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Evaluating the Efficacy of an Alternative Warfarin Bait Formulation in Controlling Wild Pigs (*Sus scrofa*) in North Texas

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ABSTRACT: Wild pigs have one of the widest global distributions of any invasive species, and damage associated with them has been documented for centuries. Previously, a field trial was performed in 2015 to evaluate the use of paraffin bait formulations containing warfarin to control wild pigs. While the results of this study were satisfactory, prior research suggests that a cracked combased alternative could successfully deliver warfarin to wild pigs and the authors indicated caveats associated with their study that should be addressed. For the current study, a field trial was conducted (2017), as a continuation of the prior research, to evaluate the use of a 0.005% warfarin cracked corn bait in reducing feral hogs, utilizing an alternative feeder type. Results indicated warfarin bait could reduce wild pig consumption and presence at feeders by 92-96.4%. The results also indicated that the feeder use during the current trial was superior in keeping non-targets from accessing the bait, yet potentially more difficult for wild pigs to utilize than previously used commercial feeders. Results further indicated that wild pig presence at feeders was noticeably greater during the current field trial. The availability of an alternative warfarin bait formulation could provide an additional tool to managers to control wild pigs in North Texas and the remainder of ever-expanding wild pig habitat in the U.S and potentially globally.

KEY WORDS: agricultural damage, anticoagulant, feeder conditioning, field trial, invasive species, pest control, *Sus scrofa*, toxicant bait, warfarin, wild pigs

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INTRODUCTION

Wild pigs (Sus scrofa) have one of the widest global distributions of any invasive species (Jones et al. 2009) and are one of the most destructive species within the U.S. (Vitousek et al. 1996). Wild pig damage has been reported for centuries (Mayer and Brisbin 2009) and their numbers in North America continue to expand (Bieber and Ruf 2005, Sales et al. 2017). Wild pig populations in the United States are estimated at 6 million (USDA 2018) and are confirmed in at least 44 U.S. States (Fogarty 2007). Toxic baits are considered an option for reducing wild pig numbers, with advantages including cost-effectiveness and potential for largescale delivery and fast-acting results (Massei et al. 2011). Pesticides such as compound 1080 (Cruz et al. 2005), warfarin (McIlroy et al. 1989, Choquenot et al. 1990), and sodium nitrite (Cowled et al. 2008, Snow et al. 2017) have all been evaluated in Australia, which has previously been estimated to have 13-23 million invasive wild pigs (Spencer and Hampton 2005). Compound 1080 is approved for use in Australia but is restricted to toxic collars used for covote control in the U.S and has been banned for use against rodents since 1989 (USEPA 1995).

The only toxicant federally approved for use in wild pigs by the U.S Environmental Protection Agency (EPA) is a bait containing 0.005% warfarin (Kaput[®] Feral Hog Bait). Warfarin has proven highly toxic to pigs (McIlroy et al. 1989, Saunders et al. 1990, Poché et al. 2018a, 2019a) with acute and chronic oral LD_{50} estimated to be 3 mg/kg and 1 mg/kg, respectively, which is substantially lower than that needed to control other vertebrate pests such as rats and mice (Eason and Ogilvie 2009, McLeod and Saunders 2013). Pen testing has indicated the potential of baits formulated at warfarin concentrations ranging from 0.025-0.005% to eliminate 100% of wild pigs (Poché et al. 2019a). A field trial performed in north Texas in 2015 (Poché et al. 2018a), with paraffin block warfarin formulations, indicated 100% removal of radio-collared pigs exposed to 0.005% warfarin and a 97.8% reduction in consumption at feeders, with no non-target fatalities being observed. The findings resulted in USEPA registration of Kaput[®] Feral Hog Bait (Reg No 72500-26) in 2017. The product is formulated with 0.005% warfarin, 80% less than the concentration recommended by the USEPA for use in commercial rodenticide baits (USEPA 1991, Poché and Poché 2012).

While the results of the above field study were satisfactory, pen studies conducted in north Texas suggested wild pigs showed a significant preference for loose corn relative to paraffin-based baits at a ratio of roughly 6:1 (Poché et al 2019a). Additional benefits of a loose corn bait formulation would be that it would be a simpler and less costly manufacturing process. However, the efficacy and environmental safety of a 0.005% warfarin cracked corn bait have not been previously evaluated under field conditions.

