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2021

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James C. Beasley

University of Georgia, Savannah River Ecology Laboratory, beasley@srel.uga.edu

Lindsay M. Clontz

University of Georgia, lindsay.clontz@uga.edu

Allison Rakowski

University of Georgia

Nathan P. Snow

National Wildlife Research Center, nathan.p.snow@aphis.usda.gov

Kurt C. Vercauteren

APHIS, kurt.c.vercauteren@aphis.usda.gov

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Beasley, James C.; Clontz, Lindsay M.; Rakowski, Allison; Snow, Nathan P.; and Vercauteren, Kurt C., "Evaluation of a warfarin bait for controlling invasive wild pigs (*Sus scrofa*)" (2021). *USDA Wildlife Services - Staff Publications*. 2457.

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Evaluation of a warfarin bait for controlling invasive wild pigs (*Sus scrofa*)

James C Beasley,^{a,b,*}  Lindsay M Clontz,^{a,b} Allison Rakowski,^a Nathan P Snow^c  and Kurt C VerCauteren^c



Abstract

BACKGROUND: Wild pigs (*Sus scrofa*) cause widespread environmental and economic damage, and as a result are subjected to extensive control. Current management strategies have proven insufficient, and there is growing interest in use of toxicants to control invasive populations of this species. In 2017 a low-dose warfarin bait was federally approved for use in controlling wild pigs in the United States. However, no states have allowed use of this bait due to unanswered questions regarding welfare concerns, field efficacy, and non-target impacts.

RESULTS: All captive wild pigs fed 0.005% warfarin baits in no choice feeding trials succumbed in an average of 8 days from exposure. Behavioral symptoms of warfarin exposure included vomiting, external bleeding, abnormal breathing, incoordination, and limping. Postmortem examinations revealed hemorrhaging in organs and muscles, particularly the legs, gastrointestinal tract, and abdomen. Warfarin residues in tissues averaged 1.0 mg kg⁻¹ for muscle, 3.9 mg kg⁻¹ for liver, and 2.8 mg kg⁻¹ for small intestines. Field testing revealed wild pigs required extensive training to access bait within pig-specific bait stations, and once acclimated, exhibited reluctance to consume toxic baits, resulting in no mortalities across two separate field deployments of toxic bait.

CONCLUSION: Our results suggest wild pigs are susceptible to low-dose warfarin, and warfarin residues in pig tissues postmortem are generally low. However, although warfarin-based baits are currently approved for use by the US Environmental Protection Agency, further improvements to pig-specific bait delivery systems and bait palatability are needed, as well as additional research to quantify efficacy, cost, and non-target impacts prior to widespread implementation.

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Supporting information may be found in the online version of this article.

Keywords: anticoagulant; bait station; non-target; population control; rodenticide; toxic bait; toxicant

1 INTRODUCTION

Wild pigs (*Sus scrofa*)¹ have been introduced globally as a food source and for hunting, where they have subsequently established invasive populations throughout much of the Americas, Australia, New Zealand, Africa, and numerous island nations.^{2,3} Once established, invasive wild pig populations have expanded in size and distribution due to a combination of their behavioral plasticity, high reproductive potential, and further introductions by humans (both intentionally and accidentally).⁴ These increases have corresponded with a surge in the magnitude and scope of ecological and economic damages to natural and anthropogenic ecosystems caused by wild pigs.^{5–7} Wild pigs also are reservoirs for numerous diseases transmissible to humans, wildlife, and livestock, including foreign animal diseases (e.g. classical swine fever, African swine fever, foot and mouth disease) that have had catastrophic consequences to livestock industries globally.^{6,8} As a result, management of invasive wild pigs has emerged as a priority of global concern.

Within North America, there has been a substantial increase in the number and distribution of wild pigs since the late 1980s,^{9,10} where they now represent the most abundant medium- to large-sized invasive vertebrate.¹¹ The economic consequences

of expanding wild pig populations are not fully described, but damage and control costs alone exceed \$1.5 billion annually in the United States.⁵ Extensive population control efforts have been implemented to reduce populations and associated damages. These efforts have varied, ranging from recreational hunting and trapping to organized sharpshooting, aerial shooting, and trapping/snaring by wildlife professionals.¹² The most common form of management, recreational hunting, has proven ineffective at controlling wild populations in many circumstances.^{13,14} Even organized efforts by wildlife damage management professionals often only have localized impacts. Thus despite extensive

* Correspondence to: JC Beasley, Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, 29802, USA. E-mail: beasley@srel.uga.edu

a Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, USA

b Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, USA

c United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, CO, USA

population control measures, wild pig populations have continued to increase throughout much of their invasive range over the last few decades.

To address rapidly expanding wild pig populations globally, new tools are needed for controlling this invasive species. In particular, there is now widespread interest in use of toxic baits to control wild pig populations, as they could be a cost-effective or less labor-intensive option for reducing damages.¹⁵ However, for a toxic bait to be widely accepted, it must meet necessary safety, humaneness, and efficacy criteria.^{12,16,17}

Wild pigs are particularly vulnerable to warfarin exposure,¹⁸ and there have been multiple efforts to produce a warfarin-based toxic bait for wild pigs. Warfarin is a first-generation anticoagulant rodenticide that has been used to control rodent populations globally since the 1950s, and is considered the least toxic of the anticoagulant rodenticides.¹⁹ Experimental assessments of warfarin-based toxic baits containing 0.09–0.13% warfarin in Australia have demonstrated a high degree of efficacy in wild pigs (e.g. 94–99% of the target population eliminated)^{20,21}; although concerns over humaneness and non-target impacts have prevented operational use. Recent formulations in the United States have yielded high rates of lethality in wild pigs consuming baits containing as little as 0.005% warfarin ($\geq 95\%$ less warfarin than previous tests and 80% less warfarin than in rodent baits).^{22,23} Such a low concentration of active ingredient could reduce risks to non-target species who often are less vulnerable to warfarin exposure than pigs.^{22,23} A low-dose warfarin formulation was approved in 2017 for use in controlling wild pigs in the United States [Environmental Protection Agency (EPA) Reg. No. 72500-26, Decision No. 510475]. However, despite research by the bait manufacturer indicating high lethality for pigs and low risk for non-target species,^{22,23} as of this writing no states have approved its operational use because of remaining concerns regarding symptoms, efficacy, and non-target impacts.

In this study, we present a broad assessment of a US EPA approved low-dose warfarin bait for wild pigs, which will provide critical data to managers and lawmakers to facilitate informed decisions regarding the potential utility of warfarin-based baits for controlling wild pigs. Through a combination of controlled pen trials, laboratory analyses, and field studies, our objectives were to: (i) assess lethality and symptoms in wild pigs following consumption of low-dose warfarin bait under controlled captive conditions, (ii) quantify the assimilation rate of blue dye incorporated into warfarin baits within the subcutaneous fat of wild pigs, (iii) quantify warfarin residues within tissues of wild pigs that succumbed to the bait to determine potential warfarin exposure risks to scavengers, (iv) quantify the efficacy of low-dose warfarin bait when deployed in the field as per specified EPA label guidelines, (v) determine spillage at bait stations designed specifically for use with low-dose warfarin baits, the extent to which non-target vertebrates are able to access baits in bait stations and spillage, and (vi) quantify effects of bait deployment to non-target species through monitoring of survival.

2 METHODS

2.1 Study area

The study was conducted at the Savannah River Site (SRS) in South Carolina, USA. The SRS is a ~800 km² restricted access site managed by the US Department of Energy. Approximately 50% of the site was comprised of upland pine (*Pinus* spp.), 25% of bottomland hardwood forest, 10% of shrub/herbaceous dominated areas, and 8% of upland hardwoods, with the remaining land area

consisting of mixed forest, developed areas, and barren land. Wild pigs have been managed on the SRS through trapping and hunting since the early 1950s when an active live-trap and removal program was initiated to reduce damage caused by wild pigs as well as reduce pig-vehicle collisions.^{24,25} The diversity of habitats on the SRS, combined with limited public access and high wild pig densities,²⁶ make the site a suitable location to study the efficacy of wild pig toxicants.

2.2 Pen study

2.2.1 Wild pig capture and housing

We trapped 41 wild pigs (males = 15, females = 26) weighing 21–95 kg throughout the SRS from November 2018 through February 2019 using box and corral traps baited with corn. We chemically immobilized wild pigs using a combination of butorphanol tartrate, azaperone tartrate, and medetomidine hydrochloride (BAM, 0.0064 mL kg⁻¹; Wildlife Pharmaceuticals Inc., Fort Collins, CO, USA) and Ketamine (2.2 mg kg⁻¹; Wildlife Pharmaceuticals Inc.) administered intramuscularly via a dart rifle (X-Caliber, Pneu-Dart Inc., Williamsport, PA, USA).²⁷ While under anesthesia, we assigned each pig an individual identification number, recorded sex and weight, and transferred them via truck to a captive facility on site.

We housed wild pigs separately in 2.5 m × 3 m pens consisting of chain-link walls and a concrete floor. Each pen was outfitted with a surveillance video camera (SWNVR-885808, Swann Communications Ltd, Santa Fe Springs, CA, USA) to allow for continuous monitoring of animal behavior and activity. We placed wild pigs captured within the same sounder (i.e. social group) in adjacent pens to allow for visual contact. We placed a barrier on walls between pigs that were not caught in the same group so they could not see or interact with each other to minimize stress among unfamiliar pigs housed beside one another. We allowed each pig a minimum of 4 days to acclimate to the new environment. During this time, we provided water *ad libitum* and fed each pig 2–3 kg of Blue Ribbon 12% maintenance grain daily, depending on body weight. The maintenance grain was mixed specifically for this study without a vitamin pack to ensure treatment animals did not consume vitamin K, an antidote for warfarin, during the acclimation period. The acclimation period lasted up to 11 days, and acclimation was considered the point when an individual ate > 75% of its maintenance feed during two consecutive or non-consecutive days. If acclimation occurred before 4 days, pigs were continued on maintenance feed through the fourth day.

