, farrowing cohorts are established and managed based on sows having
231 similar breeding dates. During the few days prior to their anticipated farrowing date, a cohort is
232 moved into a dedicated farrowing room that has been thoroughly cleaned, disinfected, and
233 contains no other animals. The cohort is allowed to farrow and lactate, often for three to four
234 weeks at which point weaning occurs and all sows and pigs are removed from the room. The
235 room is then cleaned and disinfected prior to another farrowing cohort being moved in to occupy
236 the space. This management technique is called 'all-in, all-out (AIAO)' pig flow. Use of AIAO
237 in farrowing minimizes the opportunity for pathogens, including parasitic larvae and eggs, to be
238 transmitted between cohorts. However, additional steps can be taken to manage vertical
239 transmission of parasites from dam to her offspring including treatment of sows with an
240 appropriate anthelminthic one to two weeks prior to farrowing to minimize shedding of eggs into
241 the farrowing environment. The same AIAO principles (including cleaning and disinfection
242 between groups) can be instituted across the entire farm to minimize transmission of parasites.

243 Procedures described above are imperative for control of internal parasites once a farm is known
244 to be infected. However, once a control program has been established biosecurity procedures
245 need to be implemented to minimize the risk of introducing parasites from outside the farm.
246 Maintaining a 'closed-herd' (no introductions of live animals, only semen entry is permitted),
247 quarantining and testing for parasites in new pigs that are to be introduced to a farm, and

prophylactic deworming prior to new pigs entering a farm can effectively eliminate the risk of introducing parasites into a herd.

A combination of strategic deworming, use of AIAO pig flow with good cleaning and disinfection procedures, keeping pigs free from their dung through use of perforated flooring, and establishing robust biosecurity procedures can reduce the parasite load of a farm to negligible levels. Indeed, elimination of ascarids, nodular worms, and whipworms is achievable for motivated farmers though many find reassurance in an on-going anthelminthic program with appropriate disease monitoring to ensure any subclinical level of infection is not allowed to reach a clinically significant level.

Farms that rear all or some of their pigs outdoors are likely to require on-going anthelminthic-based control programs to manage internal parasites. While there is evidence that whipworms can be eliminated from outdoor herds through a combination of strategic, intensive deworming and relocation to a known non-contaminated site, nodular worms and particularly ascarids are not likely to be completely eradicated through this same strategy.

Currently, there exists a range of safe and effective anthelminthic drugs available for use in pigs. These drugs are grouped into classes based on similar mechanisms of action, each of which is associated with a unique spectrum of activity against different parasites, its effect on adult and larval stages of these parasites, and its safety profile. Depending on country, anthelminthic drug classes that are available for use in pigs may include benzimidazoles and probenzimidazoles, salicylanilides and substituted phenols, imidazothiazoles, tetrahydropyrimidines, organophosphates, macrocyclic lactones, and more recently the amino-acetonitrile derivatives, cyclic octadepsipeptides, and spiroindoles.[32]

The precise mode of action of many anthelminthics is not fully understood but in principle, parasites must actively ingest nutrients in order to maintain an appropriate energy state for managing their reproductive processes, maintaining homeostasis, and combatting the immune response of the host, all of which require maintenance of an appropriate energy state and proper neuromuscular coordination. The pharmacologic basis of the anthelminthic drugs therefore generally involves interference with one of these core metabolic functions of the parasite and leads to starvation, paralysis, death, and expulsion or digestion of the parasite.

Benzimidazoles and probenzimidazoles (which are metabolized *in vivo* to active benzimidazoles and thus act in the same manner), salicylanilides and substituted phenols, and clorsulon act to impair structure or integrity of the parasites cells and thus have lethal effects on the worms.[33] The benzimidazoles are characterized by a broad spectrum of activity against many nematodes and have a wide safety margin. Common molecules in this class include flubendazole, fenbendazole, albendazole, thiabendazole, and thiophanate. Most benzimidazoles are poorly soluble in water and so are generally given orally as a suspension or paste (for application through drinking water or feed), or as a bolus in ruminants. The effect of these drugs is not immediate on the parasite and so contact time is important. For this reason, either repeat dosing, prolonged exposure through the feed or water supply, or bolus application is desirable. Most benzimidazoles and pro-benzimidazoles are highly effective against *A. suum* and *Oesophagostomum* spp. in pigs; they are less effective against *T. suis* though can be part of a control program if used at higher dose levels. Salicylanilides and substituted phenols, and clorsulon are primarily used for treatment of liver fluke and will not be described here.

Other drug classes act on parasites by impacting neuromuscular coordination of the worm, rather than impairing cellular function in the parasite. Most do this by inhibiting, mimicking, or

enhancing the action of neurotransmitters. These mechanisms typical have the effect of causing paralysis of the worm which in turn allows the parasite to be expelled from the gut by normal peristaltic action of the intestines. Common drug classes that rely on this mechanism include imidazothiazoles, amino-acetonitrile derivatives, macrocyclic lactones (avermectins and milbemycins), piperazine, and organophosphates (dichlorvos, coumaphos, trichlorfon, others).[32] Levamisole is the most common the imidazothiazole class used in livestock and has good efficacy against most swine nematodes except *T. suis*.[34] Amino-acetonitrile derivatives are a recently developed class of dewormer that tend to have high activity against most nematodes, including isolates resistant to all other commercially available broad-spectrum anthelmintic classes. They are effective against adult and larval stages of most nematodes but to date their use has primarily been in ruminants though some work in pigs has been reported.[35] Macrocyclic lactones were introduced in the early 1980s and a number of derivatives and competing commercial products have since been developed. They have a broad antiparasitic spectrum and tend to have good efficacy against adult and larval stages of many nematodes; uniquely, this class also has activity against a number of external (arthropod) parasites of livestock.[36] The macrocyclic lactones are well absorbed when administered orally or by injection. The class has excellent activity against most nematodes of swine except *T. suis*. Piperazine was one of the first modern classes of anthelminthic, developed in the 1950s. It is very safe as an orally administered product in pigs but has limited spectrum of activity, used primarily for control of ascarids.[37] Many organophosphates anthelminthics have been marketed over the years but due to their narrow margin of safety, limited efficacy against larval stages of nematodes, and high potential to create persistent environmental contamination through fecal excretion, their use has declined. However, dichlorvos remains in use in pigs in many parts of the world. Dichlorvos for pigs was