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[†]Contributed to this publication in his personal capacity; the views and opinions expressed are his own and do not necessarily represent the views of the National Wildlife Research Center, the USDA, or the U.S. Government.

OBJECTIVES

To evaluate the efficacy of 0.005% warfarin loose corn bait (warfarin corn bait), a field trial was conducted in 2017. The objectives of this research were to determine 1) the deliverability and efficacy of a warfarin corn bait in controlling wild pigs, utilizing a more explicit procedure than during Poché et al. (2018a) for measuring efficacy, under field conditions; and 2) how a wild pig feeder might promote feeding by wild pigs and reduce risk to non-target wildlife. A USEPA Experimental Use Permit (72500-EUP-3) was obtained prior to study initiation.

MATERIALS AND METHODS Study Area

This study was conducted in the north Texas Panhandle in Briscoe, Flovd, and Motlev counties (34°17'28" N latitude, -100°59'26" W longitude), approximately 172 km northeast of Lubbock, TX. Local weather data over the course of the study was obtained from the National Oceanic and Atmospheric Administration (https://w2 .weather.gov/climate/xmacis.php?wfo=lub). Vegetation included a mixture of rotational agriculture and pastureland, as well as uncultivated areas of wet and dry riverbeds, mesquite shrublands, and grassland. The study was conducted on private property and landowner permission was obtained prior to study initiation. One treatment plot and one control plot were selected. Each test plot (Treatment and Control) was approximately 5 km² in size and was selected based on evidence of hog presence (Twigg et al. 2005). Ten (10) feeder clusters (each containing three feeders) were selected within each test plot (n=30 feeders /plot). Plot perimeters were ~10 km apart to prevent overlap.

Wild Pig Warfarin Corn Bait

The bait matrix was developed during a previous pen study which indicated that it was more palatable to wild pigs than paraffin bait (Poché et al. 2019a). As with the paraffin bait (Poché et al. 2018a), a fat-soluble blue dye was incorporated into the warfarin corn bait. The nominal concentration of warfarin in the bait was 0.005% (50 ppm). The concentration was confirmed using a validated High-Performance Liquid Chromatography method. The mean concentration of warfarin (±SD) was 53.3 ± 1.8 ppm (106% recovery; 3.4% coefficient of variation).

Study Periods

Conditioning

Feeder doors were secured open and commercial corn was used to condition pigs to eat from feeders. Wild pigs were conditioned to feeders within the test areas for 22 days.

Pre-treatment

Feeder doors were lowered at initiation of pretreatment. Placebo corn (placebo) was applied to feeders within each plot (25 days) to 1) continue conditioning wild pigs to use feeders; and 2) establish a baseline for feed consumption as defined by Poché et al. (2018a).

Treatment

Warfarin corn bait replaced placebo in feeders within the treatment plot (35 days). Feeders within the control plots continued to be provided placebo.

Post-treatment

Warfarin corn bait in feeders within the treatment plot was replaced with placebo (14 days). Feed consumption after the baiting period was compared with consumption during the baseline to indicate bait efficacy against wild pigs.

Feeders and Bait Presentation

Warfarin corn bait and placebo were presented in a total of 10 feeder clusters over the course of the study. Ten (10) feeder clusters were positioned within each plot in areas with evidence of wild pig activity. Each feeder cluster consisted of three wild pig feeders separated by approximately 0.3 m with each feeder being presented \sim 22 kg warfarin corn bait or placebo. Feeders were either wired to T-posts or trees or anchored directly to the ground with T-posts. As was conducted by Poché et al. (2018a), feeder clusters were positioned in areas with evidence of wild pig activity such as rooting damage. To further mitigate access by nontarget wildlife such as raccoons, a different feeder type was used during the current field trial. Cubical feeders (Hog Stopper[™], Scimetrics Ltd. Corp., Wellington, CO) were utilized and equipped with guillotine-style ~7.7 kg doors, spanning the length of front and back side of the feeder. Feeders were checked at 1 to 5-day intervals and refilled ad libitum. Prior to refill, the cluster area was scanned for warfarin corn bait spilled by wild pigs. Any spilled bait was immediately collected, weighed to the nearest 0.1 g, and removed from the field. The amount of warfarin corn bait applied to each feeder at each interval was weighed to the nearest 0.1 kg. At the end of each study period, all remaining feed from each feeder was removed and weighed (nearest 0.1 kg) to measure consumption by wild pigs.