We randomly assigned pigs into four experimental groups: control ($n = 9$), 24-h exposure ($n = 6$), 72-h exposure ($n = 5$), and full-term treatment ($n = 20$). Once acclimated, we converted all treatment wild pigs to a diet exclusively consisting of Kaput® feral hog bait (Scimetrics, Ltd Corp., Wellington, CO, USA), which consisted of 3.2 cm × 3.2 cm × 1.3 cm paraffin bait blocks containing a fat-soluble blue dye (Fig. 1(A)). We fed each pig an acute median lethal dose (LD₅₀ = 3.0 mg kg⁻¹)¹⁸ of warfarin plus an additional 10% to account for spillage daily in the morning. We fed pigs Kaput 1–10 days depending on treatment type. We replenished Kaput in pens every morning by weighing the amount of Kaput left and adding the amount that had been consumed the day before. We also estimated the amount of Kaput spilled on the ground to more accurately quantify consumption. The purpose of the 24- and 72-h treatment pigs was to determine onset of internal evidence of warfarin consumption determined through postmortem examinations, and evaluate for presence of blue

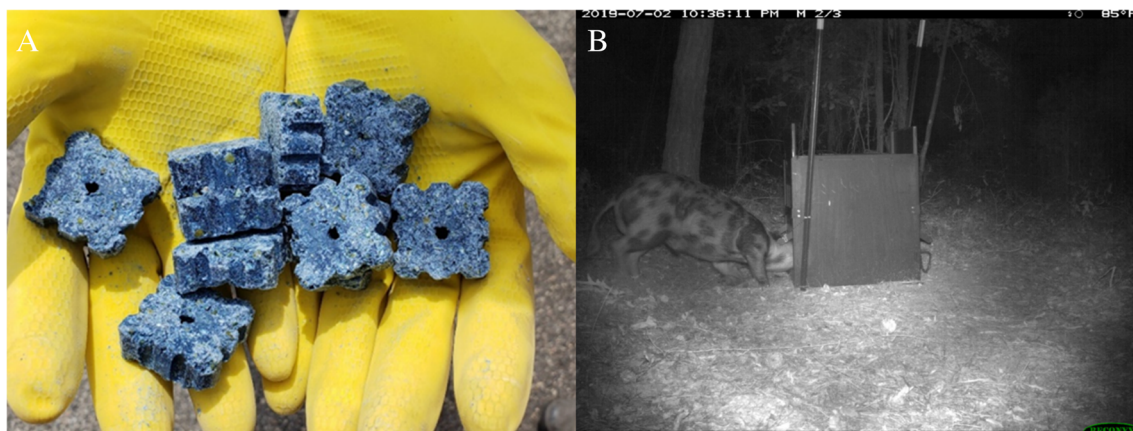


Figure 1. (A) Paraffin bait blocks containing 0.005% warfarin, and (B) guillotine-style bait station containing corn during the pre-baiting phase of trials, illustrating difficulties encountered by wild pigs attempting to feed from bait stations.

dye in subcutaneous fat. Therefore, we fed 24-h treatment wild pigs a single acute LD₅₀ dose and then euthanized them via a gunshot to the head²⁸ approximately 24 ± 6 h after consuming the acute dose. Similarly, we fed 72-h treatment pigs an acute LD₅₀ dose daily for 3 days and then euthanized them approximately 72 ± 6 h after consumption. We used surveillance cameras to determine when the acute dose was consumed for each pig. We provided full treatment pigs an acute dose daily for 10 days or until death if before day 10. Pigs still present on the 11th day were converted back to maintenance feed. Control pigs were fed maintenance feed for ≥ 16 days to allow comparison over time to treatment pigs. Similar to treatment pigs, we fed control pigs in the morning and estimated the amount of maintenance food consumed the previous day. All control pigs were euthanized at the conclusion of the study. All capture, handling, and procedures associated with this research was conducted in compliance with the University of Georgia's Animal Care and Use Committee (Permit: A2018 08-013).

2.2.2 Behavioral observations

We visited pens twice daily, once in morning and late afternoon to conduct behavioral observations on all treatment and control pigs. Observations included assessing for incoordination, breathing abnormalities, lethargy, changes in food/water intake, abnormal posture or temperament, vocalization, vomiting, diarrhea, external bleeding, and blood in feces. We quantified each metric on a scale including minor, moderate, and severe symptoms, with minor being no or slight symptoms, moderate being noticeable symptoms but short-term, and severe being heavy onset of symptoms. In addition to daytime observations, we used surveillance camera footage to include a daily nighttime observation at 10 p.m.

2.2.3 Postmortem examinations

Upon death, we conducted necropsies on all treatment and control pigs to determine presence and extent of internal symptoms caused by warfarin exposure, as well as confirm presence of blue dye within subcutaneous fat and collect samples for warfarin residue analysis. Because warfarin is an anticoagulant that causes internal bleeding, we quantified presence of any hemorrhages and free blood in muscles, joints, stomach, intestines, and other organs during necropsy. We collected samples of muscle (hind-quarter), liver, small intestine, and feces, and stored them at -80 °F in plastic bags until tested for warfarin residue. We rinsed small

intestine samples with water to remove any bait residue prior to freezing.

2.2.4 Bait/tissue evaluation

All frozen samples were shipped to the US Department of Agriculture (USDA) National Wildlife Research Center (Fort Collins, CO, USA) and prepared for analysis using a dispersive solid-phase extraction. Each sample was analyzed for residual warfarin concentrations using high-performance liquid chromatography with tandem mass spectrometry. This method was validated by fortifying 6–8 control samples of each sample type with 0.0000303%, 0.0000303%, and 0.000253% of warfarin, respectively. The efficiency of warfarin recovery averaged 99.8% [standard error (SE) = 0.91%] for muscle, 101.3% (SE = 0.95%) for liver, 100.2% (SE = 1.87%) for small intestine, and 113.5% (SE = 2.8%) for feces. The method limit of detection was 0.0023 mg kg⁻¹ for muscle, 0.0036 mg kg⁻¹ for liver, 0.0029 mg kg⁻¹ for small intestine, and 0.008 mg kg⁻¹ for feces. For all samples, if warfarin was not detected we reported the method limit of detection for that sample type.

2.2.5 Statistical analysis

We examined variation in number of days to mortality between male and female full-term treatment pigs using one-way analysis of variance (ANOVA). We further used two-way ANOVA to examine how treatment (control *versus* full-term) and sex affected weight changes during the study. We compared residual levels of warfarin among treatment groups using ANOVA in program R. We examined all pair-wise comparisons using *post hoc* Tukey's honest significant difference test. We considered differences significant at $P = 0.05$.

To assess influence of pig variables (sex, starting weight, and amount of Kaput consumed) on the number of days until mortality, we developed a suite of a priori linear regression models in program R, including each of our variables of interest as well as additive and interactive effects. We ranked models using an Akaike information criterion (AIC) model selection framework, and candidate models $\Delta\text{AIC} \leq 2$ within the top model were considered to have similar explanatory power.²⁹

2.3 Field study

We selected a study area within the SRS for the field component of this study where wild pigs were abundant and widely

distributed. Habitat composition within the study area was consistent with the dominant habitat types on the SRS.

2.3.1 Wild pig captures

We deployed baited corral traps equipped with a combination of remote operated and tripwire mechanisms throughout our study area to capture wild pigs from January to May 2019. We monitored traps using remote cameras (Reconyx PC900, Holmen, WI, USA) to identify and target whole wild pig sounders and independent males. We anesthetized captured pigs as described earlier, and while under anesthesia recorded sex and morphological measurements, attached uniquely identifiable ear tags, and assessed age through an examination of tooth eruption. We fit the largest adult female in each sounder and all adult males with an iridium global positioning system (GPS) collar (Telonics Gen4 GPS/Iridium System, Telonics, Inc., Mesa, AZ, USA; Vectronics GPS PLUS Globalstar-3, Vectronic Aerospace, Coralville, IA, USA). All other individuals received VHF (very high frequency) collars or ear transmitters depending on body size (Advanced Telemetry Systems, ATS, Isanti, MN, USA). We reversed anesthetized wild pigs with a combination of atipamezole (25 mg mL⁻¹; Wildlife Pharmaceuticals Inc.) and naltrexone (50 mg mL⁻¹; Wildlife Pharmaceuticals Inc.) at the trap site. GPS collars were programmed to record locations at 30-min intervals and were equipped with a mortality sensor.

2.3.2 Non-target captures

To assess potential impacts of warfarin baits on non-target species, we captured and fit radio-transmitters to raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), and rodents (cotton mice – *Peromyscus gossypinus* and cotton rats – *Sigmodon hispidus*). We captured raccoons and Virginia opossums using cage traps (Tomahawk Live Trap Co., Tomahawk, WI, USA) baited with commercial cat food and sardines beginning in March 2019. We placed traps systematically within the vicinity of anticipated bait station locations (see later). We anesthetized raccoons and opossums with a combination of ketamine and xylazine administered at 20 and 4 mg kg⁻¹ of estimated body weight, respectively. We attached ear tags and collected morphological measurements, sex, weight, and age via tooth-wear. We fitted the raccoons > 2.6 kg with either GPS (LiteTrack Iridium 150 Lotek, Seattle, WA, USA) or VHF collars (Advanced Telemetry Systems, ATS). We fitted all opossums > 2 kg with VHF collars (Lotek, Seattle, WA, USA).