316 formulated as a volatile component in a vinyl resin pellet. The pellet could be conveniently
317 administered to individual sows (just prior to farrowing, as an example) or blended into
318 completed diets for herd treatment. The dichlorvos in this form is released slowly from the inert
319 pellets as they pass through the gastrointestinal tract.[38]

320 Thiophanate is an anthelminthic drug classed as a probenzimidazole. Probenzimidazoles are
321 converted to active benzimidazoles by metabolic processes in the host animal and it is the active
322 metabolites that are responsible for its anthelmintic action.[39] The mechanism of action of
323 thiophanate has not been specifically characterized but mebendazole and flubendazole, members
324 of the benzimidazole class, have been shown to disrupt cytoplasmic microtubules of the
325 intestinal cell walls of nematodes, particularly ascarids. Functionally, this results in a loss of the
326 ability of these cells to take up glucose leading to starvation of the parasite and eventual death if
327 in contact with the molecule long enough;[40-43] thiophanate is presumed to have the same or
328 similar action.

329 Thiophanate made its commercial appearance as a dewormer for livestock in the early 1970s.
330 Early work in cattle and sheep proved it to be a safe and effective dewormer for cattle and sheep
331 with good efficacy shown against *Haemonchus contortus*, *Ostertagia circumcincta*, *Ostertagia*
332 *ostertagi*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Nematodirus* spp. and
333 *Cooperia oncophora*.[44,45] Subsequently, the product found use as an effective anthelminthic in
334 pigs when used to treat infections of *A. suum*, *Oesophagostomum* spp., and *Hyostrongylus*
335 *rubidus*, with less effectiveness against *T. suis* and *Metastrongylus apri*.[46-48] Notably, these
336 studies found that in addition to control of the adult stages of these worms, the molecule also
337 appeared to have some larvicidal and ovicidal activity. Regimens were developed to allow oral
338 in-feed dose over periods of 14 days and even as a continuous low-level inclusion in order

accommodate mixing the product into complete diets for whole-herd treatments.[49,50] Work has also been published documenting the safety and efficacy of feeding thiophanate for control of *A. suum* and *Oesophagostomum* spp. as a single-dose treatment for breeding sows just prior to farrowing.[51]

No toxicity studies of thiophanate administered to pigs could be identified in a search of the published scientific literature cited in PubMed and Web of Science. However, authors in the efficacy studies in pigs and sheep described above frequently emphasized the lack of any feed refusal or transient minor adverse effects such as reduced appetite or diarrhea in the treated animals. Toxicity studies in sheep and cattle have been reported and in these species, the product was dosed orally by drench and found to be very safe at the standard dose of 75 mg/kg of bodyweight (single dose) and had no measurable adverse effects in dosages up to 1,000 mg/kg of bodyweight.[52,53] Doses in sheep between 2,000 and 10,000 mg/kg of bodyweight were associated with adverse effects, which varied both with dose and at the individual animal level. Typical responses to doses greater than 2,000 mg/kg occurred in as little as a few days and up to three weeks following exposure and included anorexia, loss of rumen sounds, diarrhea, and an appearance of becoming dull and listless; rarely animals at very high doses died. The most consistent post-mortem findings in sheep that died were a 'generalized ammoniacal odor of the tissues, characteristic of uremia'. The authors suggested this was related to terminal stage kidney failure though microscopic lesions of renal tubular dilation or other kidney pathology was not consistently observed. Other authors have cited toxicological work with thiophanate in mice and rats suggesting the compound was well-tolerated by oral, parenteral, and cutaneous exposures; the oral LD_{50} in this study was quoted as greater than 15,000 mg/kg of bodyweight [Hashimoto

Y, Makita T, Mori T, Nishibe T, Noguchi T, Tsuboi S, Ohta G. (1970). *Pharmacometrics,*1970;4:5 was cited but could not be located for verification].[52]

Toxicoses in swine have been reported, but pigs raised in a commercial indoor environment, and consuming only manufactured feed have limited opportunity to encounter many of the toxic substances (and plants) to which feral or outdoor-reared may be exposed. Pigs can be discerning eaters and their inclination for feed refusal in the face of toxic exposures is an important protective measure innate to the species. Feed refusal has been reported for pigs exposed to toxic levels of carbadox antibiotic[54], pigweed,[55] and mycotoxins (T-2 toxin,[56] deoxynivalenol[57]) as examples.

When CNS signs are observed in indoor-housed pigs and the cause is suspected to be a toxicosis, the potential routes of exposure and list of possible sources of the toxicants can be substantially reduced by considering some epidemiological and practical aspects surrounding the occurrence. The appearance of clinical signs in all or most of a herd (or cohort) and the initiation of these signs over a short period of time (minutes or hours) typically leads one to consider routes of exposure related to either the feed or water supply. Most farms have a single water supply to the site so if clinical signs are limited to only one cohort of animals on the site, the likelihood of a water-borne toxin is reduced. However, the likelihood of a feed-borne toxin is increased because each animal cohort on the farm (as defined by age or stage of production) is usually fed a different diet. Discerning the cause of CNS signs due to toxic exposure can be worked through rationally based on the nature of the clinical signs and the body systems affected.

