Effects of Rhodamine B on Palatability of Invasive Wild Pig Baits

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ABSTRACT: Biomarkers have been used to quantify consumption of toxicants and other pharmaceutical baits by free ranging wildlife populations. Previous research has tested the efficacy and persistence of Rhodamine B (RB) as a biomarker in invasive wild pigs. However, little information is available about effects of RB on palatability of baits meant for invasive wild pigs, and studies have shown that the addition of RB to otherwise palatable baits reduces consumption by some species. HOGGONE® has been identified as an effective Sodium Nitrite-based oral toxicant for invasive wild pigs in trials conducted in captive pen trials. We simultaneously conducted five separate 2-choice tests to examine potential differences in consumption between HOGGONE® placebo paste (standard placebo) and HOGGONE® placebo paste containing 0.5% RB (RB placebo) in five groups of three invasive wild pigs. Each group was simultaneously presented with equal amounts of standard placebo and RB placebo paste for one night and monitored with remote cameras. Remaining bait was weighed and subtracted from the initial weight of both feed types to calculate consumption. There were no differences in the total amount of bait consumed or the time spent feeding between the two bait types across all five groups. Results of this study suggest that the addition of RB does not negatively impact consumption of HOGGONE® placebo paste by groups of invasive wild pigs. Thus, we provide more evidence that RB will be a useful tool for research on wild pigs, such as estimating proportions of free-ranging populations consuming baits that contain toxins or pharmaceuticals.

KEY WORDS: bait, biomarker, feral swine, palatability, Rhodamine B, *Sus scrofa*, vertebrate pest control, wild boar, wild pig

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INTRODUCTION

Invasive wild pigs (*Sus scrofa*) are one of the most destructive invasive species in the world (Lowe et al. 2000). Pimentel (2007) reported that each invasive wild pig has an associated annual cost of $300, which means a hypothetical invasive wild pig population of 5 million could cost $1.5 billion annually. Most of the economic damage done by invasive wild pigs is to the agricultural industry, however, the ecological impacts of this species are widespread and impossible to quantify (Pimentel et al. 2002, Seward et al. 2004). Populations of invasive wild pigs are projected to continue growing and expanding their ranges, which will lead to increased economic and ecological costs associated with damages (Timmons et al. 2012, Snow et al. 2017c).

Trapping, shooting, aerial gunning, snaring, and other lethal means of population control have been found to be effective in the temporary reduction of localized invasive wild pig populations across the world (Choquenot et al. 1993, Mayer and Brisbin 2009, Campbell et al. 2010). These methods, though, have not been effective at reducing the invasive wild pig population long term or at a broad scale (Dickson et al. 2001). Additional methods of lethal population control, to be used in conjunction with the methods above, are necessary for large-scale reduction of invasive wild pig populations and their associated damages (Beasley et al. 2018).

A multidisciplinary, international team of scientists is currently working to develop HOGGONE® (Animal Control Technologies Australia P/L, Somerton, Victoria, Australia), a sodium nitrite-based toxic bait for the lethal control of invasive wild pigs (Snow et al. 2017a). HOGGONE® placebo has been found to be highly palatable to invasive wild pigs in both pen and free range studies (Snow et al. 2016, Snow et al 2017b). The toxic bait has also shown to be highly lethal to groups of invasive wild pigs in a pen setting (Snow et al 2017b). The use of toxicants in the United States is strictly regulated by the United States Environmental Protection Agency (EPA) and at the time of this study, the efficacy of toxic HOGGONE® bait was yet to be tested in a free range setting in the US. Following completion of pen trials in 2016 and prior to initiation of free range toxic trials under an Experimental Use Permit from the EPA in 2018, a simulated free range toxic deployment was scheduled to be conducted using HOGGONE® placebo containing a biomarker (N. P. Snow, USDA-APHIS National Wildlife Research Center, unpubl. data). The addition of a biomarker to HOGGONE® placebo in a free range deployment could simulate consumption and associated mortality rates of both invasive wild pigs and non-target species during a toxic deployment, and provide

researchers valuable information prior to toxic field trials (Savarie et al 1992, Snow unpubl. data).

Rhodamine B (RB) is a fluorescent dye that has been used as a biomarker for wildlife species for decades (e.g., Evans and Griffith 1973, Lindsey 1983, Farry et al. 1998, Webb et al. 2000, Mascari and Foil 2009). The addition of RB to food items which were known to be palatable to particular species, such as coyotes (*Canis latrans*) and brush tailed possums (*Trichosurus vulpecula*), has been shown to reduce the palatability of those baits and in some cases resulted in rejection of baits entirely (Johns and Pan 1981, Morgan 1981). Recent studies have shown the efficacy of RB as a biomarker in invasive wild pig baits (Beasley et al. 2015, Webster et al. 2017), however, only two allowed for the conscious consumption of RB baits and they did not directly address palatability of RB when added to baits known to be palatable to invasive wild pigs (Fleming et al. 2000, Baruzzi et al. 2017).

