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Authors: Fletcher, W. O., Creekmore, T. E., Smith, M. S., and Nettles, V. F.

Source: Journal of Wildlife Diseases, 26(4) : 502-510

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-26.4.502

Journal of Wildlife Diseases, 26(4), 1990, pp. 502–510

A FIELD TRIAL TO DETERMINE THE FEASIBILITY OF DELIVERING ORAL VACCINES TO WILD SWINE

W. O. Fletcher,[1,2] T. E. Creekmore,[3] M. S. Smith,[4] and V. F. Nettles[3]

[1] Georgia Department of Natural Resources, Game and Fish Division, Sapelo Island, Georgia 31327, USA
[2] Present address: School of Forest Resources, The University of Georgia, Athens, Georgia 30602, USA
[3] Southeastern Cooperative Wildlife Disease Study, Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA
[4] E.I. Du Pont De Nemours and Company, Incorporated, Sabine River Works, Post Office Box 1089, Orange, Texas 77631, USA

ABSTRACT: A field study was conducted on Ossabaw Island, Georgia (USA) to determine the feasibility of delivering oral vaccines to wild swine (*Sus scrofa*). Baits were made of polymer-bound fish meal and contained a gelatin capsule as a potential vaccine chamber. Two biomarkers, iophenoxic acid and tetracycline, were incorporated into each bait, and soured chicken mash was used as an attractant. Baits (n = 1,980) were distributed in a grid pattern on a 405-ha test site and monitored for animal disturbance. Within 72 hr, 88% of 393 monitored baits were gone, and observations of track-beds surrounding 100 baits indicated that at least 52% were taken by wild swine. Subsequent testing of 80 wild swine for the biomarkers revealed that 95% of the animals had consumed bait. Track-bed observations indicated that raccoons (*Procyon lotor*) were the only non-target animal that frequently took baits. Biomarker analyses indicated 44% of 16 raccoons tested had eaten bait. It was concluded that oral vaccine delivery to wild swine should be considered as a feasible method of control or eradication of pseudorabies and/or swine brucellosis in wild swine if effective vaccines become available.

Key words: Oral baiting, wild swine, *Sus scrofa*, biomarker, seromarker, field study.

INTRODUCTION

Wild swine (*Sus scrofa*) populations occur over much of the southeastern United States and in California and Hawaii (Hanson and Karstad, 1959; Wood and Barrett, 1979), with a nationwide population estimate of about 1 million animals (Nettles and Erickson, 1984). Some diseases of domestic swine have been reported in wild swine (Wood et al., 1976; Essey et al., 1981; Clark et al., 1983; Nettles and Erickson, 1984; Corn et al., 1986; Stallknecht et al., 1986), and wild swine probably are susceptible to the same diseases as domestic swine (Pullar, 1950; Hanson and Karstad, 1959; Brugh et al., 1964). Of particular concern to wildlife interests and the domestic livestock industry is that some wild swine populations are infected with pseudorabies and brucellosis.

Although chronically infected adult domestic swine are considered to be the principle reservoir of pseudorabies virus (PRV) (Shope, 1935; McFerran and Dow, 1964), wild swine frequently are infected. Serum neutralization test results from wild swine indicate that seropositive animals are present in at least nine of 18 states where wild swine exist (Clark et al., 1983; Nettles and Erickson, 1984; Corn et al., 1986). Pseudorabies virus has been isolated from wild swine in California (California Department of Food and Agriculture, unpublished data) and Florida (Nettles and Erickson, 1984). The prevalence of animals with antibodies to PRV in infected wild swine populations ranges from 11 to 60% (Southeastern Cooperative Wildlife Disease Study, unpubl.).

Swine brucellosis is a chronic disease of swine, and adverse effects of the disease are sterility and abortion in sows, orchitis in boars, and piglet mortality (Blood et al., 1983). The U.S. Cooperative State-Federal Brucellosis Eradication Program has made

substantial efforts to remove swine brucellosis from domestic swine. In 1986, approximately 3.2 million swine were tested for this disease (Nelson et al., 1986), and currently 33 states are considered brucellosis-free. At this time, wild swine populations with confirmed infections of *Brucella suis* include California, Florida, Georgia, Hawaii, Louisiana, South Carolina and Texas (Zygmont et al., 1982; Nettles, 1984; Corn et al., 1986). In addition, serologic evidence of brucellosis among wild swine has been found in Arkansas (Zygmont et al., 1982). If brucellosis eradication efforts aimed at domestic swine are successful, wild swine will become the last reservoir for *B. suis* in the United States.