Volumes of information are available on toxins and their mechanism of actions that relate to occurrence of CNS signs. Following is a brief review of key aspects of the topic with emphasis

on toxicities due to exposure to compounds with anticholinesterase activity.[58] Transmission of nerve impulses to muscle cells is mediated by neurotransmitters; acetylcholine and catecholamines are examples. Acetylcholine acts on two different types of receptors: muscarinic and nicotinic. Muscarinic receptors mimic the effect of parasympathetic nerve stimulation (slow heart rate, pupillary constriction, sweating and salivation, and smooth muscle stimulation leading to diarrhea and urination). Nicotinic receptors are located at the junction of voluntary nerves and skeletal and their (over)stimulation can lead to muscle tremors and fasciculations. Catecholamines neurotransmitters (principally norepinephrine with related actions produced by epinephrine) act in the sympathetic nervous system and may act on alpha-adrenergic or beta-adrenergic receptors on smooth and cardiac muscles. Stimulation of alpha-adrenergic receptors can lead to mydriasis, vasoconstriction (increased blood pressure), piloerection, etc. – all reactions associated with the classic 'fight or flight' response. Stimulation of beta-adrenergic receptors stimulates increased force and rate of heart contractions, and peripheral vasodilation. When faced with a toxic insult that compromises neurotransmitter function and thus smooth and skeletal muscle function, the clinical picture is rarely as clear as might be expected based on the discrete actions described above. As body physiology becomes disrupted by a toxic event, the body activates compensatory mechanisms in an effort to maintain homeostasis which often clouds the clinical picture from a diagnostic perspective. Further complicating the diagnosis is the fact that different toxins can act to either block, or stimulate, receptors which can lead to either downregulation up upregulation of the action associated with the receptor.

Toxic exposure to organophosphate (OP) and carbamate insecticides (and dewormers) classes of drugs are described for most livestock species including pigs. However, use of these products in livestock has been reduced over time in favor of products with better spectrums of activity and

406 safety profile. These two classes of drug are known as anticholinesterases. After acetylcholine
407 has been released from a neuron and bound to the receptor on a muscle cell, acetylcholinesterase
408 is the enzyme that degrades acetylcholine and allows stimulation of the muscle cell to cease.
409 Anticholinesterase toxins therefore, prevent the action of cholinesterase allowing stimulation of
410 nicotinic and/or muscarinic receptors to persist. Carbamate and OPs competitively inhibit
411 acetylcholinesterase by binding with the molecule and prevent it from performing its normal
412 role. The affinity of the binding varies based on which OP is involved. In some cases, the bond
413 will 'age', in effect strengthening the bond and making therapy difficult or impossible.

414 Specific clinical signs of OP toxicity depend on the extent to which nicotinic, muscarinic, or both
415 receptors are affected. Muscarinic effects in poisoned animals often leads to excess salivation,
416 vomiting and diarrhea, micturition, dyspnea (from excess pulmonary secretions and
417 bronchoconstriction), and slowing of the heart rate. Death comes as a result of hypoxia from the
418 combined cardiac and pulmonary effects described. Nicotinic effects are most often associated
419 with stimulation of skeletal muscles cascading from minor muscle twitching to generalized
420 tetany, and finally to weakness and paralysis (as muscle cells eventually fatigue). Death is often
421 brought about by respiratory paralysis and failure.

422 Unequivocal diagnosis of OP toxicity can be challenging in the absence of exposure to a known
423 toxin. Some recommend determination of whole blood cholinesterase as an indicator with lower
424 than normal values indicating significant OP exposure. Others suggest administering a low dose
425 of atropine then monitoring for a rapid return towards normal values for heart rate and pupillary
426 size. Treatment for known OP poisoning often relies on administration of atropine as a
427 competitive antagonist for the actions of acetylcholine on cardiac musculature to improve and
428 manage cardiac output,[59,60] or administration of pralidoxime which acts to free

acetylcholinesterase from the OP molecule in situations where the OP binding has not aged.[61] Pralidoxime is often not available in large enough quantities, quickly enough, for practical use in herd-level exposures of food animals. Other treatments for OP toxicity are supportive in nature and may include artificial respiration, seizure management (anticonvulsant medication), and fluid therapy to manage acid-base disruptions.

Other toxins can create CNS signs in a group of pigs housed in an indoor pork farm.[62] High levels of selenium, arsanilic acid, roxarsone (3-nitro-4-hydroxyphenylarsonic acid), dimetridazole, and others have been described as causing a combination of CNS, pulmonary, or cardiac signs but most of these are also accompanied by gastric upset, vomiting, or diarrhea in response to the relatively high concentrations that must be consumed in order to create the toxicity.

Clinical Report

Adverse reactions to the oral administration of thiophanate anthelminthic containing feed were observed in gestating and lactating sows on a commercial pork farm in 2014. The farm was comprised of a breeding herd of 200 sows and all downstream production (total pig inventory of approximately 2,000 pigs from birth to 20 weeks of age). The farm raised pigs for commercial slaughter, was located on a single-site, and pigs were housed completely indoors. Breeding females in gestation and lactation facilities were housed on partially-perforated metal or concrete flooring. Post-weaning age pigs were housed in barns of various design but included both solid-concrete-floored pens and partially-perforated concrete-floored pens. The breeding herd was known to be infected with *A. suum* and negative for *T. suis* with unknown but clinically negligible occurrence of other nematodes. The farm was affected by atrophic rhinitis and known

to be infected with swine influenza virus, *Mycoplasma hyopneumoniae*, and *Actinobacillus pleuropneumoniae* type 7. The country in which the farm was located was free of many significant viral infections of pigs including porcine reproductive and respiratory syndrome, transmissible gastroenteritis, porcine epidemic diarrhea, classical swine fever, foot and mouth disease, and African swine fever. The farm had low biological performance relative to national averages (Table 1 and Table 2).