Due to the short lifespan of rodent collars, we captured rodents following deployment of bait stations during the pre-baiting period (see later). We only deployed rodent traps within ~200 m of bait stations collared pigs were actively using. We captured all rodents using Sherman traps (Tallahassee, FL, USA) baited with commercial oatmeal, sunflower seeds, and peanut oil. We anesthetized rodents with an injection of ketamine and xylazine. We weighed, ear-tagged, and fitted individuals with VHF transmitters (M1420, Advanced Telemetry Systems, ATS).

2.3.3 Bait stations

We established bait station sites by creating a 750 m grid across our study site, and selected a single location within each grid cell to receive bait stations, for a total of 46 bait sites. All bait sites were set a minimum of 500 m apart and were within 150 m from streams (but a minimum of ~50 m away) and 100 m from roads to target areas likely used by pigs that were also accessible for bait station deployment.

We pre-baited all 46 bait station locations with 11 kg of whole corn placed on the ground to attract and acclimate pigs to bait station locations. We monitored bait stations with remote cameras for 1 week. After the pre-baiting period, we reviewed images to determine the number of pigs visiting each location. We then deployed one to three Hog Stopper™ (Scimetrics, Ltd Corp.) bait stations per location based on a ratio of one bait station to every five pigs as per product label instructions. Hog Stopper bait stations were steel boxes weighing 63.5 kg with two opposing guillotine doors, each weighing 7.7 kg. Based on wild pig activity at bait station locations, we deployed 54 bait stations across our study area: 40 locations received one bait station, four locations received two, and two locations received three bait stations. We secured bait stations using T-posts. We monitored bait stations with one to two sets (depending on bait station number) of camera pairs mounted on a T-post or tree approximately 5 m away. The top-mounted camera was programmed to take images triggered by motion while the lower camera captured time-lapse images every 5 min.

To acclimate wild pigs to bait stations, we followed EPA product label instructions and deployed 22.0 kg of whole corn in all 54 bait stations on May 27, 2019. We propped bait station doors open approximately 12–15 cm using pins to allow pigs to acclimate to feeding out of bait stations for 4 weeks. During the acclimation period, we checked bait stations regularly to replenish corn and review camera images to determine if more bait stations were necessary at specific sites. During the pre-baiting period, we added four bait stations.

On June 20, we removed corn from the bait stations, replaced it with ~22.0 kg of Kaput, and removed the pin to activate bait stations as per product label instructions. Once activated, we visited bait stations daily to identify and weigh spillage to the nearest 0.1 g. Immediately after activation, wild pig use of bait stations ceased, except for two pigs that opened bait stations and consumed a minimal number of bait blocks (i.e. a non-lethal dose; see Results). Wild pigs did continue to visit bait stations initially without attempting to open bait station doors, but visits precipitously declined. After several days, almost no pigs were visiting bait stations, and no pigs were accessing bait within stations (Fig. 2). So, on June 27 and 28 we removed warfarin baits from all bait stations and on June 28 we placed corn back into bait stations to determine if pigs would re-acclimate to using bait stations. To further facilitate wild pig use of bait stations, based on guidance by the bait manufacturer, on July 9 we replaced corn in half of bait stations with non-toxic lure (Kaput® Feral Hog Lure) developed and provided by the bait manufacturer (Scimetrics, Ltd. Corp.). During this second pre-baiting period, we initially tied bait station doors completely open for 3 weeks to allow pigs to have less obstructed access to bait, after which we dropped doors to pin height (July 19). After observing dead wild pigs and other species near sites where lure was deployed, on August 2 we ceased operations at all bait stations containing lure, and had the lure tested for presence of toxicants. We discovered the lure had been inadvertently contaminated with second generation anticoagulants during manufacturing at the production plant, and subsequently killed numerous wild pigs and other animals that fed at bait stations. Therefore, we pulled stations from all sites where lure was provided for the remainder of the study, and excluded these sites from further analysis. We also excluded an additional 16 bait stations that had been baited with corn but were no longer visited by pigs after the first warfarin bait trial. For the remaining bait stations (nine bait stations across seven

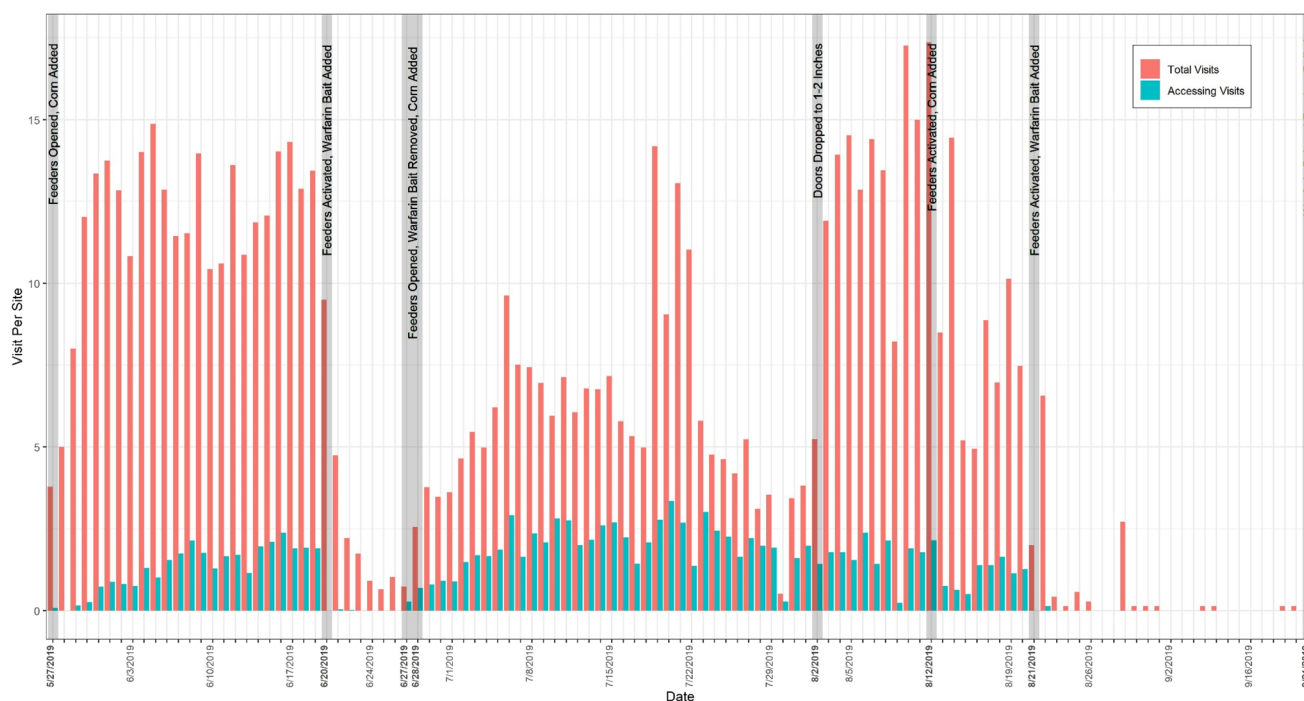


Figure 2. Timeline of field trials evaluating the efficacy of low-dose warfarin baits placed in pig-specific bait stations depicting the average number of visits by wild pigs (*Sus scrofa*) and visits where pigs were accessing baits from bait stations from May to September 2019 in South Carolina, USA.

sites), on August 12 we lowered doors to a 2–5 cm opening. After 10 days, we closed bait station doors with corn inside (i.e. activated bait stations) and monitored and replenished these bait stations for 9 days to ensure wild pigs were able to operate bait station doors when completely closed. On August 21, we removed the corn and added 22.0 kg of Kaput (Fig. 2). We left Kaput in bait stations for 30 days. During this time, we visited sites daily to measure spillage to the 0.1 g and occasionally removed and refreshed Kaput due to blocks molding or melting together. After 30 days, we removed Kaput and bait stations from the field.

2.3.4 Radio telemetry

We monitored all collared wild pigs and non-target species via radio telemetry throughout the pre-baiting periods 1 to 6 days per week. Throughout both Kaput deployments, we tracked pigs 5 to 7 days per week and recovered any mortalities to determine cause of death. We assessed whether any mortalities were due to Kaput through investigation for the presence of blue dye within the subcutaneous fat layer.

2.3.5 Image analysis

We used CPW Photo Warehouse³⁰ to categorize time-lapse remote camera images by (i) species, (ii) whether individuals were marked or unmarked (i.e. ear-tagged or collared), and (iii) for pigs whether they were accessing or not accessing the bait station (i.e. head in the bait station, raising door with their head, standing at door chewing, eating from or opening bait station). When calculating average numbers of visits and accesses, we counted each 5-min time-lapse photograph as a unique record. We also documented any instances of non-targets operating bait station doors. We summarized the number of pig visitations and accessing visits to each station daily relative to each phase of the study (i.e. pre-baiting, toxicant deployment, post-toxicant pre-baiting, second toxicant deployment).