Strategies such as quarantine, test and slaughter, and total depopulation, as implemented to eradicate pseudorabies and brucellosis from domestic swine, would be largely ineffective if applied to wild swine due to the wide geographic distribution of infected populations and the inherent difficulties encountered when dealing with thousands of free-roaming wild animals. Baer et al. (1971) immunized a gray fox (*Urocyon cinereoargenteus*) and red foxes (*Vulpes vulpes*) against rabies by the oral route and suggested using baits to orally vaccinate wildlife. Black and Lawson (1973) showed that immunization against rabies could be achieved by giving vaccine-laden baits free-choice to foxes. Oral vaccination technology is advancing rapidly, and this approach may have great promise when applied to wild swine. Control of rabies by oral vaccination of wild red foxes has been successful in some European countries (Steck et al., 1982; Schneider et al., 1983). Recent technological advances in genetic engineering have resulted in vaccinia/rabies glycoprotein vaccines intended for use in wildlife (Wiktor et al., 1984, 1985). Experimentation is in progress for vaccines against other diseases, and genetic recombinant technology may be applied to producing a swine pox/pseudorabies vaccine. An oral vaccine using *Brucella suis* strain 2 has been produced in China since 1971, and 30 to 40 million doses are used each year (Xin, 1986).

Along with vaccine development, concomitant work is needed to produce and test oral baits that would be used in the delivery of vaccines to animals such as wild swine. Therefore, the objective of this field trial was to determine the percentage of wild swine that would consume bait designed to deploy oral vaccines.

MATERIALS AND METHODS

Study area

Ossabaw Island is a barrier island owned by the Georgia Department of Natural Resources (Atlanta, Georgia 30334, USA) and is located in Chatham County, Georgia, approximately 32.3 km south of Savannah (31°47′N, 81°07′W). Uplands and fresh water marshes comprise 4,775 ha of the island, with the remainder being salt marsh. Johnson et al. (1974) gave a detailed description of forest associations and other ecologic attributes of the Georgia barrier islands. The study area was a 405-ha section covered almost entirely by climax maritime forest on the southeast end of the island. The northern and southern ends of the study area were bordered by roads, the east side by tidal creek and ocean, and the west side by continuous salt marsh (Fig. 1).

White-tailed deer (*Odocoileus virginianus*) are numerous on the island, with population estimates exceeding one deer/3.7 ha (Georgia Department of Natural Resources, unpublished data). Other common wild mammalian species include raccoons (*Procyon lotor*), gray squirrels (*Sciurus carolinensis*), eastern fox squirrels (*Sciurus niger*) and cotton mice (*Peromyscus gossypinus*). Mammalian and avian communities on Ossabaw Island are similar to those on the mainland, with the most noted exceptions being the absence of bobcats (*Lynx rufus*), red foxes (*Vulpes vulpes*), gray foxes (*Urocyon cinereoargenteus*), opossums (*Didelphis virginianus*), skunks (*Mephitis mephitis*), armadillos (*Dasypus novemcinctus*), and bobwhite quail (*Colinus virginianus*).

Ossabaw Island historically had free-ranging domestic cattle (Vanstory, 1981); however, cattle are now confined to fenced pastures. Some cows occasionally do escape, and eight to 10 were seen in the study area during this field trial. A unique resident of Ossabaw Island is the feral donkey (*Equus asinus*). Eight to 10 animals introduced in the early 1960's flourished, and the free-ranging donkeys now number 80 to 90