457 **Table 1. Breeding herd performance relative to national averages.**

		National average[a]	
Production metric	**Case herd**	**Indoor sows**	**Outdoor sows**
Litters/sow/year	2.2	2.3	2.3
Born alive/sow/litter	10.2	12.1	11.9
Pre-weaning mortality %	15.2%	12.9%	19.1%
Pigs weaned/litter	8.6	10.6	9.6
Average age at weaning (days)	27	25.9	24.9
Pigs weaned/mated female/year	18.9	24.3	22.6
Female replacement rate %	32%	43%	45%
Female culling rate %	30%	36%	40%
Female death rate %	6%	7%	5%

[a] National averages obtained from levy-funded farmer association [identification deleted]

458

459 **Table 2. Growing pig herd performance relative to national averages.**

		National average[a]	
Production metric	**Case herd**	**25th percentile**	**75th percentile**
Post-weaning mortality %	5.5%	5.30%	1.80%
ADG 4-9 weeks (g/day)	NA	470g	540g
FCR 4-9 weeks (live)	NA	1.65	1.5
ADG 25-90 kg (g/day)	NA	870g	1120g
FCR 25-90 kg (live)	NA	2.52	2.31
ADG 8-90 kg	680	700	750
FCR 8-90 kg (live)	2.85	2.81	2.73

[a] National averages obtained from levy-funded farmer association [identification deleted]
NA Data not available

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As part of an ongoing internal parasite control program, 24 adult breeding sows (Landrace-Yorkshire-Duroc composite breed) were individually treated with a single oral dose of medicated feed additive containing thiophanate[a] on the morning of July 11, 2014 (67.5 mg of thiophanate per kg of bodyweight). Each of the 24 doses was mixed individually by the farmer into a 3-kg meal comprised of a barley-soybean meal-based diet, formulated to meet the nutritional requirements of a lactating sow, which was then immediately fed to each sow along with an equal volume of water. Mixing feed with water in this way was the normal practice on the farm when feeding medicated and non-medicated diets; identical procedures for deworming with thiophanate had been implemented approximately every six months for the previous 2.5 years with no adverse effects noted. Sows ranged from Day 114 of gestation to Day 22 of lactation at the time of treatment. It was normal practice on this farm for sows between Day 110 of gestation and farrowing to be limit fed at a rate of 3 kg per head per day, while sows that had already farrowed were offered feed in an amount near *ad libitum* over two to three meals during a day. Any unconsumed feed from the previous day was typically removed from feeders just prior to delivering the first meal in the morning.

In this case, all 24 sows demonstrated immediate high levels of feed refusal after delivery of the medicated feed, though most sows were seen to consume at least some of the medicated diet during the next eight hours. The unmedicated diet into which the thiophanate feed additive had been mixed, was prepared by farm staff in a stationary on-farm feed mill two-days before and had been fed to these same sows during the two-days prior and had been consumed readily by each of the sows. Aside from reluctance to consume the medicated feed, no obvious adverse reactions or clinical signs were noted by the farmer on the day the thiophanate containing diet was fed.

On the morning of July 12, 2014 farm staff noted general malaise amongst 10 to 20% of the sows that had been medicated the day before and while most sows appeared to have consumed some of the medicated feed, there was substantially more 'wasted' feed than was typical for the population. Knowing that it was important that the sows consume the entire dose of medicated diet for the dewormer to be effective, new feed and fresh water were added to each of the 24 individual feeders without first removing the day-old feed, in an effort to induce the sows to consume the medicated feed from the day before. The feeders were constructed in such a way that the sole water supply to each sow was integrated into the feeder and required that the sow dispense water into the feed pan itself in order to drink; access to water was freely available but not without the sow first adding it to the feed pan. Addition of fresh feed and water stimulated consumption by many sows though staff reported subsequently that significant feed remained in most feeders.

At approximately 8 am on the same morning (July 12), staff different than those managing the farrowing area, were following instructions by the farm manager to implement thiophanate deworming to the remaining 124 pregnant sows on the farm, located in a separate gestating building. These sows were fed using a computer controlled, liquid-feeding batch system that relied on mixing 'batches' of feed in a central processing unit which could then be delivered to locations around the farm site by a combination of computer controlled valves and pumps.[b] A total dose of 9.3 kg of thiophanate-containing medicated feed additive (225 g thiophanate per kg of feed additive) for the population was calculated based on the assumption that an average sow weighed 250 kg and would be treated orally at the rate of 67.5 mg of thiophanate per kg of bodyweight, with the total dose to be split over two daily feedings delivered approximately eight hours apart. To achieve this, half of the total dose (4.65 kg) was added to the feeding system in

the morning which had been programed to mix and deliver 1.5 kg of dry feed to each sow. It was customary for the farm to split the total daily feed allocation for each sow (3 kg per head per day) into a morning and afternoon meal. The gestation diet (formulated to meet the nutritional requirements of gestating sows) had been blended three days before and had been fed to the sows during the interim period with normal intakes. Upon delivery of the medicated diet to the 124 sows, substantial and immediate feed refusal was noted by the staff, though not to the extent observed in the lactating sows the day prior. It was customary to limit-feed gestating sows in order to manage body condition and therefore feed refusal to any degree was typically quite noticeable. Over the next one to two hours, most sows were observed to eat at least some of the medicated feed. However, sows shared a common feed trough so the extent to which any particular sow consumed or did not consume the feed was not obvious.

By 10:30 am, clinical signs of an adverse event were occurring in both the population of lactating sows treated the day before and the gestating sows treated that morning. One sow was discovered acutely dead in the farrowing area and staff reported that 15 other sows in farrowing were showing increased respiratory rates with foam/froth accumulation around their mouths, lethargy and reluctance to rise to a standing position, skeletal muscle fasciculations over the torso, and hyperesthesia when touched. Twenty-one of the 124 sows in gestation were showing similar clinical signs. A local non-pig-specialist veterinarian was called at that time and arrived at the farm at approximately 2:30 pm. By this time, four sows had died (two each in farrowing and gestation) and farm staff had removed and disposed of all the remaining medicated feed that was in front of the treated sows. Due to this action, unfortunately no records of the actual amount of medicated diet consumed by each sow were available though the farmer estimated that perhaps 15 to 20% of the medicated diet that had been delivered to the two groups had been

consumed. The local veterinarian examined the affected sows and case records from the event reported body temperatures of 40.0 to 41.0C (normal 38.7C ± 0.3)[63] and respiratory rates of 60 to 80 (normal 13 to 18)[63] breaths per minute amongst 15 of the most severely affected animals. As the source of the exposure had already been removed by the farm staff, the veterinarian elected to treat all sows that had been exposed to the medicated feed with single intramuscular injections of meloxicam[c] at a dose of 0.4 mg per kg of bodyweight, dexamethasone[d] at a dose of 0.1 mg per kg of bodyweight, and flunixin meglumine[e] at 2.2 mg per kg of bodyweight, in an effort to reduce pyrexia and non-specific inflammation.

