TESTING THE EFFECTIVENESS OF ONE-SHOT IMMUNOCONTRACEPTIVES ON WHITE-TAILED DEER AT FRIPP ISLAND, SOUTH CAROLINA

2008 PROGRESS REPORT
to the South Carolina Department of Natural Resources and the Fripp Island Property Owners Association

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SUMMARY

In 2008, a research team consisting of personnel from The Humane Society of the United States (HSUS) and Tufts-Cummings School of Veterinary Medicine (TCSVM), with logistic and field support from the Fripp Island Property Owners Association, carried out the fourth year of a field test of the effects of long-acting contraceptive vaccines on reproduction and population growth of white-tailed deer on Fripp Island, SC. The objectives of this study are to examine the relative effectiveness, longevity, safety, and practicality of several different preparations of long-acting porcine zona pellucida (PZP) contraceptive vaccines, and to examine the effects of one-shot, multi-year contraceptive vaccines deer population dynamics on Fripp Island.

Both distance-sampling and mark-resight methods indicated a decline in deer densities and fawn-doe ratios on Fripp Island in 2008. Between 2006 and 2008, deer numbers appear to have declined between 21-35% on Fripp and fawn:doe ratios have declined by 38-56%. Both measures remained roughly stable on the Hunting Island control site during that period. There is also a trend for increased male:female ratios over the course of the study on both sites, for reasons that are not well understood.

During February-March 2008, 25 new deer and 54 previously tagged deer were captured on Fripp Island using chemical immobilization, averaging approximately 4.6 person-hours/deer captured. Annual survivorship of tagged females averaged 82%. All 25 new deer were treated with an emulsion of 100µg PZP + modified Freund's Complete Adjuvant (mFCA) plus PZP incorporated into three lactide-glycolide pellets engineered to be released at one, three, and 12 months.

Pregnancy tests on the blood serum of captured females using Pregnacy-Specific Protein B (P-SPB) assays provided additional support for high levels of effectiveness after one and two years of SpayVac® (16% and 25% pregnancy rates, contrasting with 83% pre-treatment) and for the PZP/adjuvant pellet preparations (19% and 20% pregnancy rates, contrasting with 74% pre-treatment). Remotely-delivered boosters administered to treated does that had previously given birth were highly effective in restoring contraception the year after treatment (13% pregnancy rates), regardless of initial treatment.

Highly significant differences between pregnant and non-pregnant does were detected in steroid levels in fecal samples collected in February-March 2008. Levels overlapped in both steroids measured, however; further analysis will be needed to discover whether fecal steroid measurements can be substituted for P-SPB diagnosis of pregnancy.

INTRODUCTION

Conflicts with white-tailed deer have become commonplace in residential areas throughout the eastern and Midwestern United States. These conflicts include damage to ornamental plantings, deer-vehicle collisions, undesirable ecological impacts on natural areas, and an association with tick-borne zoonotic diseases including Lyme disease and ehrlichiosis. Although a variety of techniques exist for mitigating these conflicts, the public often views deer population control as an essential component of a comprehensive deer conflict resolution program. However, traditional deer population control methods such as public hunting may be unsafe, inappropriate, or publicly unacceptable in residential areas experiencing deer conflicts, and alternative methods are being explored.

Immunocontraception has proven to be a promising approach to deer population control in cities, towns, suburbs, and heavily used parks. Previous studies have shown that the porcine zona pellucida (PZP) vaccine dramatically reduces pregnancy rates in treated female white-tailed deer, and can stabilize and gradually reduce deer populations over limited areas (Turner et al. 1992; Naugle et al. 2002; Rutberg et al. 2004; Rutberg & Naugle 2008). However, previous studies of PZP on free-ranging white-tailed deer have employed and tested vaccines that require two initial shots and annual boosters to achieve full effectiveness. The need for repeat treatments significantly increases stress on treated animals, technical and logistical challenges of applying the vaccine, and cost of delivery, and limits the scope of potential management applications. Consequently, long-acting effectiveness with a single shot is extremely desirable for management purposes.

Three technologies for single-shot effectiveness have now emerged in recent years. One, named Spay-Vac® (ImmunoVaccine Technologies, Halifax), uses a liposome preparation of PZP (with adjuvant) to boost antibody effectiveness and longevity, and has shown long-term effectiveness in a variety of species, including white-tailed deer (Brown et al. 1997; Fraker et al. 2002; Locke et al. 2007). A second, which uses timed-release pellets to simulate booster injections of PZP, has produced single-treatment, multi-year contraceptive effectiveness in wild horses and now possibly deer (Turner et al. 2007; Turner et al. 2008; and see below). Finally, GonaCon®, a GnRH-based vaccine, has shown evidence of single-shot effectiveness in wild horses and other species (Killian et al. 2008; Fagerstone et al. 2008).