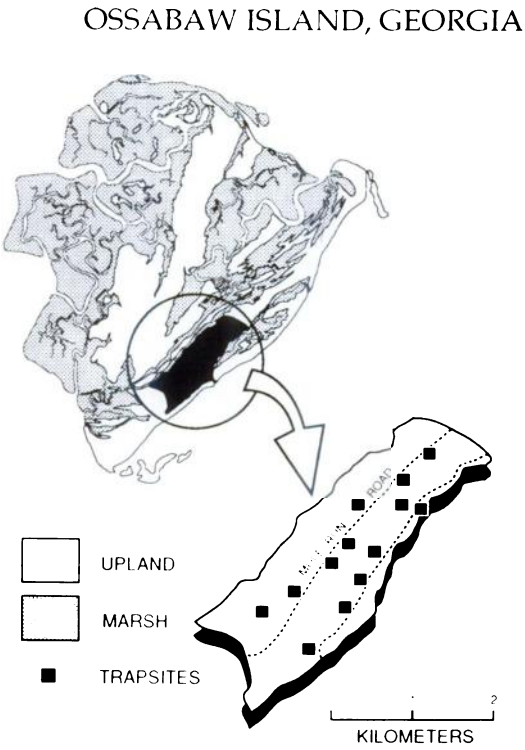

FIGURE 1. Map depicting site of pilot study to determine the feasibility of delivering oral vaccines to wild swine on Ossabaw Island, Georgia.

animals. Donkeys were seen frequently on the study area during this field trial.

Swine were reportedly introduced to Ossabaw Island during the 1500's when the Spanish were attempting to settle coastal Georgia. Various breeds of domestic swine have been introduced over the years in attempts to improve the stock; however, the animals have retained characteristics typical of wild swine throughout the southeastern United States. In recent years wild swine on Ossabaw Island have been controlled by trapping, and 400 to 600 animals are removed annually.

Bait preparation

A polymer fish meal bait (E.I. Du Pont De Nemours and Company, Inc., Ethylene Polymers Research, Sabine Research Laboratory, Orange, Texas 77630, USA) consisting of 10% polymer binder, 15% fish oil, 73.3% fish meal, and 1.7% tetracycline hydrochloride (150–200 mg/bait) was selected for use in the field study. Baits were cylindrical in shape, approximately 3.8 cm long and 3.2 cm in diameter with a 1.4 cm hole through the center. The tetracycline hydrochloride, incorporated into the baits during manufacture, served as a biomarker (Johnston and Voigt, 1982), since this antibiotic chelates with bone upon absorption in the blood stream and concentrates in newly formed calcium apatite (Buyske et al., 1960). Its presence is indicated by a characteristic golden-yellow fluorescence when teeth or bone sections are viewed under ultraviolet light (Milch et al., 1957, 1958).

In order to simulate vaccine delivery, a gelatin capsule filled with the seromarker iophenoxic acid (IA) (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin 53233, USA) (Larson et al., 1981; Baer et al., 1985; Follmann et al., 1987) was inserted into the cavity of each polymer bait. Iophenoxic acid was dissolved in corn oil heated to 90 C at a concentration of 10 mg IA/ml of corn oil. Torpac® size 13 lock ring gelatin capsules (Torpac, New York, New York 10019, USA) were filled with 2 ml of the IA/corn oil solution and placed in the cavity of each polymer fish meal bait.

Evaluation of IA as a seromarker

Prior to field distribution of IA-labeled baits, a small experiment was performed to estimate the required intake of IA to effectively mark wild swine. Two adult, two subadult and two juvenile wild swine were trapped on Ossabaw Island, bled via anterior vena cava puncture, and held in separate wooden pens. One pig of each age class was force-fed 20 mg of IA dissolved in 2 ml of corn oil. The corresponding animal in each age class was offered free choice a polymer fish meal bait containing 20 mg of IA in 2 ml of corn oil. A small amount of soured chicken mash attractant was placed on top of the bait. Captive wild swine given IA were bled 7 days post ingestion.

Determination of baseline serum iodine levels

In order to determine normal serum iodine levels in the free-ranging wild swine population, blood samples were taken via anterior vena cava puncture from 20 wild swine captured on Ossabaw Island prior to the distribution of marked baits (27 April 1988 to 29 July 1988). Serum samples also were obtained from five raccoons collected by shooting at sites more than 1.6 km from the study area between 4 October 1988 and 17 October 1988.

Marked bait trial

To insure even distribution of baits, transects on a compass heading of 130 degrees were spaced at 91.4 m intervals on the study area. Transects were flagged and numbered where they crossed a centrally located road (Mule Run Road) for

easy reference (Fig. 1). On 20 August 1988, 1,980 polymer baits containing both IA and tetracycline were distributed over the 405-ha study area. Baits were hand placed every 22.9 m along each transect to provide a density of 4.9 baits/ha. Approximately 22.5 ml of soured chicken mash was added as an attractant to the top of each bait at time of distribution. Bait sites along every fifth transect were marked with vinyl flagging and numbered. Also, at each fourth bait site along these flagged transects an approximately 2 m area surrounding the bait was raked to bare soil. Baits along flagged transects were checked daily for 3 days following distribution. Records were made of presence or absence of bait, condition of bait, tracks present in raked areas, and weather conditions.