Post-mortem examinations were conducted on the four deceased sows by the local veterinarian. Case records showed the veterinarian observed non-specific changes to the lung including edema, presence of frothy exudate in the bronchi and trachea, and diffuse hemorrhagic congestion in the lung parenchyma. Livers were slightly swollen and congested with blood, exuding substantial frank hemorrhage on cut section. Subsequent histologic examination of formalin-fixed tissues that were retained from the post-mortem examinations showed a variety of lesions consistent with acute non-specific inflammation. Lungs from all four sows had evidence of mild to moderate perivascular and peribronchial lymphoid hyperplasia associated with chronic, resolving bronchointerstitial pneumonia (likely due to *M. hyopneumoniae* infection and unrelated to the adverse event). The lungs also had severe inter- and intralobular edema with alveolar spaces and minor airways filled with proteinaceous fluid. The myocardial cells were slightly swollen but there was no evidence of necrosis or noticeable inflammatory cellular response. The liver, spleen, and kidney were examined and found to be unremarkable.

From July 13 to July 16, 2014, the prevalence and clinical signs steadily improved and no further animals died. Piglets born during this period from affected dams, appeared somewhat

lethargic and weak but it was unclear if this was a primary effect of the toxic exposure or secondary to an effect the toxin may have had on milk production or quality. No excess preweaning mortality was observed in these litters and weight gain of the litters prior to weaning was not measured. Over the next two-week period, all exposed females appeared to make a full return to health. No abortifacient effect was observable in the computerized records for the farm nor were there appreciable differences (relative to prior years) in numbers of pigs born alive per litter from the affected sows; there was no contemporary 'unexposed' cohort upon which to statistically assess the production data, so the conclusions must be drawn with care.

Aside from strongly suspecting a toxic insult, the list of potential causes was limited and non-specific. While the muscle tremors and salivation are hallmarks of toxic exposure to an OP insecticide, other common signs such as vomiting, diarrhea, miosis, and bradycardia did not occur according to notes written by the local veterinarian (though specific clinical signs can vary depending on the specific OP involved). Thiophanate itself appears to have a wide safety margin making it an unlikely cause of the toxicity and further, the clinical signs exhibited by the sows were inconsistent with the related literature on the topic. An unknown number of other toxic substances may also have been included in the feed additive but neither clinical signs nor lesions in the deceased pigs were particularly useful in refining the differential list further.

The farmer was motivated to seek financial compensation for his losses from the thiophanate distributor. The distributor was approached initially but was not receptive to the farmer's request for compensation unless clear evidence was produced that the thiophanate containing product was defective and responsible for the adverse reaction observed in the sows. To assist, a veterinary epidemiology consultant[f] with experience in pig disease and management was contacted in early August 2014 to assist in generating evidence acceptable to the product

distributor and support the farmer's claim for compensation. On August 10, 2014, a retrospective report of an adverse event related to a registered animal medication was made to the government agency responsible for such matters. The report included a brief summary of the events that occurred on July 11 to 13, 2014 as well as the specific product name and lot number involved.

During conversations with the distributor during this period, the distributor revealed that they were receiving concentrated thiophanate base from an importer, who in turn was purchasing from a thiophanate manufacturer in China. The distributor was blending the thiophanate with an inert carrier, then on-selling the diluted product as a medicated feed additive for use by farmers or veterinarians. The distributor located their retained samples of the same lot number of thiophanate feed additive that had been sent to the farm and submitted it to a third-party laboratory for analysis. The laboratory indicated the concentration of thiophanate in the retained sample was within 5% of the expected value. Next, the distributor was asked to disclose what other chemicals or products were being blended in their facility that might have been a source of contamination in the feed additive product and it was determined that oxytetracycline hydrochloride, sulphadimidine, tiamulin hydrogen fumarate, dimetridazole, furazolidone, and 3-nitro-4-hydroxyphenyl arsenic acid were also being blended in the same facility as the thiophanate; the facility was registered with the government and permitted to undertake these activities. When assayed using thin layer chromatography and melting point analyses, the retained sample of the thiophanate feed additive was determined to be free of contamination with these molecules. A retained sample of each of these potential contaminants was also tested for purity by mass spectrophotometry and found to be within normal limits for purity.

Through the course of discussions with the farmer, his staff, the distributor, and the analytical chemists involved in the testing it became apparent that the lot number of the thiophanate feed

600 additive that was in question had a distinct and strong ‘chemical’ odor. The farm staff believed
601 the odor was much more noticeable than historical lot numbers they had received and used
602 (though they had no historical samples for comparison). Staff at the distributor had a similar
603 opinion and confirmed this by comparing it with other retained samples to which they had
604 access. The Chinese manufacturer of the thiophanate was contacted by the importer to determine
605 steps involved in synthesis of the thiophanate base molecule with hopes of establishing a list of
606 potential contaminates, related to the manufacturing process, that could be assayed for in the
607 suspect lot number. The manufacturer described the main reactions and reagents (sodium
608 thiocyanate, methyl chloroformate, O-phenylenediamine, and sodium chloride) used in
609 manufacture of thiophanate but refused to provide a list of all reagents, reaction catalysts, and
610 washing solvents. Their justification for this position was that these were trade secrets and they
611 were unwilling to disclose them, even under a confidentiality agreement. The suspect lot of
612 thiophanate feed additive was assayed by thin layer chromatography to determine if any of the
613 known reagents listed above were present but results were negative.