In February 2005, we began a study on the effectiveness of one-shot PZP preparations on Fripp Island, South Carolina. The primary objectives of this study were (1) to evaluate the relative effectiveness, safety, and ease-of-use of four different one-shot vaccine preparations, including two forms of Spay-Vac® and two forms of timed-release pellets; and (2) to examine the effects of PZP contraception on deer population density, growth, and composition, in comparison to an untreated deer population at nearby Hunting Island State Park.

The study is collaboration between The Humane Society of the United States (HSUS), TerraMar Environmental, Tufts-Cummings School of Veterinary Medicine (TCSVM), Holterra Wildlife

Management, Fripp Island Property Owners Association (FIPOA). It is being carried out under INAD 8840 from the FDA Center for Veterinary Medicine, belonging to the HSUS, and Protocol #G940-08 approved by the TCSVM Institutional Animal Care and Use Committee. Chemical immobilizing (CI) drugs used for capture are provided by and accounted for by Mark Guilloud, D.V.M. at the Animal Hospital of Lady's Island (Drug Enforcement Administration (DEA) number BG 3699026). Drug storage on site is provided by and overseen by the Fripp Island Police.

FIPOA and the Fripp Island Resort provide technical assistance, transportation and amenities. Housing is graciously provided by residents and property owners of Fripp Island. Funding for the immunocontraception project is provided by the generous residents of Fripp Island and by The HSUS. Numerous residents also volunteered to help with fieldwork.

METHODS

Population studies. During the winters of 2005-2008 Holterra Wildlife Management conducted surveys of deer population density and group composition at Fripp Island and Hunting Island State Park. Counts were conducted using distance sampling methods described by Burnham et al. (1980) and developed for deer by Gogan et al. (1986), Underwood et al. (1998), and Focardi et al. (2002).

Transects for sampling were selected randomly on Fripp Island and Hunting Island State Park. Salt marshes were not sampled in either location. In 2008, sampling on Fripp Island included 1485 acres, 49% of its 3000 acre total, and in Hunting Island State Park 2000 acres, 40% of the 5000 acre total. Surveys were performed during nighttime (2000-0530 hr) hours. Observers rode in the cab of a pickup truck and located deer with two million-candlepower hand-held spotlights. Observers determined distance to the center of deer clusters with a laser rangefinder and determined angles to the center of each cluster with a truck-mounted compass. Clusters were separated using nearest-neighbor criteria (LaGory 1986), location of deer, and their behavior in yards. In general, deer traveling together on the same or adjacent residential properties were considered one cluster.

All field data were entered into the computer program DISTANCE 5.1 (Laake et al. 1992) and density was derived from the model with the best Akiaiki Information Criteria and detection function with the lowest coefficient of variation.

During these surveys, we also attempted to determine buck:doe ratios and fawn:doe ratios.

To supplement the distance-sampling transect data, we carried out daytime mark-resight surveys in August 2006, November-December 2006, and August 2009. During these surveys, the observer noted all does, bucks, and fawns, and noted whether deer carried ear-tags. Because 99% of the ear-tagged deer were adult or yearling females, we applied a Lincoln-Peterson index to estimate the total number of females in the population, using the formula:

N = M*n/R where

N = number of females in the population
M = number of marked deer in the population
M = total number of females sampled on surveys
R = number of marked deer located on surveys

The total population was then estimated by summing the estimated number of females, the number of fawns estimated from the observed fawn/doe, and the number of bucks estimated from buck/doe ratios.

Deer captures. Deer on Fripp Island were captured during February-March, 2008, using a combination of Xylazine HCl (at approximately 2.2 mg/kg) and Telazol HCl (at approximately 4.4 mg/kg). The drug combination was loaded into self-injecting 1 cc Pneu-Dart, Inc. transmitter darts with 1" needles and double wire barbs or Palmer Cap-Chur transmitter darts with 1" needles and single wire barbs. Darts were delivered intramuscularly in the hip from a Dan-Inject CO2 powered Blo-jector at ranges of 3-12 meters. Dart transmitters had a tracking range of approximately 1 kilometer and were tracked with a Telonics TR-4 receiver and Yagi antenna.