3 RESULTS

3.1 Pen study

All 20 full-term treatment pigs succumbed following daily consumption of low-dose warfarin. Mortality averaged 7.95 ± 0.28 (SE) days and ranged from 6 to 11 days after consuming the first chronic LD_{50} dose (0.05 mg kg^{-1} ; Fig. 3). Full-term treatment pigs consumed an average of 14.68 ± 1.18 kg of warfarin bait. Females consumed an average of 15.56 ± 1.95 kg, while males consumed 13.81 ± 1.36 kg. There was no difference in time to mortality between males and females ($F_{1,18} = 0.266$, $P = 0.613$). We tested for differences in pig weight between the start and conclusion of pen trials and found the difference in pig weight differed between control and full-term treatment pigs ($F_{1,27} = 11.08$, $P = 0.003$). On average, control pigs gained 8.55 ± 2.57 kg and full-term pigs lost 1.09 ± 1.55 kg. Sex ($F_{1,27} = 0.002$, $P = 0.96$), and the interaction between sex and treatment ($F_{1,27} = 0.544$, $P = 0.544$) did not affect pre- and post-pen pig weight (Supporting Information Fig. S1).

Linear regression results indicated two models best explained the number of days until mortality (Supporting Information Table S1). The top model included the interaction of sex and the amount of warfarin consumed ($t_{16} = -3.32$, $P < 0.01$), and suggested the more warfarin males consumed, the sooner they reached mortality, while the opposite was observed for females (Fig. S2). The second model included starting weight ($t_{17} = -2.22$, $P = 0.04$) and amount of warfarin consumed ($t_{17} = 2.01$, $P = 0.06$), although the amount of warfarin consumed was not significant in this model. The starting weight of pigs had a significant effect on days until mortality, with heavier pigs not surviving as long (Fig. S3).

3.1.1 Behavioral observations

Full-term treatment pigs had the highest proportion of symptoms associated with warfarin toxicity, followed by 72-h treatment pigs,

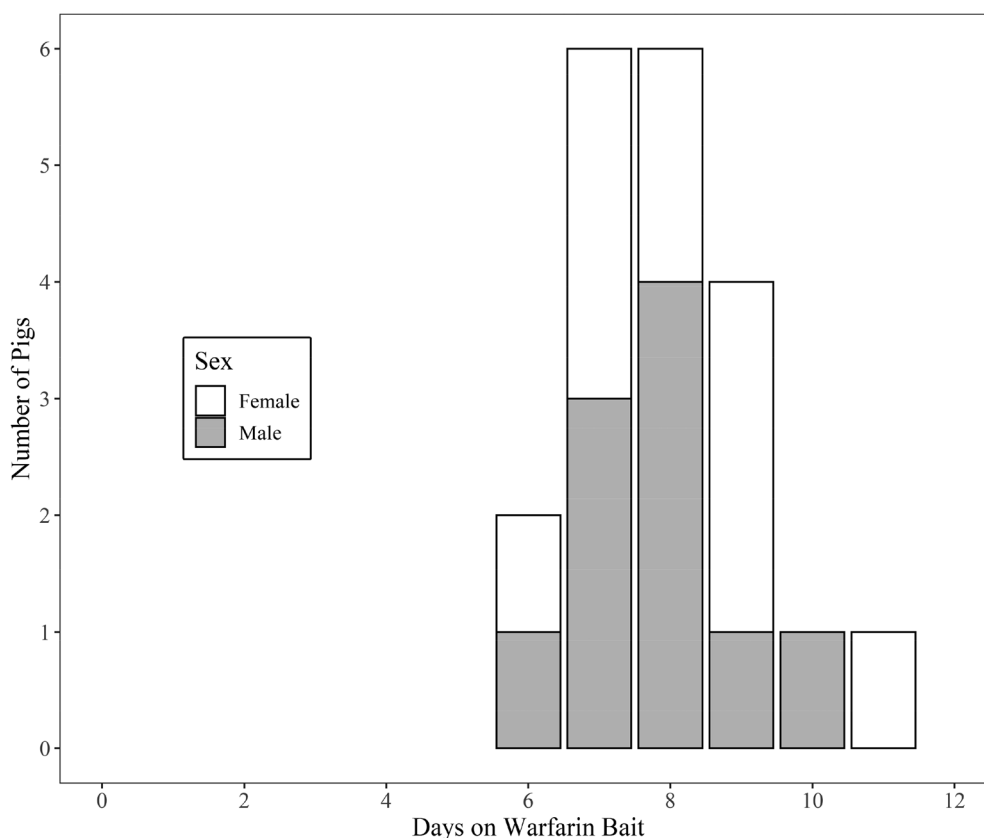


Figure 3. Number of days it took wild pigs (*Sus scrofa*) to reach mortality after consuming the first chronic LD₅₀ dose of low-dose warfarin baits in trials of captive wild pigs during November 2018 through January 2019.

24-h treatment pigs, and control pigs, respectively (Fig. 4). Vomiting was the most frequent symptom observed in full-term pigs (40%) followed by abnormal breathing and limping (35%), and then bleeding from an orifice and incoordination (30%). We also recorded bouts of major lethargy. However, 97.5% of all pigs exhibited major lethargy, likely due to fact that wild pigs were captured and brought to the pens for holding and observation. Use of surveillance cameras showed there was a period of duration of high distress (i.e. pigs falling over and paddling or exhibiting other similar behavior) for full-term treatment pigs preceding mortality that lasted 12–318 min (Fig. S4), although the duration of high distress was < 60 min for 65% of pigs and < 90 min for 80% of pigs. We were unable to verify whether pigs were conscious during this time.

3.1.2 Postmortem examinations

Necropsies revealed blue dye was present in all pigs that consumed warfarin baits (Fig. 5). Many treatment pigs exhibited hemorrhaging in organs and muscles, free blood in organs, foam in the trachea, and blood and swelling in joints. Full-term treatment pigs exhibited the highest frequency of symptoms, with the most prevalent being free blood in organs (90%), blood in lungs (85%), hemorrhaging in legs (65%), hemorrhaging in gastrointestinal tract (60%), and hemorrhaging in abdomen (55%). Furthermore, 72-h treatment pigs displayed the next highest frequency of symptoms, followed by 24-h treatment pigs. We observed two similar symptoms in control pigs that were not related to warfarin exposure, including: hemorrhaging in kidney

(10%) and blood in lungs (40%). The latter likely was resultant from euthanasia (Fig. 4).

We found 50% of full-term pigs had free blood from internal hemorrhaging in the abdominal cavity and the large intestine followed by 40% with free blood in the small intestine, 20% in the thorax, and 5% in the urinary bladder. Twenty percent of 72-h treatment pigs had free blood in the abdominal cavity, and 17% of 24-h treatment pigs had blood in the small intestine. No control pigs had free blood in any organs (Fig. S5).

3.1.3 Bait/tissue evaluation

We found no differences in mean concentrations of residual warfarin amongst treatment groups within each sample type, with the exception that control animals had significantly lower concentrations (Fig. 6). Excluding control animals, muscle samples averaged $1.0 \pm 0.08 \text{ mg kg}^{-1}$ of warfarin, livers averaged $3.87 \pm 0.21 \text{ mg kg}^{-1}$, small intestines averaged $2.78 \pm 0.20 \text{ mg kg}^{-1}$, and feces averaged $2.90 \pm 0.29 \text{ mg kg}^{-1}$.

3.2 Field study

We captured 94 wild pigs (28 GPS, 64 VHF, two ear tag only; 50 female, 44 male), 43 raccoons (32 male, 11 female), 18 opossums (12 male, 6 female), 28 cotton mice, and eight cotton rats for this study. During the first pre-baiting period, there was an average of 16.84 pig visits per day per active bait station, which decreased to 4.67 pig visits during the first deployment of warfarin baits. The average number of accesses per day per active bait station also decreased 99.5% from 1.89 during the pre-bait period when doors were open to 0.01 during the first bait station

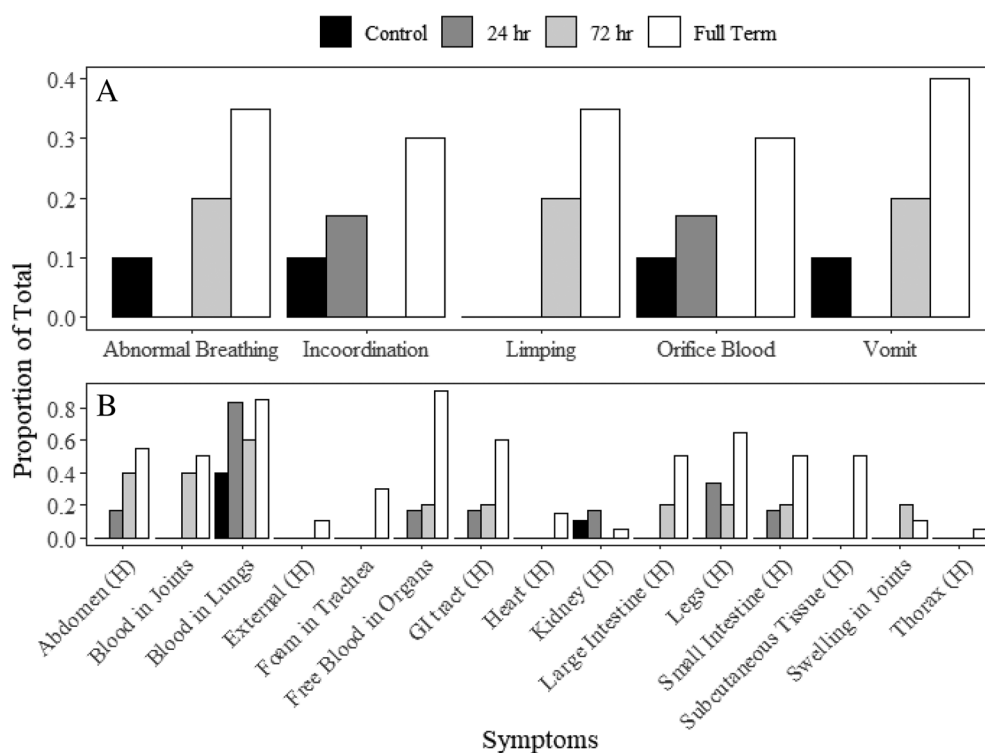


Figure 4. Frequency of (A) behavioral symptoms based on live observations and (B) hemorrhages (H) and free blood in organs based on necropsy examination of captive wild pigs (*Sus scrofa*) by treatment type. Full-term treatment pigs were fed low-dose warfarin baits for 10 days or until mortality, while 72- and 24-h treatment pigs were fed low-dose warfarin baits for 72 and 24 h, respectively and then euthanized. Control pigs were fed a mixed feed that contained corn, oats, molasses, and soybean oil. Trials were conducted during November 2018 through January 2019 in South Carolina, USA.