614 At this point, both the distributor and importer still refused to settle the financial claim of the
615 farmer’s loss. The farmer had retained his own sample of the suspect lot number which provided
616 an opportunity to purposefully expose pigs to the product under controlled conditions to see if
617 the adverse reaction could be reproduced and thus generate the required evidence. A protocol
618 was developed to orally dose six culled breeding females with the suspect retained product and
619 was submitted for approval to an institutional animal care and use committee for feedback and
620 approval. After some negotiation including appointment of a third-party veterinary expert to
621 oversee welfare, ethics, and animal care aspects of the proposed study (to be done on farm),
622 approval was granted.

At 8:00 pm on October 11, 2014, six healthy, non-pregnant, breeding females (previously identified for culling and not involved in the adverse event of July 2014) were fed a single dose of thiophanate (33.75 mg per kg of bodyweight) that had been blended into 1 kg of gestation diet along with two liters of water; two cohort females had been previously identified and were fed unmedicated diet at the same time to serve as untreated controls (Table 3). A second identical exposure occurred 12 hours later, to replicate the exposure sequence experienced by the gestating sows on July 12, 2014. This dosing schedule resulted in delivery of a total dose of 67.5 mg per kg of bodyweight over two feedings.

632 **Table 3. Allocation of sows to treatment groups in thiophanate exposure study.**

ID	Weight (kg)	Source of thiophanate[a]	Dose of active ingredient (mg per kg bodyweight)[b]	
			Feeding 1[c]	Feeding 2[d]
3	128	Unmedicated control	Nil	Nil
4	128	Unmedicated control	Nil	Nil
5	118	Farmer retained feed additive	33.75	33.75
6	123	Farmer retained feed additive	33.75	33.75
7	131	Distributor retained feed additive	33.75	33.75
8	138	Distributor retained feed additive	33.75	33.75
9	127	Thiophanate base (unblended)	33.75	33.75
10	141	Thiophanate base (unblended)	33.75	33.75

[a] All thiophanate sources originated from the same manufacturer's lot of unblended base molecule.
[b] Values reflect amount thiophanate active ingredient added to 1 kg of feed.
[c] Fed at 8 pm on October 11, 2014
[d] Fed at 8 am on October 12, 2014 (+12 hours after initial feeding)

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To manage the pig's welfare, all sows were provided access to a separate source of fresh water at all times during the study. Whole blood samples in EDTA were collected from each sow just prior to feeding and heart rate (HR), respiratory rate (RR), and rectal temperature (RT) were measured and recorded every four hours over the course of the 48-hour observation period. Animals were observed every two hours during the study for clinical signs resembling those that occurred during the previous adverse event. The veterinary overseer was prepared with equipment and supplies (atropine,[g] corticosteroids, meloxicam, and flunixin meglumine) in the event emergency care was required for any of the study pigs. He was also equipped with a penetrating captive bolt gun to euthanize any pig if the need arose.

By 8 am on October 12, 2014 (+12 hours after initial exposure), all pigs except numbers 7 and 8 had consumed their entire allocation of feed. Though HR, RR, and BT were within normal limits, all treated pigs showing some adverse reactions compared to the control pigs which remained normal. Common amongst most of the six treated pigs was a reluctance to stand and rapid return to a lying position when stirred, clear nasal discharge, oral frothing, and skeletal muscle tremors. At this time, sows were also fed the second half of their thiophanate dose mixed into one kilogram of fresh feed as done previously.

By 8 pm on October 12, 2014 (+24 hours after initial exposure), the clinical picture remained very similar despite almost complete refusal across all thiophanate-treated pigs to consume the second exposure meal. On welfare grounds, the medicated feed was removed from all sows and replaced with two kilograms of fresh unmedicated feed.

At 8 am on October 13, 2014 (+36 hours after initial exposure), clinical signs in all treated sows had worsened significantly. Sow numbers 5 and 6 were lying in awkward positions and had the

appearance they were in some discomfort. With encouragement and assistance, these sows were able to rise but appeared to have severe muscle soreness or stiffness in addition to muscle fasciculations and tremors. Clinical signs in sows 7 through 10 were similar to those reported the previous day. Throughout the study, HR, RR, and RT remained in the normal range for all treated pigs and the unmedicated control sows remained clinically normal with normal appetite. A summary of clinical signs observed during the study is presented in Table 4. In addition to written records of the study, a video log of each sow's clinical behavior was recorded at periodic intervals. At this point in this study, enough information had been collected to document recreation of the adverse events experienced by sows on the farm in July 2014. With little more information to be gained by carrying the study forward for the entire planned 48-hour observation period and to better manage the welfare implications of the exposed sows, the study was terminated at 8 am on October 13, 2014.

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671 **Table 4. Summary of clinical signs exhibited by controls and sows orally exposed to thiophanate.**

ID	Treatment	Time 0	+12 hours[a]	+24 hours[a]	+36 hours[b]
3	Unmedicated control	Normal	Normal FR (0%)	Normal FR (0%)	Normal FR (0%)
4	Unmedicated control	Normal	Normal FR(0%)	Normal FR (0%)	Normal FR (0%)
5	Farmer retained feed additive	Normal	RTS, MT, ND FR (0%)	RTS, MT, ND FR (99%)	RTS, MT, ND, OF FR (100%)
6	Farmer retained feed additive	Normal	Restless FR (0%)	Hyperaesthetic FR (98%)	RTS, MT, ND, OF FR (100%)
7	Distributor retained feed additive	Normal	RTS, ND FR (60%)	RTS, MT, ND, OF FR (40%)	RTS, MT, ND, OF FR (94%)
8	Distributor retained feed additive	Normal	RTS, ND FR (60%)	RTS, MT, ND, OF FR (92%)	RTS, MT, ND, OF FR (100%)
9	Thiophanate base (unblended)	Normal	RTS, ND FR (0%)	RTS, MT, ND, OF FR (95%)	RTS, MT, ND, OF FR (100%)
10	Thiophanate base (unblended)	Normal	RTS, ND FR (0%)	RTS, MT, ND, OF FR (88%)	RTS, MT, ND, OF FR (97%)