Trapping, Handling, and Radiotelemetry

Wild pigs were trapped and fitted with radio tracking devices, with mortality sensors, to monitor movement and mortality using methods described by Poché et al. (2018a). Pigs estimated to weigh \geq 55 kg were chemically immobilized (Telazol + xylazine: 1.0 cc/18 kg) and fitted with GPS Collars (Lotek Wireless, Inc., Newmarket, ON, Canada). To ensure the GPS collars were not removed, pigs were fixed with a fabricated harness attached to the GPS collar. All trapped animals were safely released at the trap site when fully alert after chemical immobilization. Pigs <55 kg were fitted with VHF ear tags (Advanced Telemetry Systems, Isanti, MN). When a mortality signal was detected, the transmitter was tracked to the location, and it was determined if mortality occurred or if the transmitter detached from the pigs.

Trail Cameras

Motion-sensitive trail cameras (Primos Hunting, Flora, MS), were positioned at each feeder cluster using methods described by Poché et al. (2018a) to record visitations by wild pigs and non-target species. Wild pig and non-target wildlife presence and behavior at feeder clusters were examined within all study plots to confirm, 1) animal presence at feeders and 2) the ability of wild pigs and non-targets to open the feeder doors to access feed. Wild pigs and non-target wildlife visiting feeder clusters were moni-

tored daily during the pre-treatment, treatment, and posttreatment periods through observations made using motion-sensitive cameras. Observations are defined as a 30-minute interval during which wild pigs or non-target species are observed on camera (e.g. 08:00-08:29, 08:30-08:59, etc.). A visitation was defined as each individual wild pig or non-target animal observed on camera. More specifically, the number of visitations were estimated by calculating the maximum number of individuals per species observed on camera within each observation. For each observation, the date and time of first appearance, species, individual numbers, and behavior (approaching, opening feeder doors) were recorded.

Carcass Search for Target and Non-Target Species

Within, and outside of the periphery of all plots, carcass searches were conducted in all habitat types using a systematic grid design described by (Snow et al. 2017). Searchers were separated by ~10 m and walked in parallel lines across the area to look for target and non-target carcasses. Open areas were carefully scanned for carcasses using binoculars and were additionally accessed using 4wheel drive vehicles. All animals recovered within plots were necropsied for signs of warfarin corn bait consumption (e.g. blue dye, hemorrhaging organs). Liver samples (~50 g) were retained for warfarin residue analysis, because the liver is expected to contain the highest concentration of warfarin (Meehan 1984). Liver samples were analyzed for warfarin concentration using a validated HPLC method. Carcasses of animals experiencing nonwarfarin related mortality were excluded from analysis.

Data Analyses

We evaluated the efficacy of the bait in reducing 1) radio-tagged wild pigs, 2) consumption at feeder clusters, and 3) wild pig presence at feeder clusters by a minimum of 70% based on USEPA guidelines (Schneider 1982). Warfarin bait was considered efficacious if radio-tagged wild pigs were removed within the treatment plot and showed signs of bait consumption and toxicity. Wild pigs which died of non-warfarin related causes (e.g. hunting, roadkill), or lost their transmitters, were excluded from analysis (Poché et al. 2018a). Differences in the number of wild pigs remaining at study termination, relative to the number initially included in the analysis, were compared using a Pearson's x^2 test. Warfarin corn bait was considered efficacious if we observed a reduction in placebo consumption during post-treatment relative to pretreatment. The average daily consumption during pretreatment was compared with post-treatment within control and treatment. Warfarin corn bait was additionally considered efficacious if we observed a reduction in the number of wild pig visitations per day during posttreatment relative to pre-treatment. The effectiveness of warfarin corn bait in reducing 1) radio-tagged pigs, 2) consumption, and 3) feeder visitation was estimated using a standard equation which adjusts efficacy in response to changes within the control group (Henderson and Tilton 1955): *Efficacy (%)* = $\left[1 - \frac{(n C \text{ pre } * n \text{ T post})}{(n C \text{ post } * n \text{ T pre})}\right] \times 100$ where n= average daily consumption; C = control; T = treatment;