Deer that failed to become fully sedated or were difficult to restrain were given supplemental injections of Ketamine HCL (at approximately 5 mg/kg). When a subsequent darting was necessary either 1 or 2cc standard Pneu-Dart darts with 1" needles and single wire barbs were used. These darts were fired from either a Dan-Inject CO_2 Blo-jector or a Pneu-Dart Blo-jector.

To reverse the effects of xylazine, Tolazoline was given intravenously and/or intramuscularly at approximately 4mg/kg when the effects of the Telazol and/or Ketamine diminished (blinking, swallowing, tail twitching, etc.).

Deer were either captured as they were encountered along community streets while driving a vehicle or at 3 bait stations set up on empty lots with minimal exposure and risks to residents or visitors. Each bait station consisted of a single 30 gallon (114 liter) barrel feeder suspended from a tree branch. Each feeder had an automatic timer and electronic dispensing unit and was programmed to dispense approximately 3-5 pounds (1.4-2.3 kg) of whole corn one or two times daily depending on local deer activity.

Upon capture, each deer's eyes were treated with an ophthalmic ointment and the head and eyes covered with a cloth hood. Heads were elevated and vital signs (heart rate, respiration rate, body temperature, and coloration of mucosa) were checked at 10 minute intervals. When possible, heart rate and respiration were monitored continuously using a handheld pulse-oxymeter with the sensor attached to the tongue or ear. Deer showing any signs of respiratory distress (SpO2 < 90%) were given approximately 0.3-0.5 mg/kg of Dopram V at 20 mg/ml intravenously. Deer in severe respiratory distress (SpO2<80%) were given Dopram V and/or the antagonist Tolazoline.

Once a deer was stabilized darts were removed and wounds were treated with a triple antibiotic topical ointment (Neosporin). Deer were then examined for injuries related and/or unrelated to the capture process. Deer were then ear-tagged with a large, red, plastic, individually numbered cattle tag in the right ear. Tags were labeled on the back with "EXPERIMENTAL ANIMAL, DO NOT CONSUME" and a telephone number where information could be obtained in the event an animal was killed or found. A small round, yellow hog tag with a corresponding number and label also was placed in the left ear.

Measurements were then taken on girth, hind-foot length, and body length. Each deer was weighed, and age (adult, yearling, fawn) was determined when possible by examining tooth wear. Deer were then assessed for any prior injuries and mammary glands were visually examined for signs of lactation. After examination each deer was injected subcutaneously with 2-3 ml Liquamycin (LA-200) antibiotic.

Pregnancy testing. At the time of capture, we collected 10 ml of blood from each deer when possible for determination of pregnancy. Blood was cooled for a minimum of 30 minutes and centrifuged. After centrifugation the serum was poured off, labeled, and immediately frozen. Samples were sent to Biotracking (Moscow, Idaho) for pregnancy testing using ELISA tests for the presence of Pregnancy-Specific Protein B (P-SPB; see http://www.biotracking.com for more information).

Steroid assays. We also obtained fecal samples from as many females as possible at time of capture. Fecal samples were frozen and shipped to the Science and Conservation Center in Billings, MT, for enzyme immunoassay (EIA) analysis of estrone conjugate (E1C) and non-specific immunoreactive pregnanediol 3-glucuronide (iPdG) following the methods of Stoops et al. (1999). Fecal steroid levels were then compared to pregnancy diagnoses using P-SPB to determine whether collection of fecal samples could substitute for capture and blood sampling for pregnancy diagnosis.

PZP treatments. After all standard procedures were completed; new deer captured in 2008 were hand injected in the hip with the following treatment: 100µg PZP in 0.5 ml PBS emulsified with 0.5ml mFCA plus 550µg PZP and 500µg QA-21 prepared in three "heat-extruded" pulsed-release lactide-glycolide pellets engineered to release at 1 months, 3 months, and 12 months. These three pellets were loaded into14 gauge needles and hand injected in the hip along with an emulsification of PZP in mFCA using a trochar syringe supplied by Dr. John Turner of the Medical University of Ohio, Toledo.

The PZP for primers and pellet preparations was produced at the Science and Conservation Center, Billings Montana. The PZP/QA-21 heat extruded pellets were prepared in the laboratory of Dr. Douglas Flanagan, College of Pharmacy, University of Iowa.