RTS: Reluctant to stand, MT: Muscle tremors, FR: Feed refusal (% refused), ND: Nasal discharge, OF: Oral frothing

[a] Represents 12-hour period immediately after first exposure (FR = control and medicated diets)

[b] Represents 12-hour period immediately after second exposure (FR = control and medicated diets)

[c] Represents 12 to 24-hour period after second exposure (FR = all unmedicated diets)

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At +36 hours following the initial exposure, whole blood samples (in EDTA) were collected from each of the eight sows and one sow from each treatment and control group was euthanized for post-mortem examination and harvest of tissues for histopathological examination. Consistent with observations of sows affected in July during the initial adverse event, gross lesions were not specific and were limited to very minor edematous changes in the lungs which were later confirmed histologically. Whole blood samples were centrifuged, plasma harvested, then submitted for analysis of analysis of erythrocyte acetylcholinesterase levels at a regional human reference laboratory using a published benchtop method.[64] Erythrocyte (and plasma) cholinesterase concentrations fall sharply when an animal is acutely exposed to high levels of OPs. Reference values were not available for pigs, so each pig's pre-exposure level was used as a baseline to determine the proportional change in the value 36 hours post-exposure to the first feeding. Human literature suggests that erythrocyte cholinesterase levels need to fall to below 30% of baseline value before appreciable changes in neuromuscular transmissions occur.[65] In this case, erythrocyte cholinesterase levels did not change appreciably during the study providing some evidence OPs were not responsible for the adverse reaction to the thiophanate (Table 5).

689 **Table 5. Erythrocyte cholinesterase activity pre- and post-exposure to thiophanate.**

		RBC cholinesterase (U/g of hemoglobin)		
ID	**Treatment**	**Pre-exposure**	**+36 hours**	**Change**
3	Unmedicated control	3.6	4.0	11.1%
4	Unmedicated control	5.0	5.2	4.0%
Avg.		**4.2**	**4.6**	**+9.6%**
5	Farmer retained feed additive	4.2	4.5	7.1%
6	Farmer retained feed additive	3.8	3.8	0.0%
7	Distributor retained feed additive	4.1	4.2	2.4%
8	Distributor retained feed additive	3.8	3.9	2.6%
9	Thiophanate base (unblended)	4.9	2.8	-42.9%
10	Thiophanate base (unblended)	4.6	4.8	4.3%
Avg.		**4.3**	**4.2**	**-2.4%**

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Though the exact cause of the adverse event was not determined, the combination of observations from the initial clinical event, the distinct and unusual 'chemical' odor of the fed and retained thiophanate products, and especially the results of the prospective exposure study enabled the farmer to reach a financial settlement with the thiophanate distributor.

Two other veterinarians work exclusively in the pig industry in the country (servicing around 90%+ of the commercial pig industry). At the time of the adverse event, they were contacted directly to determine if they had experienced any adverse reactions with the thiophanate product in the recent past. Both replied negatively and confirmed they had no clients that were even using the product. The low market volume of the product and perhaps the results of this adverse event and exposure trial ultimately led to the product being deregistered for use in the country approximately two years later.

Discussion

Internal parasitism in pigs is an on-going problem, even in modern confinement production systems where pigs can be raised in an environment free from contact with soil, bedding, and most effluent that could otherwise harbor parasites and their eggs. Numerous parasitic nematodes have been identified in pigs but three species appear to occur most persistently around the world: *A. suum*, *Oesophagostomum* spp., and *T. suis*. Along with control of risk factors that contribute to infection with these parasites such as poor hygiene and biosecurity, and use of bedding, use of strategic use of anthelmintic drugs is a key component of most parasite control and elimination program.

There is an abundance of anthelminthics available from which choices can be made based on the actual parasites(s) present on a farm, whether control of adult or larval stages is desired, cost and

availability, safety, and efficacy (particularly considering anthelminthic resistance patterns). When using anthelminthics on large modern farms, it is most practical that a product be chosen can be delivered *en masse* to the population of pigs which generally means it is incorporated into the feed or water supply. Mass-medication programs need to be well-managed as if any problem occurs such as dosage miscalculation, the wrong product is used, or if the product is tainted by a contaminant or toxin, the scale of the resulting problem can easily exceed a farmer's ability to effectively manage it. Despite the practical issue of simply how one manages the problem once it occurs, the resulting consequences to animal health, welfare, human food supply, and financial losses can be significant.

In the current example, the initial adverse reaction (feed refusal) to exposure of pigs to thiophanate containing feed was recognized quickly by farm staff. However, poor communication between farm staff in farrowing and gestation, and poor communication between staff and the farmer-owner did not permit the magnitude of the problem to be recognized quickly enough for any intervention to occur. In fact, the opposite occurred in the gestating sows on the second day which were provided with a second dose of the thiophanate containing feed despite limited information that all was not well. When the problem was recognized, a local veterinarian responded quickly and competently implemented practical steps including clinical examination of a representative number of affected sows, necropsy and sample collection for later microscopic assessment, and initiation of some basic recordkeeping.

Treatment of large numbers of adult livestock for exposure to an unknown toxin is challenging. Clinical signs in this case did not clearly point toward and specific toxic agent though it did appear they were not likely to simply be a function of an overdose of thiophanate as the product is known to be safe even at high doses and the clinical signs being expressed were not those

typically associated with experimental thiophanate toxicity. The scientific literature has little to offer in the way of specific therapy in the event of a toxicity to thiophanate or more broadly the family of benzimidazoles of which the drug is a member. Given the lack of a specific therapy in this situation, the veterinarian acted appropriately by treatment with flunixin meglumine, corticosteroids, and meloxicam which had little potential to make the situation worse and offered the possibility of help, at least from the standpoint of animal welfare and pain relief. Arguably, atropine therapy could have been attempted during the initial treatment given some of the clinical presentation was suggestive of OP toxicity. However, atropine therapy is typically applied in the event of cardiopulmonary depression (primarily an effect of muscarinic receptor stimulation), with the appropriate dose being determined by observation of the animal's response (HR) to increasing (or more frequent) doses of atropine. In this case, neither HR or RR was depressed (in fact they were slightly elevated), suggesting atropine may not have been warranted and that if it was given, there was a possibility that it could have made this situation worse. Atropine therapy is not particularly effective in countering the effects of nicotinic receptor stimulation (muscle tremors in this case).[61] To the credit of the farm staff, the contaminated feed was quickly removed once the problem was confirmed in the two groups of pigs.