pre = pre-treatment; and post = post-treatment. Comparisons were additionally made between (treatment v. control) and within (pre-treatment v. post-treatment) study plots using a student's t-test (p < 0.05) or Wilcoxon Signed-Rank test (p < 0.05). Statistical analyses were performed using JMP Statistical Software (Cary, NC, U.S.A).

Ethical Guidelines for Use of Animals

All activities involving animals for this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and followed the Animal Welfare Act and Genesis Laboratories IACUC policies (protocol #s: 16001, 17001).

RESULTS

Warfarin Corn Bait Consumption

A total of 1,041.9 kg of warfarin corn bait was applied to feeders within the Treatment plot during treatment (Table 1). The application rate was estimated to be 10.4 g warfarin /km². Warfarin corn bait consumption totaled 453 kg. Approximately 3.8 kg of spillage was collected.

Trapping and Radio Telemetry

Forty-one (41) wild pigs were trapped and fitted with radio transmitters; 23 were within the treatment plot and 18 within the control plot (Table 2). Within the treatment plot, six transmitters detached from pigs and three pigs were shot by local hunters. Within the control, four transmitters were removed by pigs and one pig died from nonwarfarin related causes.

Target and Non-Target Visitation

Wild pigs were able to lift feeder doors and consume warfarin corn bait during the current study (Figure 1). During the treatment period, wild pigs were observed lifting feeder doors within the treatment plot during 276 of 552 observations (50%). Of the observations in which feeder doors were lifted, 258 observations were of groups of pigs (93.48%). The average number of wild pigs present during individual visitations was significantly greater when feeder doors were lifted, relative to when they were not lifted (ttest: p = 0.0001), indicating that wild pigs were more likely to lift the doors and feed from the feeders when in sounders as opposed to solitary pigs. While deer (Odocoileus sp.) were most frequently observed, raccoons (Procyon lotor) were the only non-target wildlife which attempted to lift feeder doors (Table 3). Though the raccoons were present in the area during the current study, the modified design of the feeder appeared to completely deter access by raccoons as also indicated by Poché et al. (2018b).

Carcass Searches for Wild Pigs and Non-Target Species

A total of 75 carcass searches were conducted. Fortyseven (47) pigs were recovered with 43 showing signs of bait consumption and warfarin toxicity. Samples from 35 wild pig livers were collected. The mean concentration of warfarin in liver was 1.41 ± 1.31 mg/kg. Of the four which did not show signs of toxicity, three were shot by hunters, and one was badly decomposed. No non-targets were found showing any signs of warfarin toxicity.

Table 1. Application rate and consumption of warfarin corn bait.

Applied			Consumed			Spillage	
Bait (kg)	Warfarin (g)	Warfarin (g/km²)	Bait (kg)	Warfarin (g)	Warfarin (g/km²)	Bait (g)	Warfarin (g)
1,041.9	52.1	10.4	453	22.7	4.5	3843.3	192.2

Table 2. Number of trapped wild pigs within each plot fitted with radio tracking devices.

	Study Plot			
Status of Tagged Pigs	Warfarin- Treated	Untreated Control		
Transmitters Attached	23	18		
Transmitter Removed	6	4		
Non-warfarin Fatalities	3	1		
Remaining Pigs for Analysis	14	13		

Table 3. Non-target daily visitation rate.