Figure 5. Photographs depicting the incorporation of Keystone blue dye into the subcutaneous fat of wild pigs (*Sus scrofa*) that consumed low-dose warfarin baits.

activation period with warfarin bait. During the second pre-baiting period, the number of visits per day per active bait station (excluding all stations where lure was deployed and stations eliminated due to lack of wild pig presence) was 9.02 with doors fully open, 35.65 with doors open 2.5–5 cm, 23.01 with doors closed and activated with corn bait, and then decreased to 1.16 visits

per day per active bait station during deployment of warfarin baits. Average number of accesses per day per active bait station during the second pre-bait period with doors fully open was 3.03, 3.04 with doors open 2.5–5 cm, and declined to 2.38 with doors closed, and further declined to 0.01 accesses per day when bait stations were activated and contained warfarin bait (Table 1).

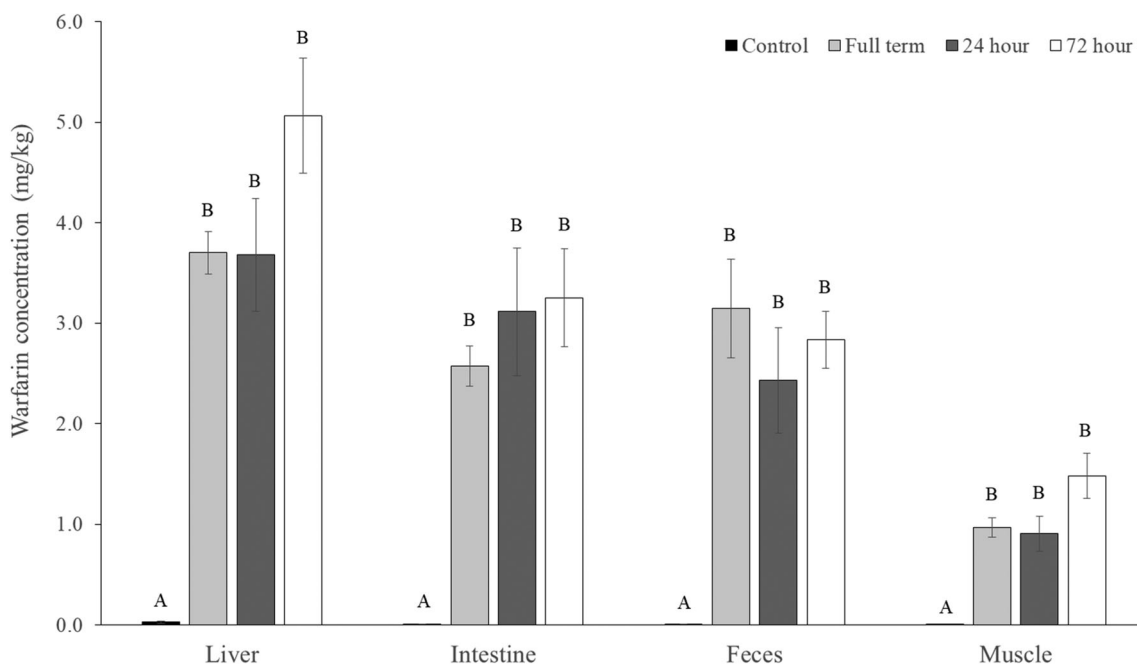


Figure 6. Mean concentrations (and standard errors) of residual warfarin from samples of captive wild pigs (*Sus scrofa*) fed low-dose warfarin bait containing 0.005% (50 mg kg⁻¹) warfarin in a one-choice test during November 2018 through January 2019. Means with the same letters are not significantly different at the level of $P = 0.05$ within each sample type, respectively.

Table 1. Summary statistics (mean \pm standard deviation) of instances where wild pigs (*Sus scrofa*) visited and accessed bait stations during multiple pre-baiting (corn) phases and deployment of low-dose warfarin bait on the Savannah River Site from May to September 2019

Trial stage	Average number of pig visits per day per feeder	Average number of pigs per visit	Average number of accesses per day per feeder
Pre-bait period 1 (doors open – pin height)	16.84 \pm 4.51	1.43 \pm 0.98	1.89 \pm 1.11
Warfarin deployment 1 (bait stations activated)	4.67 \pm 4.88	1.57 \pm 1.42	0.01 \pm 0.02
Pre-bait period 2a (doors open fully)	9.02 \pm 3.82	1.61 \pm 1.23	3.03 \pm 1.32
Pre-bait period 2b (doors open – pin height)	13.20 \pm 6.40	2.14 \pm 1.68	3.99 \pm 1.43
Pre-bait period 2c (doors open – inches)	35.75 \pm 14.99	2.82 \pm 1.90	3.04 \pm 1.19
Pre-bait period 2d (doors closed)	23.01 \pm 9.72	2.47 \pm 1.78	2.38 \pm 1.10
Warfarin deployment 2 (bait stations activated)	1.16 \pm 0.77	2.61 \pm 1.61	0.01 \pm 0.08

Visits are defined from time-lapse photographs taken at 5-min intervals as the number of photographs of wild pigs at bait stations, whereas accesses are defined as instances where pigs had their heads in the bait station, were raising doors, or were actively engaging with the bait station door.

Consumption of warfarin baits by wild pigs accessing bait stations was negligible, and thus no pigs expired from consumption of warfarin baits throughout either field deployment of toxic baits. In addition, no non-target species accessed bait stations during any periods when doors were fully closed and activated. However, squirrels, other rodents, opossums, raccoons, deer (*Odocoileus virginianus*), wild turkeys (*Meleagris gallopavo*) and other birds were detected consuming corn or lure from bait stations during pre-baiting. Raccoons were the most active non-target to visit bait stations (Table 2).

4 DISCUSSION

We evaluated low-dose warfarin baits for controlling invasive wild pigs, as evidenced through captive dosing experiments and field trials assessing access to pig-specific bait stations and mortality rates by wild pigs and non-target species. Our results suggest wild

pigs are susceptible to low-dose warfarin when consumed in large amounts, as we observed 100% mortality among captive wild pigs fed an acute LD₅₀ dose of 0.005% warfarin baits in no-choice feeding trials, consistent with Poché *et al.*²³ Further, despite the lower dose, time to mortality and symptoms of warfarin toxicity also were consistent with the literature for wild pigs and other mammalian species,^{31–33} and all wild pigs that consumed toxic baits had visible blue coloration of abdominal fat. This dye was visible in all individuals examined ~24 h after dosing, suggesting individuals exposed to warfarin baits can easily be distinguished by hunters in the field, even shortly after bait consumption.

Results from field aspects of the study were not as promising. When deployed under field conditions using pig-specific bait stations provided by the bait manufacturer and following manufacturer instruction, wild pigs required extensive acclimatization to bait stations. Once acclimated, pigs exhibited a strong reluctance to consume toxic baits, resulting in no mortalities of wild pigs

Table 2. Average number of non-target species per day per active feeder (\pm standard deviation) detected visiting pig-specific bait stations throughout pre-baiting and activation stages using low-dose warfarin baits from May to September 2019 on the Savannah River Site, where visits are defined from time-lapse photographs collected at 5-min intervals