The farmer was justified in his desire for compensation from the product distributor for his losses. Whether it was reasonable for the distributor to refuse considering the rather dramatic clinical situation is another matter. However, it did appear the distributor was willing to support further investigation of the problem which suggested that if enough evidence could be generated that excluded other possibilities for the adverse reaction, the supplier would compensate the farmer.

Significant laboratory effort was committed to identifying the presence and/or nature of any contaminant in samples of the thiophanate containing feed additive that had been retained both by the farmer and the distributor. Aside from agreement that the product had a strong chemical odor, the laboratory testing essentially ruled-out the presence of the most likely contaminants (molecules being handled concurrently in the distributor's blending facility), that the thiophanate molecule itself appeared to be of expected purity, and that the thiophanate base molecule had been blended into the feed additive at the correct level. This demonstrated an important problem when investigating clinical outbreaks of disease that appear to be related to exposure to an unknown substance. It is a relatively straightforward task for a competent laboratory to determine if a known compound is, or is not, present in a substrate (such as feed) using any number of laboratory technologies. However, in the absence of a list of suspect compounds, laboratories are essentially given the task of 'test for everything' which of course is impossible. In the current case, parties involved pursued the most likely contaminates and came away empty-handed hence the implementation of a small controlled exposure study.

The prospective feeding study was very useful in creating documentation about the event that could support a financial loss claim by the farmer and avoided the likelihood of having to go down a protracted and expensive legal tract to otherwise receive compensation. In the current case the product distributor, importer, and manufacturer were incrementally less helpful in supporting the investigation. In retrospect this is not surprising as it correlates directly with each of their proximity to the customer and need to maintain a future relationship. The study was not designed to, nor did it achieve an answer to the question of 'What caused the adverse reaction?'. However, it quickly and efficiently achieved the objective of reproducing the adverse event thereby documenting the role of the defective product and justifying financial compensation to

783 the farmer. It was not a given that this exposure study could be done. There were ethical and
784 welfare obligations that needed to be met and assistance and guidance by a recognized animal
785 care and use committee was useful.

786 In response to the incident, the farm has established standard operating protocols for retention of
787 all mass medication products that will assist in any future investigations. Also, the farmer has
788 committed to being on-site whenever mass medication events are occurring. His presence makes
789 it clear to the staff that any problems that occur as a result of mass medication are likely to be
790 significant in terms of their scale, cost, and potential consequences to public health. In the
791 current case, all animals exposed to the thiophanate containing feed additive were held on-farm
792 for at least 180 days to allow tissue clearance of any undesirable compounds. The stated pre-
793 slaughter withdrawal period for the thiophanate product used in this instance was seven days
794 after the last treatment. No official guidance was available to determine a precise withdrawal
795 period in this instance given the apparent adverse reaction and therefore the prescribed
796 withdrawal period was extended by approximately 25-fold; this in combination with the history
797 indicating all clinical signs related to the exposure had ceased gave confidence that any
798 offending compounds were either cleared by the pigs or were below a level likely to produce
799 adverse effects.

800 The farmer in this instance requested the assistance of a third-party consulting veterinarian with
801 expert knowledge in swine medicine to investigate the case. Systematic examination and
802 documentation of the clinical signs and the epidemiology of the outbreak, investigation of the
803 suspected toxicant, and ultimately reproduction of the clinical episode were necessary in order to
804 produce evidence sufficient to convince the thiophanate supplier to compensate the farmer for
805 his financial loss. While it can be helpful for the consulting veterinarian to seek continued

involvement of the referring veterinarian in management and resolution of a referred case, the farmer in this case requested that the referring veterinarian not remain involved beyond supply of information related to the initial farm visit and treatment records.

Summary

Gestating and lactating sows at a commercial farm were orally dosed over two days with a feed additive containing thiophanate. After initial refusal, sows consumed the diet over the next 12 hours then began to present with signs of an adverse reaction including weakness, hypersalivation, muscle tremors, recumbency, and death. Examination of deceased sows showed non-specific lesions of pulmonary edema but little else indicative of the cause. An investigation was undertaken to determine the presence of likely contaminants in the additive, and the purity and concentration of the product but no significant findings were identified. A prospective exposure study was implemented using retained samples of the thiophanate in order to reproduce the adverse event and provide documentation supporting a claim by the farmer for financial losses. The study was a critical step required to bring closure to the episode though the exact compound responsible for the adverse was never identified.

822 **Endnotes**

[a] Thiophanate, Nemafax Pig Wormer (225 g/litre) batch #3460706, PCL Industries Ltd, Auckland, New Zealand.

[b] ACO FUNKI, Kirkevænget 5, DK-7400 Herning, Denmark.

[c] Meloxicam, Metacam (20 mg/ml), Boehringer Ingelheim (NZ) Ltd., Auckland, New Zealand.

[d] Dexamethasone, DEXA 0.2 injection (2 mg/ml), Kela N.V., Hoogstraten, Belgium.

[e] Flunixin meglumine, Flunix injection (50 mg/ml), Bayer New Zealand Ltd., Auckland, New Zealand.

[f] ABVP candidate 6189871

[g] Atropine sulfate, Phoenix atropine injection (0.60 mg/ml), Phoenix Pharm Distributors Ltd, Auckland, New Zealand

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