Thirteen pen-style wild swine traps baited with whole corn were set inside the study area on the second day after bait distribution. Traps were examined twice daily. When feasible, additional juvenile swine were hand captured. Captured wild swine were sexed, aged (Matschke, 1967), and bled via anterior vena cava puncture. Forty of the animals were killed so that mandibles could be collected for tetracycline analysis. All other wild swine were transported to a holding facility to await removal from the island. Trapping activities were terminated after 11 days (2 September 1988).

Raccoon collections within the study area were initiated on 22 August 1988, and continued until 13 October 1988. Raccoons were shot, and blood samples and mandibles were taken. Sex, age (Johnson, 1970), estimated weight, and location were recorded for each animal.

Mandibles were collected from deer killed on the 405-ha study area during managed hunts conducted by the Georgia Department of Natural Resources during November and December 1988. Sex, age (Severinghaus, 1949), weight and location of kill were recorded for each deer.

Serum samples from wild swine and raccoons were placed in labeled tubes and frozen. Frozen serums were submitted to the Veterinary Laboratory of SmithKline Bioscience Laboratories, Ltd. (Tucker, Georgia 30084, USA) for total serum iodine determinations using Technicon Autoanalyzer methodology (Technicon Instrument Corporation, Irvine, California 92705, USA) (Henrey, 1964). Disguised duplicate samples were included in each shipment of serums to serve as quality controls.

Mandibles from wild swine, raccoons, and deer were placed in labeled plastic bags and frozen. Undecalcified canine teeth from wild swine and mandibles from raccoons and deer were sectioned using an Isomet® low speed, double blade saw (Buehler Ltd., Lake Bluff, Illinois 60044, USA). Acetate spacers were placed between the diamond Isomet® saw blades to produce sections 100–150 μm thick. Canine teeth from wild swine and mandibles from raccoons were sectioned longitudinally; mandibles from deer were sectioned transversely. Sections were mounted in glycerine on glass slides and covered with 0.17 mm cover slips. Slides were stored in the dark at −4 C to minimize exposure of samples to light since prolonged exposure greatly diminishes the intensity of fluorescence. Slides were viewed using an ultraviolet (UV) light microscope (Olympus Corporation, Lake Success, New York 11042, USA) with the exciting light at a wave length of 365 nm. Samples containing tetracycline deposits in teeth and bones emitted fluorescent light at a wave length of 560 nm, which appeared as yellow bands.

RESULTS

Evaluation of IA as a seromarker

Only one of the three penned wild swine offered the IA-containing polymer fish meal bait free choice ate the bait. Therefore, control value testing was limited to this animal and the three wild swine that were force-fed 20 mg of IA in corn oil solution. Serum samples taken before dosing with IA and 7 days post ingestion were tested for total serum iodine. Pre-dose values of the four wild swine ranged from 2.6 to 3.5 μg I/100 ml of serum ($\bar{x}$ = 3.08, SD = 0.38), whereas post ingestion values ranged from 145 to 5,100 μg I/100 ml ($\bar{x}$ = 1,539.0, SD = 2,381.6).

Baseline serum iodine values

Total serum iodine levels for the 20 wild swine sampled prior to bait distribution and used to determine baseline total serum iodine levels ranged from 2.6 to 12.0 μg I/100 ml ($\bar{x}$ = 6.5, SD = 3.04). Based on the Shapiro-Wilk statistic (SAS, 1985), these data were normally distributed (P = 0.0781). Therefore, the criterion for successful biomarking of wild swine was a value greater than 15.62 μg I/100 ml ($\bar{x}$ + 3 SD).

Five raccoons collected for baseline total serum iodine determinations had values of 12.0 to 21.0 μg I/100 ml ($\bar{x}$ = 14.4, SD = 3.78). Based on the Shapiro-Wilk statistic

TABLE 1. Results of iophenoxic acid and tetracycline analyses in animals taken from the study area after marked bait distribution on Ossabaw Island, Georgia, on 20 August 1988. Animals were collected from 22 August to 22 December 1988.