Species	Pre-bait period 1 (doors open – pin height)	Warfarin deployment 1 (stations activated)	Pre-bait period 2a (doors open fully)	Pre-bait period 2b (doors open – pin height)	Pre-bait period 2c (doors open – 2.5–5 cm)	Pre-bait period 2d (stations activated)	Warfarin deployment 2 (stations activated)
Raccoon (<i>Procyon lotor</i>)	17.02 \pm 5.85	2.90 \pm 1.72	20.71 \pm 7.22	9.27 \pm 11.06	3.42 \pm 1.44	1.01 \pm 1.21	0.19 \pm 0.52
Virginia opossum (<i>Didelphis virginiana</i>)	0.01 \pm 0.01	0.00 \pm 0.00	0.24 \pm 0.28	0.15 \pm 0.29	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.03
White-tailed deer (<i>Odocoileus virginianus</i>)	0.50 \pm 0.29	0.02 \pm 0.03	0.03 \pm 0.05	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Eastern wild turkey (<i>Meleagris gallopavo</i>)	0.35 \pm 0.22	0.32 \pm 0.31	0.04 \pm 0.04	0.05 \pm 0.04	0.25 \pm 0.30	0.39 \pm 0.45	0.00 \pm 0.00
Eastern gray squirrel (<i>Sciurus carolinensis</i>)	10.28 \pm 2.79	5.69 \pm 3.68	13.49 \pm 5.28	7.67 \pm 11.19	0.02 \pm 0.07	0.00 \pm 0.00	0.00 \pm 0.00
Fox squirrel (<i>Sciurus niger</i>)	1.79 \pm 1.01	1.36 \pm 1.06	0.61 \pm 0.37	0.56 \pm 0.30	0.75 \pm 0.48	0.23 \pm 0.38	0.00 \pm 0.00
Rodents (<i>Rattus</i> sp., <i>Mus</i> sp.)	0.66 \pm 0.33	0.48 \pm 0.30	0.89 \pm 0.40	0.70 \pm 0.61	1.01 \pm 0.69	0.62 \pm 0.60	0.58 \pm 1.83
Songbirds	3.99 \pm 1.38	2.43 \pm 1.59	5.25 \pm 2.03	8.01 \pm 5.46	9.86 \pm 3.56	9.32 \pm 4.08	0.93 \pm 2.01

across two separate field deployments of toxic baits. These results are inconsistent with reporting from a field trial of the same low-dose warfarin baits in Texas by Poché *et al.*,²² who reported 100% mortality among radio-collared wild pigs. The discrepancy may be due to differences in bait station design and geographic differences among studies, as feeders used by Poché *et al.*²² had lighter (4.5 kg) doors that pigs could flip open with their snouts. In contrast, HogStopper bait stations the manufacturer provided us had heavier (7.7 kg) guillotine doors that were difficult for wild pigs to operate (Fig. 1(B)). Similar to our results, upon activation of bait stations Duguay *et al.*³⁴ observed a substantial reduction (from eight to one bait station) in wild pigs accessing similar guillotine-style bait stations, even when filled with corn as opposed to toxic bait. Our results also may reflect the limited palatability of paraffin bait blocks compared to corn formulations of warfarin baits, as observed by Poché *et al.*²³ Thus, improvements to existing pig-specific bait delivery systems and bait palatability are needed prior to widespread implementation of low-dose warfarin baits to control wild pigs. Further, since wild pigs did not access bait stations and did not feed on toxic bait, we could not address two of our primary objectives; determining amounts of spillage left by wild pigs outside bait stations and quantifying effects of spilled bait on non-target species.

Warfarin is a latent toxicant that induces mortality through disruption of normal blood clotting mechanisms, resulting in extensive hemorrhaging throughout the body.³³ As a result, most animals are asymptomatic initially after consumption, and mortality typically occurs after several days.³⁵ Similar to other experimental trials for wild pigs,^{23,31,32} average time to mortality was approximately 8 days. However, unlike previous studies in wild pigs, time to mortality did not differ between males and females, potentially reflecting differences in sample size, dose concentrations, and experimental design among studies, although sex-related differences in warfarin toxicity are inconsistent within the broader literature. Interestingly, although the amount of warfarin consumed had no effect on time until mortality, we did observe an interactive effect with sex. This interaction revealed the more warfarin male pigs consumed, the faster they

succumbed to the toxicant, while the opposite pattern was observed for females. It is unclear whether this pattern was an artifact of sample size or physiological differences between male and female pigs. Thus, further research is needed to elucidate differences in warfarin sensitivity between male and female pigs.

Given the latent effects of warfarin exposure, we evaluated the onset of behavioral, external, and internal symptoms of anticoagulant toxicity to elucidate the timeline and severity of symptoms of exposed individuals. Most pigs were asymptomatic 1 to 3 days post-exposure, although onset of internal hemorrhaging was observed in a small number of individuals in as little as 24 h, and there was a noticeable increase in the presence of blood in the joints and abdominal hemorrhaging after 72 h. Nonetheless, the delayed onset of symptoms should facilitate consumption of toxic baits over multiple days, increasing the possibility individuals may consume a lethal dose. However, the proportion of pigs exhibiting symptoms of warfarin toxicity and the extent of symptoms increased markedly after 3 days post-exposure, as reported by O'Brien and Lukins.³² External symptoms were less common than internal pathology, although vomiting, external bleeding, abnormal breathing, incoordination, and limping each were observed in 30–40% of full treatment pigs. Among pigs fed toxic baits for the duration of the experiment that vomited ($n = 8$), 62.5% died within 24 h after vomiting, and 87.5% died within 48 h, suggesting vomiting may be a proximate cue of terminal warfarin toxicity in wild pigs.

Consistent with the findings of Hone and Kleba,³¹ postmortem examinations revealed extensive internal hemorrhaging, particularly within the digestive tract and legs. Several pigs were noticeably limping during pen trials, likely reflecting hemorrhaging and buildup of blood in leg joints. Extensive hemorrhaging also was observed in the lungs of nearly all treatment pigs; however, hemorrhaging in lungs was observed in some control, and most pigs culled 24 h after exposure (although to a much lower severity) that likely was induced during euthanasia, and thus we were unable to discern the extent of hemorrhaging due to warfarin toxicity. Although uncommon prior to 4 days post-initial exposure, free blood within organs was observed in nearly all (90%) pigs

fed toxic baits for the duration of trials, with the majority of free blood occurring in the large and small intestine and abdominal cavity. Internal bleeding within organs was often extensive, with up to 2.5 L of free blood collected from an individual during examination.

A critical component of the acceptance of toxicants for use in controlling wildlife is the ability to rapidly produce a state of unconsciousness without a prolonged period of distress.³⁶ Indeed, concerns over animal welfare prompted Australian officials to pursue alternative toxicants to warfarin for controlling wild pigs.¹⁷ Our results suggest wild pigs exposed to low-dose warfarin baits likely experience some level of chronic and increasing distress for several days prior to death. Despite the latent period until mortality and gradual onset of symptoms, the duration of obvious severe distress was < 1.5 h for the majority of pigs. Thus, the period of most severe distress appears to be relatively acute. Other toxicants used for controlling wild pigs have documented much quicker times-to-death, with mortality averaging 2 days for yellow phosphorous,³² 2–6 h for sodium monofluoroacetate,³⁷ and 2–3 h for sodium nitrite.^{17,38}

Captive wild pigs in this study were fed an acute LD₅₀ dose of low-dose warfarin daily up to the time of death. Therefore, concentrations of warfarin in wild pig tissues likely can be considered a worst-case scenario relative to carcasses from field deployment of low-dose warfarin baits. Concentrations of warfarin in pig tissues did not differ between individuals culled after 24 h, 72 h, or individuals fed toxic baits for the duration of the experiment, suggesting warfarin is rapidly assimilated within tissues and does not increase with prolonged exposure to toxic baits. Similar to Poché *et al.*,²³ concentrations of warfarin in muscle tissue were substantially lower than in liver and intestinal tissue. Coyotes (*Canis latrans*), turkey vultures (*Cathartes aura*), and black vultures (*Coragyps atratus*) are the predominant scavengers of wild pig carcasses in the south-eastern United States.³⁹ Although data for many wildlife species are unavailable, warfarin is considered of limited toxicity to domestic birds, suggesting pig carcasses may pose limited risk to vultures, although limited data are available for predatory and scavenging birds.³³ Based on an oral LD₅₀ established for dogs, a 15 kg coyote would have to consume 20 kg of liver or 75 kg of muscle daily for several days to achieve an oral LD₅₀ (5 and 50 mg kg⁻¹, respectively¹⁸). Thus, despite wild pig carcasses persisting on the landscape for several days,³⁹ warfarin residues in wild pig tissues likely pose minimal risk to vertebrate scavengers. However, adverse effects have been observed in predators/scavengers consuming carcasses that had consumed warfarin-based baits of higher concentrations (e.g. 0.025%).³⁵ The extent to which sub-lethal effects may occur from prolonged consumption of tissues containing low concentrations of warfarin is unknown though, and thus an important area of future research.³³

In addition to consumption of wild pig carcasses, during field deployments of toxicants wildlife may also be exposed through consumption of bait blocks or crumbs produced by wild pigs feeding at bait stations. Due to the lack of wild pig use of bait stations when baited with low-dose warfarin baits, we were unable to quantify potential impacts to non-target species, either through accessing spilled bait or consuming dead pigs that succumbed to the toxicant. Thus, further documentation of spillage rates and non-target consumption of spillage and or secondary consumption are needed to assess risks to non-target wildlife. We did observe cockroaches (*Blattodea* sp.), ants, and other insects maneuvering and/or carrying bait particles inside and outside feeders during toxic bait deployments. Further, our results do allow us to make some inferences regarding non-target species

that may be most vulnerable to use of low-dose warfarin bait for controlling wild pigs. We recorded raccoons, opossums, rodents, white-tailed deer, wild turkeys, armadillos (*Dasypus novemcinctus*), gray squirrels (*Sciurus carolinensis*), rabbits (*Sylvilagus floridanus*), and several other species visiting bait stations during pre-baiting, with > 50% of visits by raccoons, suggesting these species may be most vulnerable to exposure from any spillage from bait stations that is not consumed by wild pigs. However, our results suggest direct access to toxic baits by raccoons and other non-target species can be minimized through use of doors weighing at least 7.7 kg, although new bait station developments are needed prior to deployment in areas with bear (*Ursus*) populations.³⁴

In addition to spillage, wild pigs may disseminate warfarin throughout the landscape through defecation of undigested baits, as we observed warfarin residues in wild pig scat at levels (2.9 mg kg⁻¹) that approached those in liver. Given that wild boar defecate approximately four times per day⁴⁰ and individuals will likely feed on warfarin baits for several days, large eradication campaigns have the potential to expose rodents and other small mammals that forage for seeds and other foodstuffs in scat to elevated levels of warfarin,⁴¹ although a 25 g rodent would have to consume 8.6 g of wild pig scat for several days to achieve a LD₅₀ dose.¹⁸

Our results also suggest the current matrix formulation for low-dose warfarin baits (i.e. paraffin blocks) were of limited palatability among wild pigs in our study area, as across two baiting periods and several dozen bait stations no wild pigs consumed a lethal dose of bait, although Poché *et al.*²² reported high consumption and efficacy of low-dose warfarin bait blocks for free ranging wild pigs in Texas. Experimental field trials in our study were conducted during the summer, when wild pigs have abundant natural forage resources; however, this did not appear to be limiting use of bait stations by wild pigs. This was evidenced by extensive use of bait stations by wild pigs throughout this study during pre-baiting phases, and after the first deployment of warfarin baits were removed from bait stations wild pigs immediately (within 1–2 days) returned to bait stations and began feeding on corn. Collectively, our results suggest although warfarin-based baits are currently approved by the US EPA for use in the United States, additional improvements to bait delivery systems and bait palatability are needed, as well as additional research to quantify efficacy, cost, and non-target impacts of management programs implementing low-dose warfarin baits as a means for wild pig population control.