In addition, females observed over the summer with fawns and/or distended udders and deer determined pregnant by blood samples in spring-summer 2008 were given remote treatments during August 2008. Each female treated was given 100µg PZP in 0.5 ml PBS emulsified in either mFCA or FIA depending on past treatments. Treatments were delivered in 1 cc Pneu-Dart darts with 1" barbless needles fired from a Dan-Inject Blo-jector into the hip. Each dart was then recovered and examined to confirm complete discharge.

Radio telemetry. During winter captures radio collars were removed from deer in 2008 and radiotelemetry observation ceased.

Observations of study animals. During one week in June and one week in August 2008 as many tagged deer as possible were relocated and observed to gather fawning data. Deer were observed for signs of extended udders to attempt to corroborate the winter pregnancy test results.

RESULTS

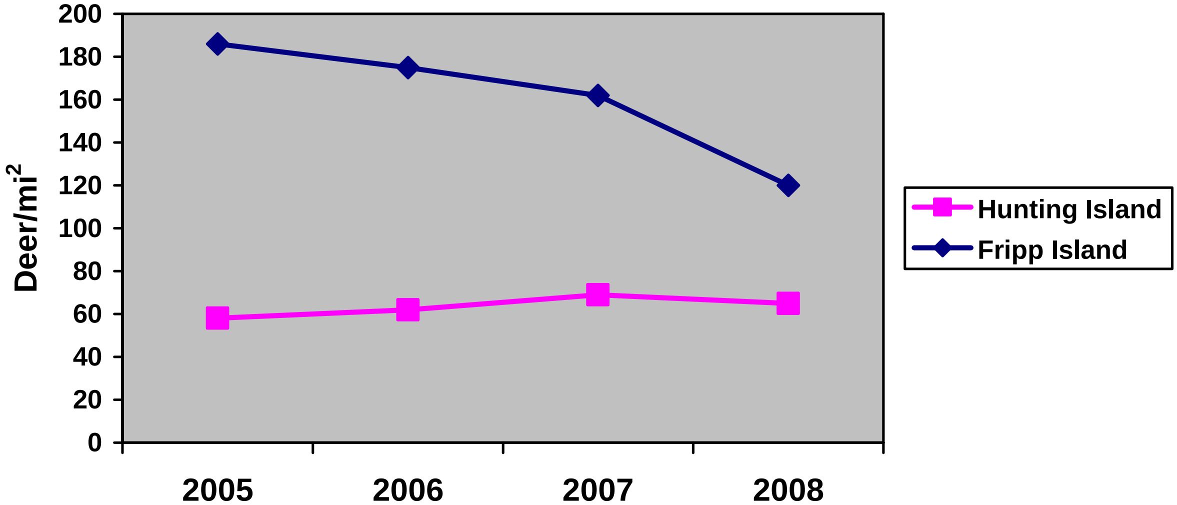
Population studies. Twenty (20) sampling sessions were conducted during 22 February – 6 March 2008. This number achieved the 60 or more deer clusters at both Fripp Island and Hunting Island State Park needed to generate a robust estimate of population abundance (Buckland et al. 1993). Investigators randomly sampled 31.9 miles of roads on Fripp Island and systematically sampled 42.3 miles of road within Hunting Island State Park. The new transect line added at Hunting Island in 2007 was retained.

A total of 274 fawn, yearling, and adult deer in 94 clusters was detected on Fripp Island, and 117 fawn, yearling, and adult deer in 66 clusters were detected within Hunting Island State Park. Deer densities for Fripp Island and Hunting Island State Park were 119.5 deer/mi^2 (90% C.I.:97.8-147.2 deer/mi^2) and 64.9 deer/mi^2 (90% CI: 41.8-82.5 deer/mi^2), respectively. The estimated sex ratio was 1M:1.44F at Fripp Island and 1M:1.70F at Hunting Island State Park (standard errors of estimates of sex ratios <15%), with 0.53 fawns per doe observed at Fripp and 1.30 fawns per doe observed at Hunting Island.