Prior to implementing largescale field trials on RB bait acceptance by invasive wild pigs, it is necessary to evaluate affects RB may have on palatability of invasive wild pig baits. The overall goal of this study was to evaluate the impacts that adding RB to baits may have on the consumption of baits previously known to be palatable to invasive wild pigs. The direct objective was to test for a difference in consumption between standard HOGGONE® placebo (standard placebo) and HOGGONE® placebo containing 0.5% RB (RB placebo).

METHODS

Our study took place in April 2017 at the Texas Parks and Wildlife Department's (TPWD) Feral Swine Research Facility located on the Kerr Wildlife Management Area (KWMA), Hunt, TX. Private landowners and USDA /APHIS/Wildlife Services personnel trapped free-ranging invasive wild pigs throughout nearby counties and TPWD employees transported them to KWMA via cattle trailer for housing and testing. While at the research facility, invasive wild pigs were fed Bluebonnet® Sow Ration Pellets (AC Nutrition, LP, Ardmore, OK) at 3-5% of group body mass, daily. Water was provided *ad libitum* from self-maintaining water troughs. All invasive wild pigs were group-housed in a 2.02 ha holding pen with naturally-growing vegetation and were provided supplemental shade structures as well as a small pond for wallowing. All experimental methods were approved by the TPWD-KWMA Institutional Animal Care and Use Committee (protocol 211072020151).

Prior to the trial, invasive wild pigs were moved through a chute system into a handling facility and three individuals were randomly selected to be placed in one of five trial pens (N = 15). Random assignment to groups was conducted under the condition that each animal's weight was ≤50 kg due to staff safety while handling the animals. Upon selection, invasive wild pigs were moved into their respective pens for the trial (pens 23, 24, 25, QP 1, and QP 2). Invasive wild pigs were allowed one day to acclimate to their new environment and were provided water *ad libitum* as well as the same daily diet ration provided in the 2.02 ha holding pen.

Each trial pen was approximately 15 × 15 m and were setup following the methods outlined in Blass et al. (2016) with the exception that we placed four 58-l rubber feeding tubs (Marshalltown Company, Fayetteville, AR) in each pen rather than two. We placed two RECONYX PC800 remote cameras (RECONYX, Inc., Holmen, WI) above each feeding station with two tubs in the frame of each camera. We placed an additional camera at the back of each pen approximately 10 m from the feeding stations to observe group feeding behavior. Cameras were set to time-lapse mode and were scheduled to take a single photo every 15 seconds.

We conducted five simultaneous 2-choice tests to compare the consumption of standard placebo and RB placebo containing 0.5% Rb. Each bait was offered at 4% of group body weight calculated individually for each pen. Bait was evenly distributed across the four feeding tubs in an alternating pattern from left to right starting with RB bait (i.e. RB placebo, standard placebo, RB placebo, standard placebo). Baits were removed from each pen and weighed 15 hours after the start of the trial.

We calculated a conservative estimate of the RB concentration required in placebo HOGGONE to effectively mark the facial vibrissae of invasive wild pigs at which time the individual consumed a dose equivalent to the known LD_{99} of 400 mg/kg for toxic HOGGONE® (Cowled et al. 2008). Given average single night consumption of placebo by free ranging invasive wild pigs of 300 grams (Snow et al. 2016), and the minimum dose for long term marking with RB 15-30 mg/kg (Webster et al. 2017), we estimated that a 75 kg pig would be effectively marked by 300 g of HOGGONE® with a 0.5% RB concentration and could be assumed dead had toxic HOGGONE® been consumed.

We compared relative preferences between RB placebo and standard placebo using two metrics: 1) the amount of each bait consumed and 2) the amount of time spent at each bait. We compared total bait consumption between RB placebo and standard placebo by measuring bait remaining after the 15 hour trial. We analyzed these data to determine if there were statistically significant differences in consumption using a Gosset's-student's T-test. We compared time spent at each type of bait in each pen with camera data indexed using 15 second time lapse photos from remote cameras. These data were analyzed to determine if there were statistically significant differences in time spent at each bait using a Gosset's student's t-test. All statistics were performed in Microsoft Excel (Windows 2007-2010) and JMP (JMP®, Version 12. SAS Institute Inc., Cary, NC).