Species	Age	Iophenoxic acid		Tetracycline	
		Number marked/ number in sample	Percent marked	Number marked/ number in sample	Percent marked
Wild swine	All ages	76/80	95	38/40[a]	95
	Juvenile	37/40	93	19/20[a]	95
	Adult	39/40	98	19/20[a]	95
Raccoons	All ages	6/16	38	7/16[b]	44
	Juvenile	0/6	0	1/6[b]	17
	Adult	6/10	60	6/10[a]	60
Deer	All ages	Not tested		0/44	0
	Juvenile	Not tested		0/5	0
	Adult	Not tested		0/39	0

[a] All individuals marked with tetracycline also were marked with iophenoxic acid.
[b] One raccoon that was marked with tetracycline was negative for iophenoxic acid.

(SAS, 1985), these data were normally distributed ($P = 0.0214$). Therefore, the criterion for successful biomarking of raccoons was a serum iodine level greater than 25.74 μg I/100 ml ($\bar{x}$ + 3 SD).

Marked bait trial

Of the 1,980 bait sites, 393 were checked daily for 3 days following bait distribution. Fifty-six percent of these baits were gone after 1 day and 88% of the baits were gone after 3 days. Most baits present after 3 days were in poor condition due to ant (Formicidae) and carrion beetle (Siliphidae) activity. In some instances, ant hills were made on top of the baits. Ants also ate the gelatin capsules, allowing the IA/corn oil solution to drain from the capsule. Damage from carrion beetles ranged from minor to near complete consumption of baits. Rainfall during the third night further damaged the baits by causing some of the fish meal portion of the baits to become soggy and by dissolving the gelatin capsule. On the fourth day, only 49 of 393 baits were found. Of these, two baits were intact; seven baits were partially eaten, with the IA-containing capsule empty; and 40 baits were undisturbed except for insects, but again the IA was not present in the gelatin capsule.

Of the 393 monitored bait sites, 100 had been raked to bare soil and were observed for animal tracks each day. Percentages of baits that had obviously been taken by swine were 44, 50, and 52% after 24, 48 and 72 hr, respectively. By the end of 3 days, 95% of the 100 baits in the raked areas were gone.

During a 10-day period following bait distribution, 100 wild swine (59 adults, 41 juveniles) were captured from the study area. Blood samples were taken from all wild swine captured; however, due to economic constraints, only 80 sera were tested. Seventy-six of 80 (95%) wild swine had elevated serum iodine levels (Table 1). Four wild swine considered unmarked had serum iodine levels that ranged from 6.0 to 11.0 μg I/100 ml. These levels were less than the upper limit for normal swine as determined by control values. Marked wild swine ranged from 19.0 to 15,000 μg I/100 ml ($\bar{x}$ = 1,809, SD = 2,138). Difference in juvenile wild swine marked with IA (37 of 40) and adult wild swine marked (39 of 40) was not significant ($P \leq 0.05$) as indicated by Chi-square analysis.

Mandibles were collected from 20 adult and 20 juvenile wild swine. Tetracycline was detected in 38 of 40 (95%) wild swine tested (Table 1). Numbers of juvenile and adult wild swine positive for tetracycline were the same, i.e., 19 of 20. Wild swine

positive for tetracycline also were marked by IA, and wild swine negative for tetracycline were not marked by IA.

Six of 16 (38%) raccoons collected from the study area after bait distribution were marked by IA (Table 1). Serum iodine levels for 10 raccoons that were considered unmarked ranged from 11 to 21 μg I/100 ml. These levels were less than the upper limit for normal raccoons as determined by control values. Marked raccoons had serum iodine values from 62 to 2,180 μg I/100 ml ($\bar{x}$ = 1,095, SD = 837). Seven of 16 (44%) raccoons were positive for tetracycline. Six of the positives were adults, and one was a juvenile. The IA and tetracycline results for each animal corresponded, with the exception of one juvenile raccoon which was positive for tetracycline but unmarked by IA.

All 44 deer taken from the study area were negative for tetracycline (Table 1). Ages of deer ranged from 0.5 to 7.5 yr with representatives present in each yearly age class.