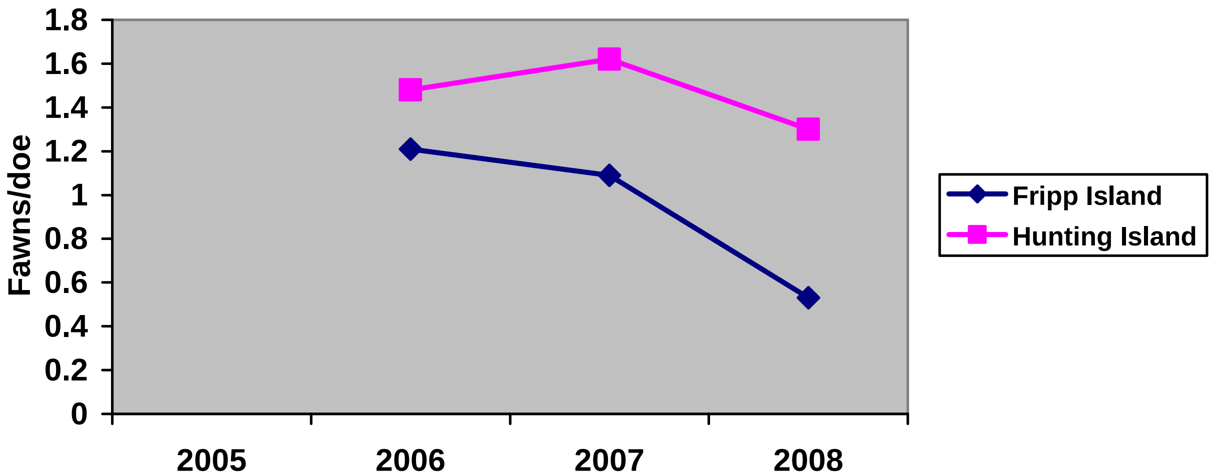
Since 2005, estimated population density has declined by about 35% on Fripp Island, while estimated population density has remained roughly stable on Hunting Island (Fig. 1a). Since 2006, the number of fawns per female has declined by approximately 56% on Fripp Island, and has declined by about 12% on Hunting Island (though it was higher in 2007 than in either 2006 or 2008) (Fig. 1b). The number of females per male has declined gently but steadily on both Fripp and Hunting Island since 2005, though somewhat more steeply on Hunting Island (Fig. 1c).

Figure 1. Deer population trends in distance samples conducted at Fripp Island and Hunting Island State Park, February-March 2005-2008. (a) Population density; (b) fawns/doe; (c) females/buck. Note that in (b), no estimate was made of fawn/doe ratio because the survey was conducted.

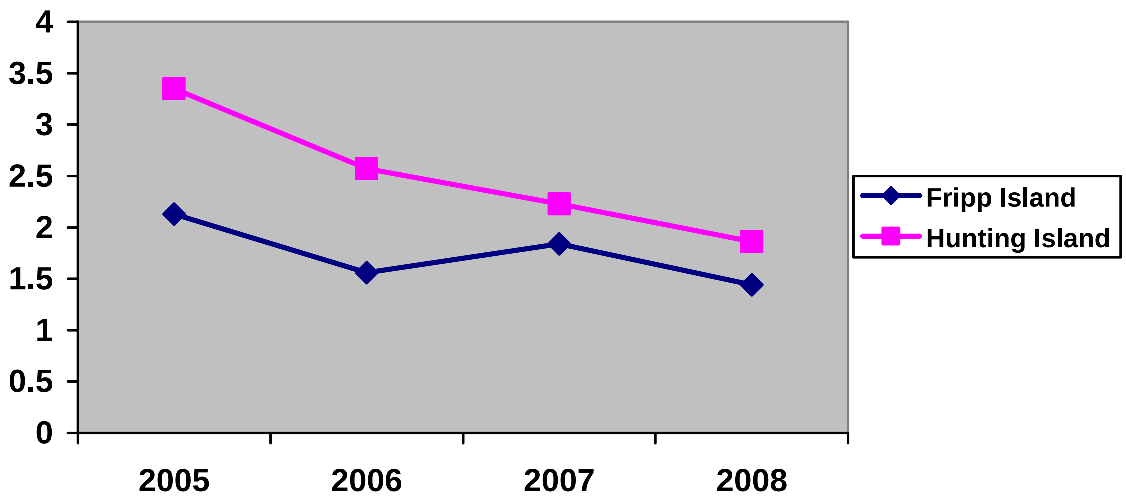
(a)



(b)



(c)



Summer mark-resight studies also suggest a notable decline in the number of adult and yearling females on Fripp Island (Table 1). Although it is difficult to interpret the November-December 2006 results, a comparison of surveys conducted in August 2006 and 2008 suggest a 21% reduction in the number of does (and a similar decrease in overall population size) over that period. The reduction in number of fawns per doe and the relative increase in the proportion of males also parallel the trends reported in the distance sampling studies.