	Pre-Treatment		Treatment		Post-Treatment	
Wildlife Observed	Number of Visitations	Lifting Feeder Doors	Number of Visitations	Lifting Feeder Doors	Number of Visitations	Lifting Feeders
Raccoon (Procyon lotor)	4.96	0	2.29	0	1.21	0
Deer (Odocoileus sp.)	21.68	0	7.2	0	4.57	0
Wild Turkey (<i>Meleagris gallopavo</i>)	0.04	0	9.57	0	24.29	0
Bird Species	2.52	0	3.31	0	6.79	0
Mammal Species	3.44	0	3.63	0	4.71	0

Efficacy Against Wild Pigs

All wild pigs found dead with evidence of warfarin toxicity had internal hemorrhaging and blue-stained subcutaneous and abdominal fat as previously reported (Poché et al. 2018a, 2019a). Efficacy results are presented in Figure 2. Of 14 hogs fitted with transmitters within the Treatment plot, nine were found dead showing signs of warfarin toxicity (64.3% mortality). All 13 radio-tagged Control pigs survived. Differences in the number of live wild pigs remaining at test termination were significant between the plots (Pearson's: p = 0.0004).

Placebo consumption by wild pigs was reduced during post-treatment relative to pre-treatment (Figure 3). Average daily consumption within the Treatment plot was significantly reduced (Wilcoxon: p < 0.0189) from 52.9 kg (pre-treatment) to 6.8 kg (post-treatment) and did not differ statistically within the Control (Wilcoxon: p = 0.112). Consumption within the Treatment plot was reduced by 98%, relative to the Control.

Wild pig visitation within the Treatment plot was significantly reduced by 92%, relative to Control (t-test: p = 0.0011) (Figure 4). Daily average wild pig visitations decreased significantly from 173.7 (pre-treatment) to 33.9 (post-treatment). The average number of visitations within the control plot markedly increased during post-treatment (91.1/day) relative to pre-treatment (37.1/day). Visitation within the Treatment plot was significantly reduced during post-treatment, relative to pre-treatment (t-test: p < 0.0001).



Figure 1. Large wild pig sounder lifting feeder doors and feeding.

DISCUSSION

Wild pigs damage agricultural areas (Choquenot et al. 1996), outcompete and predate upon various animal species (Cruz et al. 2005, Rollins and Carrol 2001), and spread diseases transmissible to wildlife (Glass et al. 1994),

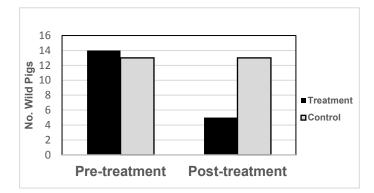


Figure 2. The efficacy of warfarin corn bait in reducing VHFtagged wild pigs. Mortality reached 63.4%.

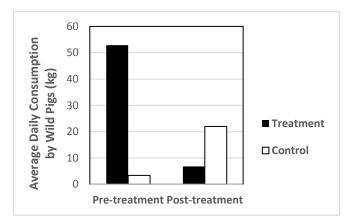


Figure 3. Average daily consumption during pre- and posttreatment within the Treatment and Control plots. Consumption within the Treatment plot was reduced 98%, relative to Control.

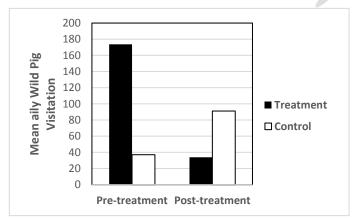


Figure 4. Average daily wild pig visitation during pre- and post-treatment within the Treatment and Control plots. Visitation was reduced 92% within the Treatment plot, relative to Control.

livestock (Bevins et al. 2014), and humans (Li et al. 2005). Toxicants present one of the more promising, ecologically appealing, potentially far-reaching mechanisms for curbing population growth and expansion. The results of the current study expand upon those of Poché et al. (2018a), suggesting an alternative warfarin bait formulation may aid in the control of wild pigs in north Texas.