DISCUSSION

As deployed, the baiting system would have delivered an oral vaccine to about 95% of the wild swine in the population within 72 hr. This conclusion was based on the almost complete disappearance of baits after 3 days and the finding of biologic markers in 95% of the swine tested.

Both the IA and tetracycline marking systems were effective in identifying swine that had eaten baits, and it is likely that many swine ate multiple baits based on the high serum iodine levels in some animals. Of the two marking systems, IA is more expensive due to the costs of the chemical and laboratory analyses. However, IA does have the advantage that only serum is needed for testing.

Results of all animals tested for both markers corresponded with the exception of one juvenile raccoon. The presence of tetracycline in the young raccoon without a corresponding elevated serum iodine level could have occurred if the animal ate part of the polymer bait without damaging the IA-containing capsule.

The range of baseline serum iodine levels of 20 raccoons from Washington, D.C. (USA) (Hadidian et al., 1989) and three raccoons from Colorado (Larson et al., 1981) were 1.9 to 9.4 μg I/100 ml and 1.4 to 12.4 μg I/100 ml, respectively. Baseline iodine levels of Ossabaw Island raccoons were slightly higher (12.0 to 21.0 μg I/100 ml). Raccoons forage extensively in salt marsh habitats consuming a variety of marine organisms (Fleming et al., 1976; Harman and Stains, 1979). Salt water and the associated marine life provide a readily available source of iodine to raccoons (Rusby et al., 1930; Osol and Pratt, 1973) and may be responsible for the higher baseline iodine levels experienced in raccoons on Ossabaw Island.

The success of this pilot study in reaching wild swine with baits could be partially attributed to seasonal timing of bait deployment. Late summer on Ossabaw Island is a period of minimum mast availability, and therefore, natural foods for swine were in short supply. If this trial had been done in the fall when acorns and palmetto berries were abundant, it is likely that bait acceptance would have been lower. Past experiences on Ossabaw Island livetrapping wild swine in pens baited with corn have provided evidence that trapping success falls dramatically once these natural foods become available.

Another important feature of this field trial that may have contributed to the high percentage of swine marked was the baiting density (4.9 baits/ha). The 100 wild swine trapped from the 405-ha study area represents a minimum density of 24.7 wild swine/km^2. Based on the number of wild swine sighted on the study area before and after trapping activities, it appeared that over 50% of the wild swine on the study area were removed. However, even if only half the population was captured, in a conservative estimate there would have been almost 10 baits available per target animal.

Our result of 44% acceptance by rac-

coons is much lower than rates found by other researchers that were deliberately targeting raccoons with the polymer fish meal bait (Hable et al., 1990). Possible explanations were that the soured chicken mash not used in other trials acted as a repellent for raccoons. An alternate hypothesis was that raccoons were more interested in the wild grapes that were ripening in the forest overstory during the study period. More work should be undertaken to learn methods of using such biologic eccentricities of non-target animals to minimize their ingestion of vaccine-ladened baits. These studies should consider not only raccoons but opossums, skunks, foxes, feral dogs, coyotes (*Canis latrans*) and many other small mammals not present on Ossabaw Island.

The present study clearly demonstrates that delivery of an oral vaccine to wild swine is feasible. However, to increase the practicality and efficiency of the delivery system, future studies should consider methods for optimizing baiting density. This encompasses problems associated with insect fouling, adverse environmental conditions, estimating population density of target animals, target animals eating multiple baits and loss of baits to non-target species. Hand delivery of baits may be practical for small areas, but a method of aerial distribution is needed for large or less accessible areas. Seasonal differences as well as immunologic status of the target population also must be given consideration when determining optimal season for bait distribution.

ACKNOWLEDGMENTS

We thank the Georgia Department of Natural Resources, Game and Fish Division for their permission to work on Ossabaw Island and for their logistical support and help during the study. The Ossabaw Foundation, especially Mrs. E. T. West and Roger Parker, provided valuable support and assistance particularly with the wild swine. E.I. Du Pont De Nemours and Company, Inc. furnished the polymer baits used in the study. Bradley Snyder, Charles Waters, Gregory Lee and William Hartley provided assistance with bait distribution, wild swine trapping, and raccoon collections. We thank Donna Wood and Geraldine Cornish for typing and editing of the manuscript.

This project was supported through Cooperative Agreement Number 12-16-93-032, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture.

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Received for publication 25 September 1989.