Table 1. Results of island-wide daytime mark-resight surveys on Fripp Island, 2006-2008. Total number of does is estimated using a simple Lincoln-Peterson index calculation; total deer population is calculated by estimating buck and fawn numbers from the estimated number of females and observed buck/doe and fawn/doe ratios.

Date (#surveys)	Number does observed	Number marked does observed	Number marked does in population	Bucks/ doe	Fawns /doe	Estimated # Does	Estimated Total Population
August 2006 (3)	76	56	147	0.46	0.32	200	357
November-December 2006 (5)	48	41	147	0.49	0.28	173	308
August 2008 (2)	53	50	151	0.61	0.20	158	285

Deer captures and treatments. During 22 days in February-March 2008, 25 new deer and 54 previously tagged deer were captured on Fripp Island using chemical immobilization. A total of 360 person-hours was spent capturing and handling the deer, at an average of 16.4 person hours/day, 3.6 deer captured/day, and 4.6 person-hours/deer captured.

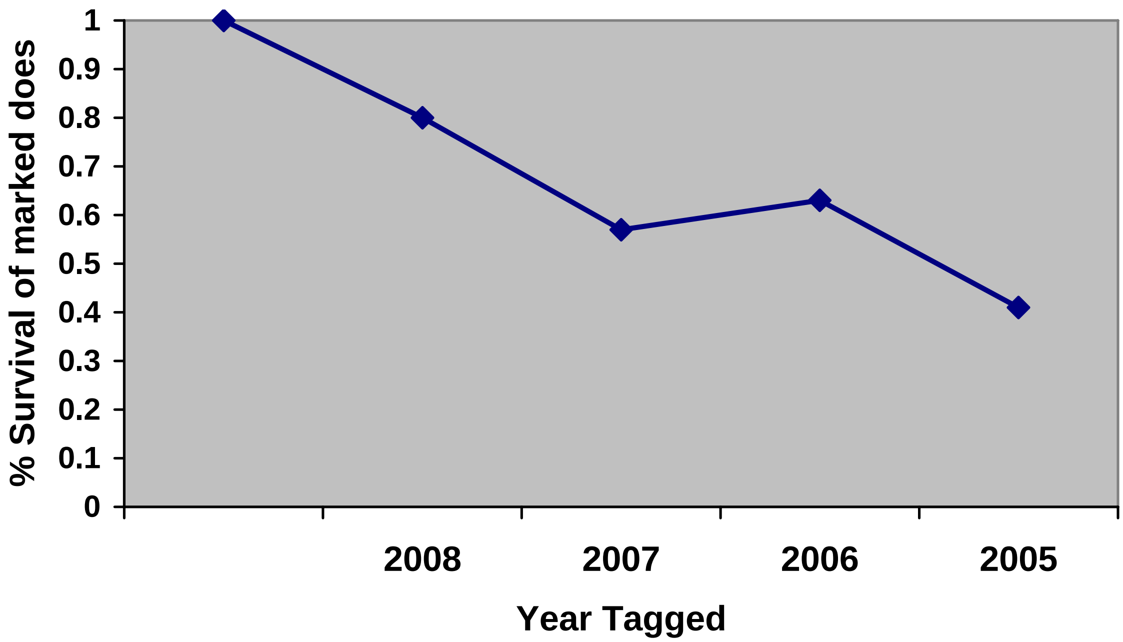
Of the 79 deer captured 7 had to be darted multiple times to insure capture. An additional 8 deer were darted and not immediately recovered. Of these, 5 were relocated within 48-60 hours after first being darted, darted a second time, and captured. The other 3 deer were not recaptured and the telemetry darts and transmitters were not recovered. In total, 94 darts hit deer with 79 deer captured for a dart/capture failure rate of 16%. Four (4) darts missed deer and all were recovered. Deer not captured carried darts ranging from 2 to 7 days. No deer sustained serious injuries as a result of darting or captures, but 1 female was found dead 24 hours post release. A necropsy revealed that the deer was very old and probably died of capture myopathy. The deer was buried on site according to protocol.

All 25 new deer captured were females. Each was treated with mFCA and 3 heat evaporated pellets + PZP emulsified in mFCA as described above. Twenty-four (24) of the new females and 54 recaptured females were bled to determine pregnancy using PSPB diagnosis. Fecal samples were also obtained from 22 newly tagged females and 52 previously tagged females.