ACKNOWLEDGEMENTS

The authors thank the numerous field technicians who assisted with this research, as well as the US Forest Service for their assistance in the capture of wild pigs used in this study and Dr C. Ellis, DVM, for guidance and assistance in conducting necropsies. The authors appreciate the research foundation built, and warfarin bait and bait stations graciously provided by R. Poché and his staff at Genesis Laboratories and Scimetrics. Funding for this investigation was provided in part by the US Department of Agriculture Animal Plant Health Inspection Service as well as the US Department of Energy under Award No. DE-EM0004391 to the University of Georgia Research Foundation.

DISCLAIMER

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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Evaluation of a warfarin bait for controlling invasive wild pigs (*Sus scrofa*)

James C. Beasley, Lindsay M. Clontz, Allison Rakowski, Nathan Snow, Kurt C. VerCauteren

Supplementary Information

Table S1. Model selection (AIC; Akaike Information Criterion) results including the number of parameters (K) and model weight from linear regression models investigating the influence of sex, starting weight (weight), and amount of warfarin bait consumed (consumed) on the number of days until mortality in wild pigs (*Sus scrofa*).

Candidate model	K	AIC	ΔAIC	Weight
Sex + Consumed + Sex*Consumed	5	67.6	0.0	0.428
Weight + Consumed	4	69.5	1.9	0.165
Null	2	70.2	2.6	0.118
Weight	3	70.6	3.0	0.096
Consumed	3	71.4	3.8	0.063
Sex	3	72.7	5.1	0.034
Weight + Consumed + Weight * Consumed	5	72.7	5.1	0.033
Weight + Consumed + Sex	5	73.0	5.4	0.028
Sex + Weight	4	73.7	6.1	0.020
Sex + Consumed	4	74.5	6.9	0.014

Figure S1. The average difference in captive wild pig (*Sus scrofa*) weights pre- and post-consumption of low-dose warfarin baits during November 2018 through January 2019. Thick horizontal lines indicate medians, boxes indicate the interquartile range, and vertical lines indicate reasonable extremes (± 1.58 times the interquartile range/ \sqrt{n}). Full-term treatment pigs were fed low-dose warfarin baits for 10 days or until mortality, while control pigs were fed a mixed feed that contained corn, oats, molasses, and soybean oil.

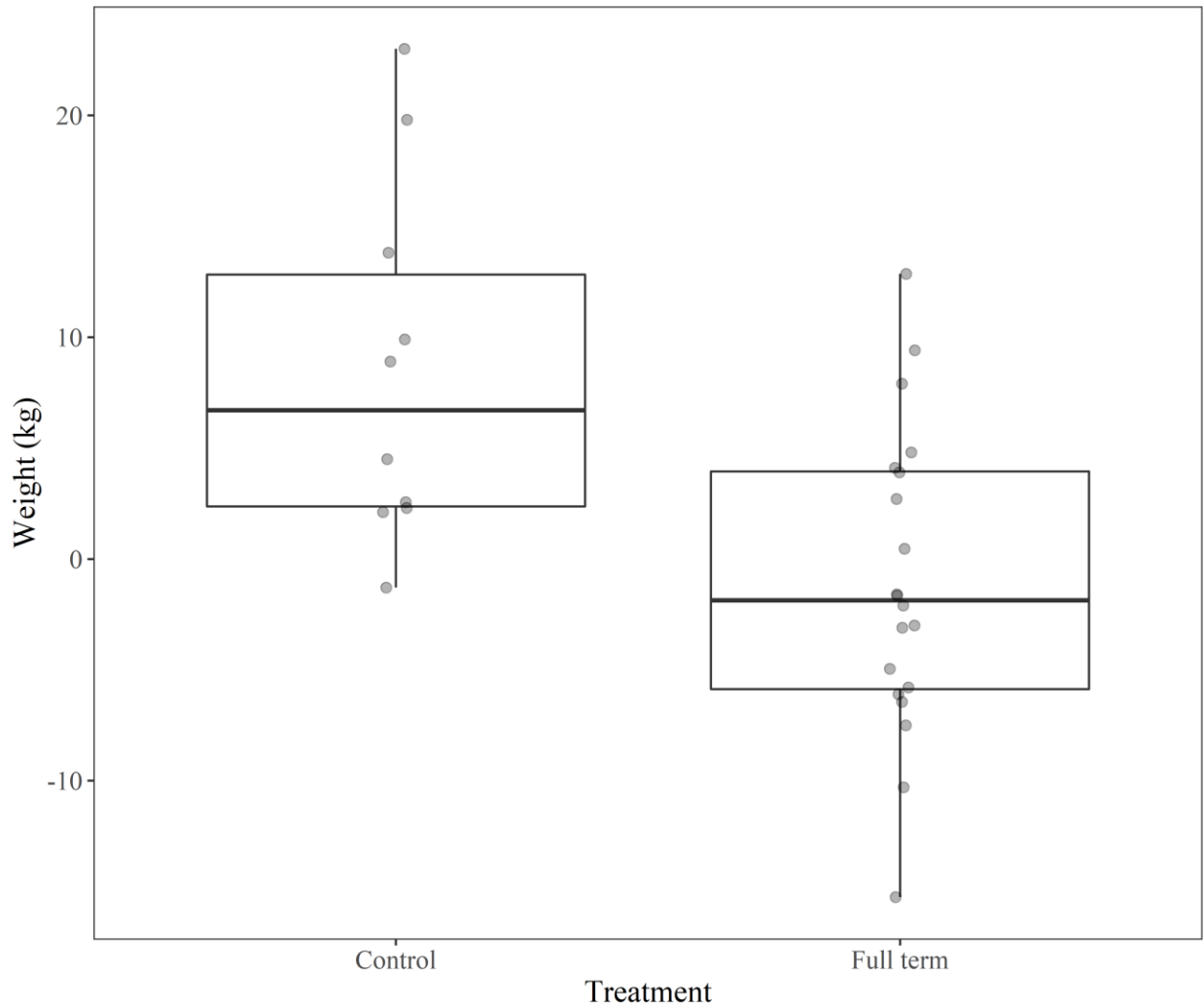


Figure S2. The number of days it took wild pigs (*Sus scrofa*) to reach mortality relative to the amount (kg) of low-dose warfarin bait consumed based on trials of captive wild pigs during from November 2018 through January 2019.