RESULTS

Statistical analyses suggest that there were no significant differences in consumption between RB placebo and standard placebo ($P = 0.90$, $DF = 8$). Of the 6.71 kg of total bait consumed in all pens, 3.51 kg was RB placebo and 3.20 kg was standard placebo (Figure 1).

Statistical analyses of camera data illustrate that total time spent at feed varied significantly among pens ($P = 0.03$, $DF = 8$), however there was no significant differences between time spent feeding at either bait type across all pens ($P = 0.89$, $DF = 8$) (Figure 2).

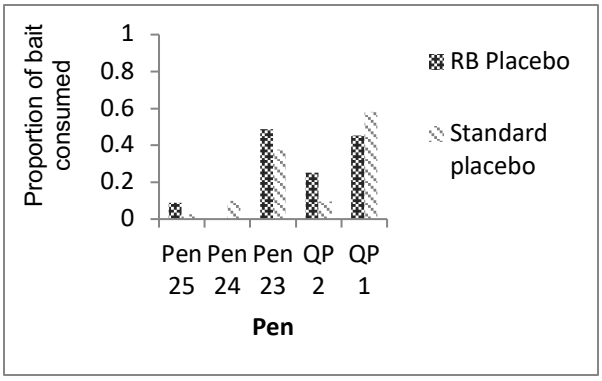

Figure 1. Proportion of RB placebo and standard placebo consumption across 5 pens of 3 captive invasive wild pigs (*Sus scrofa*) during bait preference trials in the Feral Swine Research Facility at the Kerr Wildlife Management Area, Hunt, Texas.

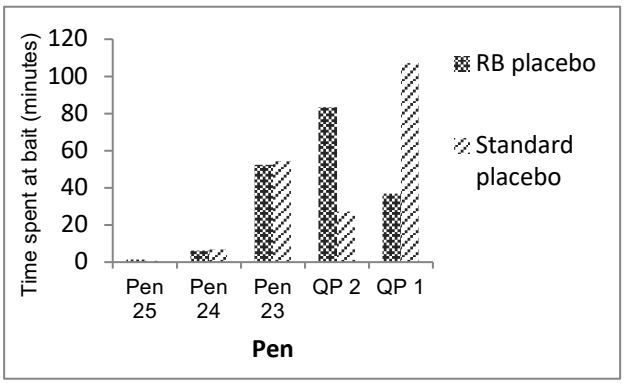

Figure 2. Total time spent at RB placebo and standard placebo by 5 groups of 3 invasive wild pigs (*Sus scrofa*) during bait preference trials in the Feral Swine Research Facility at the Kerr Wildlife Management Area, Hunt, TX.

DISCUSSION

Results of this study indicate that the addition of RB at 0.5% of total bait composition did not significantly affect consumption of HOGGONE® placebo. Proportions of baits consumed in this study were similar to consumption results reported by Blass et al. (2016). Though there was no significant statistical difference in consumption between RB and standard placebo, more RB placebo was consumed across all pens and feed tubs containing RB placebo were the first tubs visited in four out of five pens. Both standard HOGGONE® placebo and the feed tubs used in this trial were black. The color contrast between the reddish-purple RB placebo and the black feed tubs may have introduced bias in this study even though domestic swine are believed to be dichromatic and invasive wild pigs have been shown to only consistently distinguish blue from other colors (Neitz and Jacobs 1989, Eguchi et al. 1997).

Following the 15-hour trial, vomit of RB placebo was observed in two of the five pens. Vomit of RB placebo was identified by the color of each bolus. Each bolus observed contained RB and had the appearance of watered-down RB placebo. No standard placebo vomit was observed, which indicated to us that consumption of RB may lead to stomach irritation and induce vomiting in invasive wild pigs. Our literature review did not uncover any studies indicating consumption of RB directly caused vomiting in any species. Further research needs to be conducted to better understand this before any conclusions on causation can be drawn.

The need for access to research pens immediately following this trial in conjunction with TPWD Feral Swine Research Facility protocols led to the euthanasia of research subjects immediately upon completion of 2-choice trials. Thus, additional information regarding the detection and persistence of RB as a biomarker in HOGGONE® placebo was not gathered. Results from recent studies on the efficacy of RB as a biomarker in invasive wild pigs coupled with results of this study would indicate, though, that RB is an effective biomarker for invasive wild pigs (Beasley et al. 2015, Webster et al. 2017, Baruzzi et al. 2017, Snow unpubl. data).

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