Over a 4 day period in August, 2008 two persons remotely treated 29 deer previously treated with either Spay-Vac®, Adjuvac® + pellets, or mFCA + PZP and pellets with either mFCA or FIA emulsified with 100 µg PZP in PBS. All 29 females had been determined pregnant either visually (distended udder) or by PSPB diagnosis. Of the 29, 19 received treatments of FIA and 10 received treatments of mFCA. A total of 65 person-hours was spent darting at an average of 7.3 deer/day and 2.2 person-hours/treatment.

Female survivorship. During inventories of tagged females conducted during February, March, June, and August 2008, we sighted 38 of 93 (40.9%) of females tagged in 2005, 52 of 83 (62.7%) of the females tagged in 2006, 16 of 28 (57.1%) of the females tagged in 2007, and 20 of 25 (80%) of females tagged in 2008 (Fig. 2). A log-linear regression over the four year period yields a crude annual survival rate among marked adult and yearling females of 82%.

Figure 2. Survival to end of calendar year of cohorts of adult and yearling female deer eartagged since 2005.



Reproduction and vaccine effectiveness. Pregnancy diagnoses using PSPB indicated that 18 of 24 (75%) new females captured in 2008 were pregnant, and 18 of 53 females (34%) recaptured in 2008 were pregnant. Overall, from 2005-2008, 175 of 220 females (79.5%) were pregnant at the time of initial capture, presumably reflecting the baseline pregnancy rate among untreated female deer at Fripp (Table 2). Pregnancy rates among recaptured does are significantly lower than pregnancy rates among recaptures ($\chi 2=67.08$, df=1, $P<<0.001$).

The 2006 preparation of SpayVac® showed a high level of effectiveness into a second year. Among does treated with SpayVac® in 2006 and not known to have been pregnant in 2007, 4 of 16 (25%) were diagnosed as pregnant in 2008, that is, two years after treatment (Table 3). In this second year, aqueous and non-aqueous forms of SpayVac® showed identical 25% efficacy.

Heat-extruded (HE) controlled-release pellets also showed a high level of effectiveness into a second year, with hints that there might be some effectiveness in the third year as well. In 2008, 3 of 10 (30%) of does treated in 2007 with the HE pellets and mFCA were diagnosed as pregnant, and 2 of 11 does treated with HE/mFCA in 2006 were pregnant. These results are combined with the results from previous years in Table 4. No additional data were collected in 2008 on unboosted females initially treated in 2006 with cold-evaporated pellets.

Limited data are available on the effectiveness of booster treatments (Table 5). In general, however, it appears that remotely-delivered booster treatments provide at least one additional year of contraception, with no clear differences emerging among initial treatments.

Table 2. Proportion of captured does pregnant, as indicated by Pregnancy-Specific Protein B (P-SPB) in blood samples, 2005-2008.

Proportion Pregnant	2005	2006	2007	2008	Total
Newly captured does	79/92 (85.9%)	61/76 (80.3%)	19/27 (70.4%)	18/25 (72%)	**177/220 (80.5%)**
Recaptured does[1]	--	21/38 (55.3%)	12/48 (25%)	18/53 (34%)	**51/139 (36.7%)**

[1] Includes untreated and sham-treated controls

Table 3. Pregnancy rates in SpayVac® treated females, 2007-2008. Year 2 excludes females diagnosed as pregnant in Year 1.

SpayVac® Prep	Initial Capture	Year 1	Year 2
Aqueous	24/28 (85.7%)	0/9 (0%)	2/8 (25%)
Non-aqueous	20/26 (76.9%)	3/10 (30%)	2/8 (25%)
Total	**44/54 (81.5%)**	**3/19 (15.8%)**	**4/16 (25%)**

Table 4. Pregnancy rates of deer treated with a priming dose of PZP/mFCA or PZP/Adjuvac® plus cold-evaporated or heat extruded pellets in 2005-2007

	Initial Capture	Year 1	Year 2	Year 3
PZP/adj + cold-evaporated pellets	10/12 (83.3%)	2/8 (25%)	0/1 (0%)	--
PZP/adj + heat-extruded pellets	43/58 (74.1%)	3/19 (15.8%)	3/14 (21.4%)	0/2 (0%)
Total	53/70 (75.7%)	5/27 (18.5%)	3/15 (20%)	0/2 (0%)

Table 5. Pregnancy rates of remotely-delivered boosters of PZP/mFCA or FIA emulsions after one year. FIA was administered to deer that had previously received mFCA in priming doses.