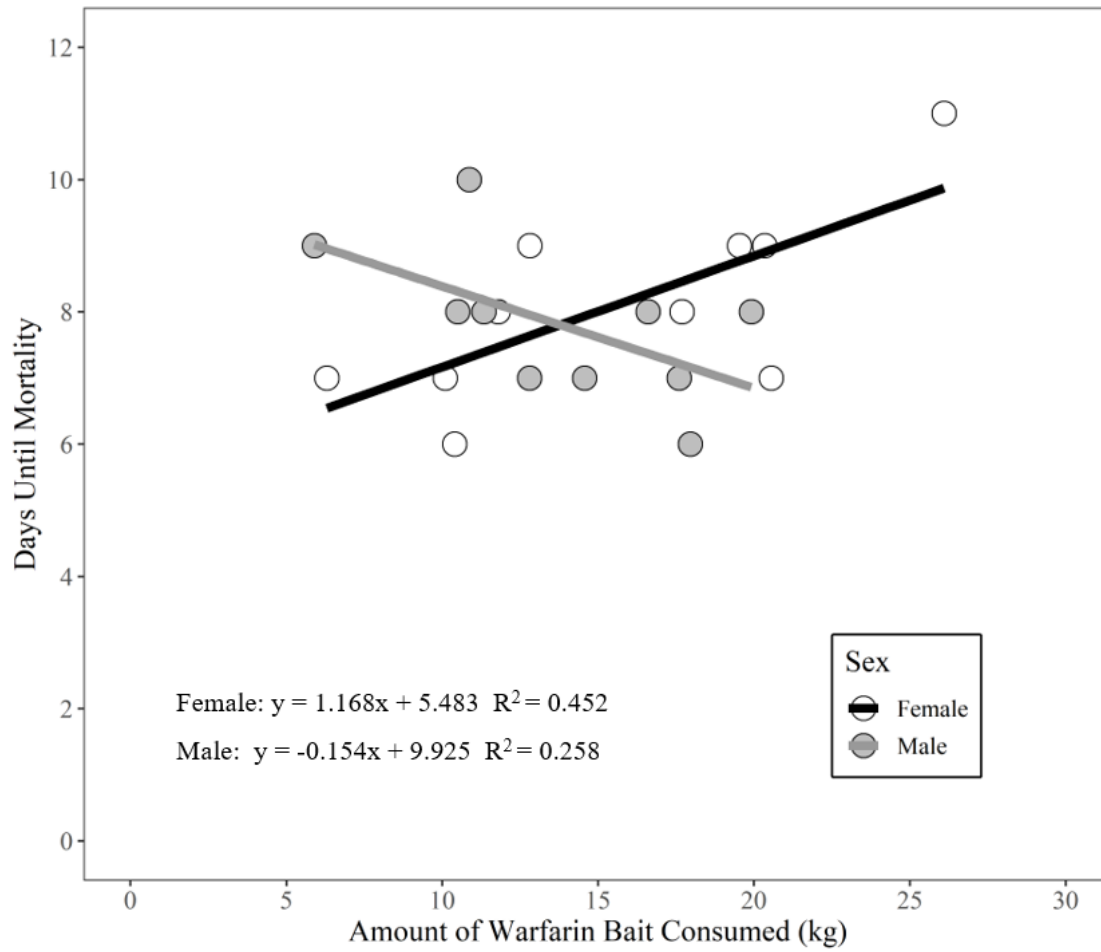


Figure S3. The number of days it took wild pigs (*Sus scrofa*) to reach mortality after consuming warfarin baits relative to their starting weight during a captive study from November 2018 through January 2019.

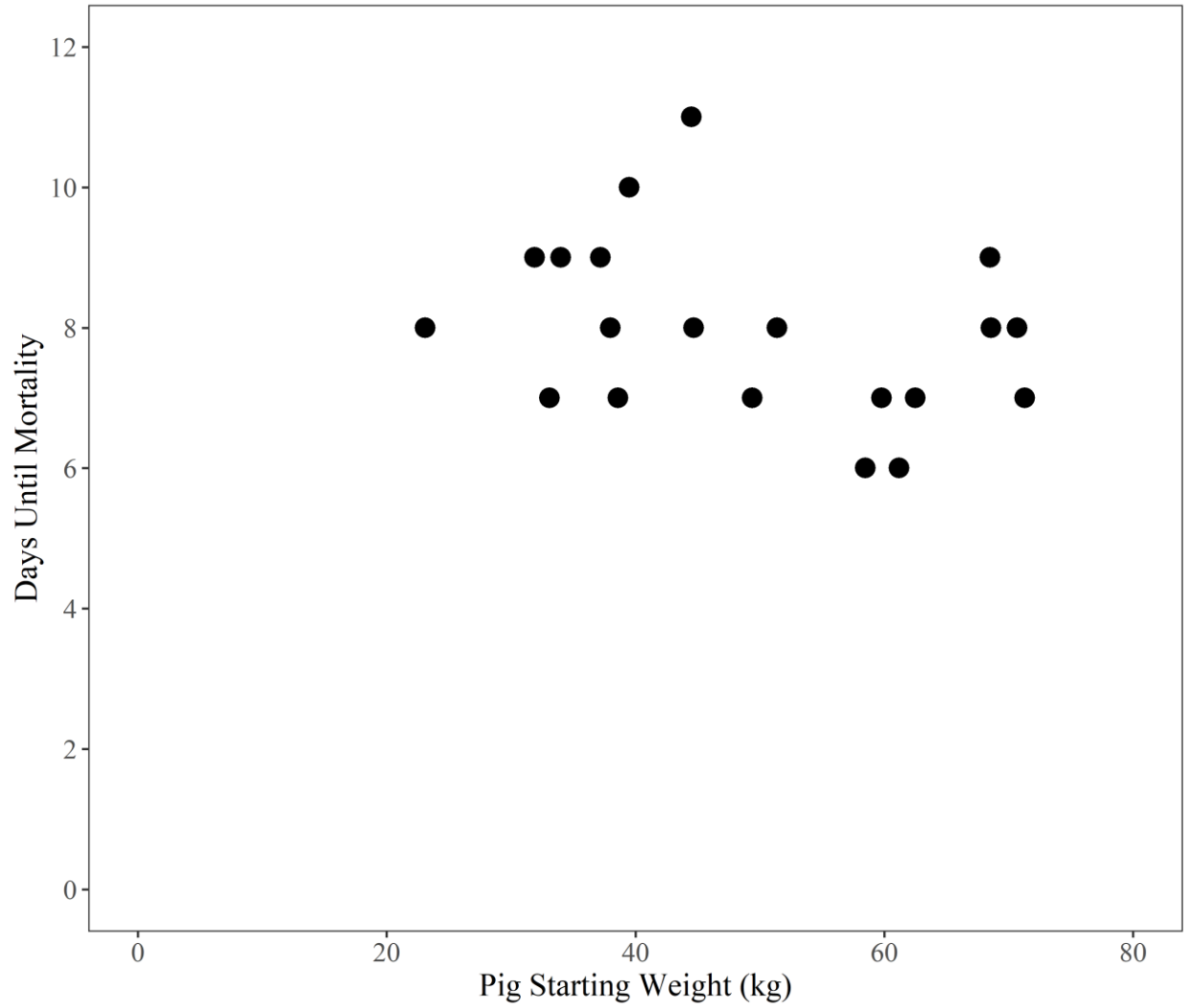


Figure S4. Duration period of perceived high distress (i.e. falling over and paddling or exhibiting other similar behavior) preceding mortality for 20 captive wild pigs (*Sus scrofa*) fed low-dose warfarin baits. We were unable to verify whether pigs were conscious during this time.

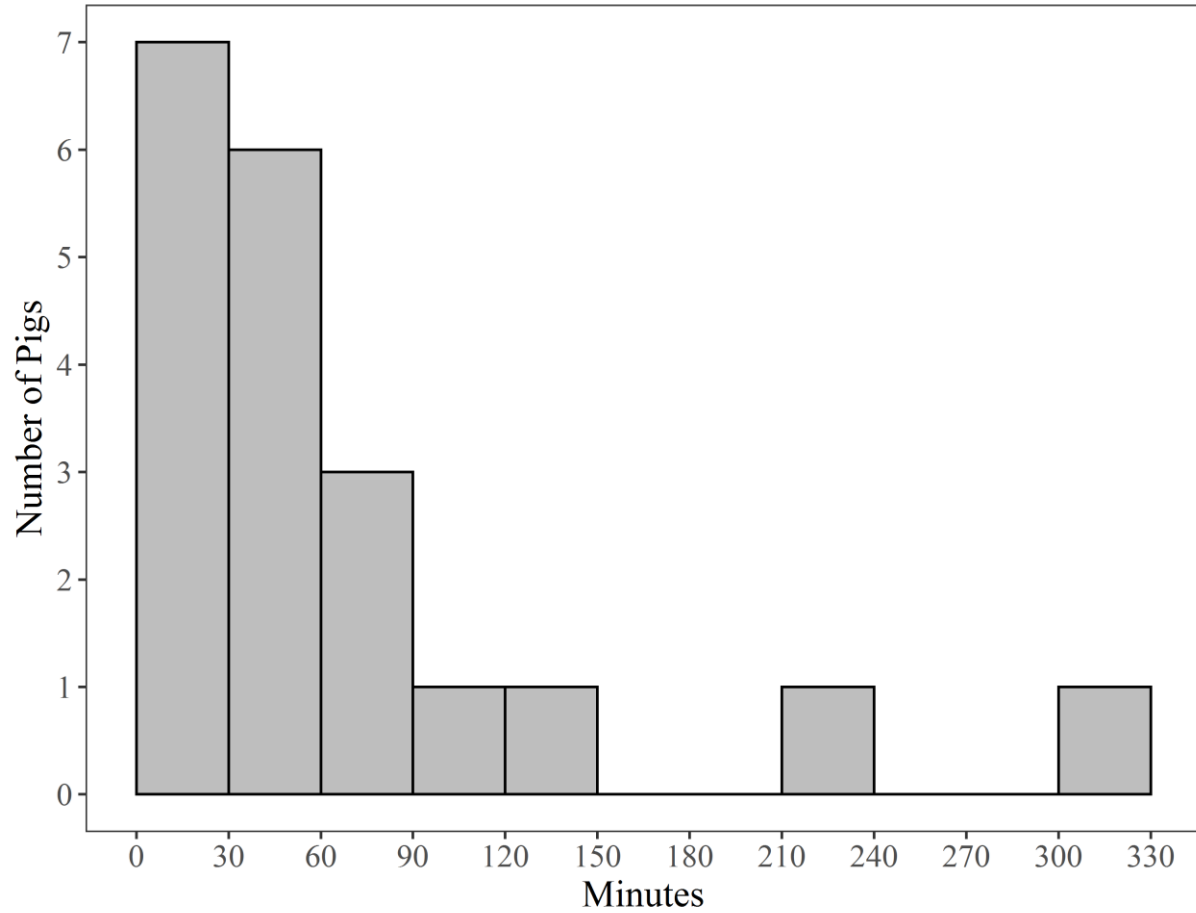


Figure S5. Distribution of free blood found in organs of wild pigs (*Sus scrofa*) relative to treatment type based on experimental trials of captive wild pigs fed low-dose warfarin baits during November 2018 through January 2019. Full-term treatment pigs were fed low-dose warfarin baits for 10 days or until mortality, while 72 and 24 hr. treatment pigs were fed warfarin baits for 72 and 24 hours, respectively and then euthanized. Control pigs were fed a mixed feed that contained corn, oats, molasses, and soybean oil.