During exposure, 453 kg of 0.005% warfarin corn bait was consumed, which is greater than three times the amount of bait consumed during a previous study using a paraffin block formulation (Poché et al. 2018a). This is supported by the results of Poché et al. (2019a) which indicated greater palatability of cracked corn relative to paraffin. Subsequently, 43 carcasses were recovered showing signs of bait consumption with only 17 being recovered by Poché et al. (2018a). Large sounders were often observed feeding from the feeders and fed far more successfully than smaller groups or individual wild pigs. The natural social grouping of wild pigs (Dardaillon 1988) would suggest the potential for all individuals within a sounder to be eliminated by warfarin bait if capable of accessing it. This suggests the potential for warfarin corn bait to reach considerable individuals in wild pig populations. However, the warfarin corn bait also has greater potential for spillage by pigs relative to paraffin bait. During the current study, >3.8kg of spilled bait was collected over the course of the treatment period, relative to 466.3 g collected by (Poché et al. 2018a). While no non-targets succumbing to warfarin were recovered during carcass searches, we cannot say with absolute certainty that no non-targets were affected. However, images of wild pigs visiting feeders indicate the vast majority of spillage was consumed by wild pigs during feeding. It's worth reiterating that the concentration of warfarin within the bait is $18-26 \times \text{less}$ than was previously used in wild pig control in Australia (McIlroy et al. 1989, Saunders et al. 1990). The total amount of warfarin in the spillage amounted to ~192.2 mg, which would be equal to 38.4 mg/km^2 within the treatment plot over the course of 35 days. In terms of risk of secondary toxicity, the average warfarin concentrations in livers collected (1.41 ± 1.31) mg/kg) were low, which is supported by Poché et al. (2018a). At the highest warfarin concentration obtained (4.48 mg/kg), a 10 kg dog would need to consume ~111.6 kg of liver to reach the estimated acute oral LD₅₀ (Meehan 1984). Prior studies have concluded reduced primary (Poché et al. 2019b) and secondary (Poché and Mach 2000) risk of warfarin bait at concentrations $5 \times$ and $10 \times$ higher than the current product.

While results suggested that application of the bait reduced consumption and visitation by the EPA-recommended >70% (Schneider 1982), we did not exceed this threshold with radio-tagged wild pigs. This is contrary to Poché et al. (2018a) in which 100% mortality was obtained for radio-tagged wild pigs within the 0.005% warfarintreated plot. In the current study, many transmitters were removed by pigs, and thus, the true number of warfarinkilled pigs was unknown. Also, some surviving wild pigs fled the area after being trapped and marked, and it is unknown if they consumed any warfarin corn bait. This work was confined to private property and therefore it was not possible to collect many surviving animals after test termination to determine whether they had consumed warfarin bait. Future studies involving this warfarin corn bait should consider advanced methods to retrieve these animals and confirm or disconfirm mortality or consumption of a sublethal dose. Although the 64.3% mortality did not satisfy the requirements of Schneider (1982), it did fall within the 50-60% population reduction estimated to be necessary to prevent wild pig populations from increasing

(Mayer and Brisbin 2009). The 43 warfarin-killed wild pigs collected suggest potential for warfarin corn bait to reduce abundance.

The ability of raccoons to lift feeder doors in Poché et al. (2018a) was eliminated by the modified feeder in the current study. These results suggest that this feeder might be best for minimizing non-target access. While the feeder prevented raccoons from lifting feeder doors, analyses suggested that it may have been slightly more difficult for wild pigs to lift as well. The conditioning period is useful in getting pigs to associate the devices with a food source, and many wild pigs that struggled to lift feeder doors were likely not exposed to them during conditioning. It appears that conditioning may be more of a necessity when dealing with the cubical feeders. Although the cubical feeder may have been slightly more difficult for pigs to use relative to the commercial feeders utilized by Poché et al. (2018a), large sounders and groups of pigs were routinely lifting the doors to feed and the results, including the 43 warfarinkilled pigs recovered, suggest that a sizable proportion of local wild pigs were removed.

CONCLUSION

This field trial was a continuation of the research performed by Poché et al. (2018a) which was the first field evaluation of a toxicant for use against wild pigs in the U.S. This study suggests the potential for use of an alternative more cost-effective warfarin bait formulation to control wild pigs. While the new formulation was readily consumed by pigs, it also resulted in a greater amount of spillage relative to the paraffin block formulation (Poché et al. 2018a), suggesting that managers should weigh the costbenefits of using either loose corn or paraffin formulations. This field trial demonstrated improvement of the feeder in restricting access by non-targets such as raccoons. The subcutaneous blue dye once again proved effective in marking wild pigs in the field. The potential availability of an alternative warfarin bait formulation may provide an additional tool to managers to control wild pigs in north Texas and the remainder of the ever-expanding wild pig habitat in the U.S and potentially globally.

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