Initial Treatment	Proportion pregnant
SpayVac 2005 Aqueous	0/6 (0%)
SpayVac 2005 Non-Aqueous	2/6 (33%)
Subtotal SpayVac	**2/12 (16.7%)**
Cold-evaporated pellets	0/2
Heat-extruded pellets	0/1
Subtotal pellets	**0/3**
Total	**2/15 (13.3%)**

Fecal steroid assays. Both fecal progesterones (I-PdG) and estrone conjugates (E1C) were significantly higher in pregnant than non-pregnant does (t-test for unequal variances; I-PdG: $t = 4.00$, $df = 52$, 2-tailed $P = 0.0002$; E1C: $t = 3.60$, $df = 32$, $P = 0.0005$; Table 6). In both measures, however, there was considerable overlap in the ranges of non-pregnant and pregnant females (Fig. 3, Fig. 4). Thus, no simple threshold value of either measurement can be used for pregnancy diagnosis.

Table 6. Mean levels of progesterones (I-PdG) and estrogen conjugates (E1C) in fecal samples collected from pregnant and non-pregnant females at time of capture. Pregnancy was diagnosed using serum P-SPB. Range in parenthesis is 95% CI.

	Non-pregnant	Pregnant
I-PdG	**161.0 ng/g (119.0 – 203.0)**	**327.9 ng/g (257.7 – 398.1)**
E1C	**2.32 ng/g (2.19 – 2.46)**	**3.95 ng/g (3.07-4.83)**

Fig. 3. Distribution of I-PdG levels in pregnant and non-pregnant females captured on Fripp Island in February-March 2008. Pregnancy was diagnosed using serum P-SPB.

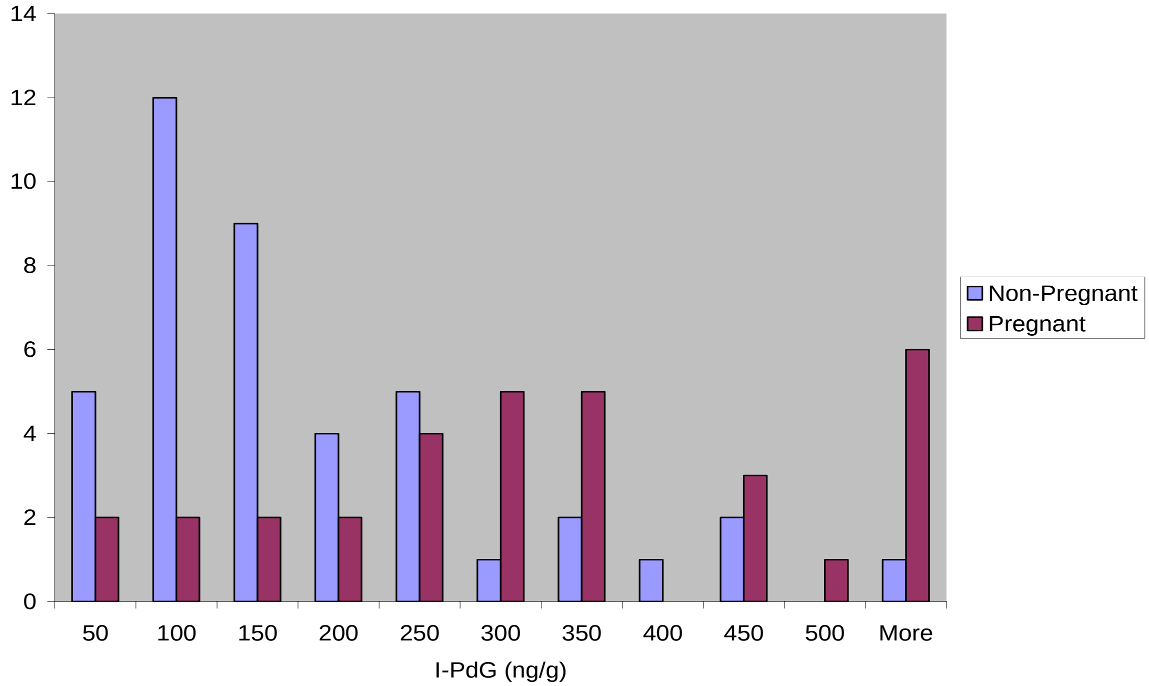
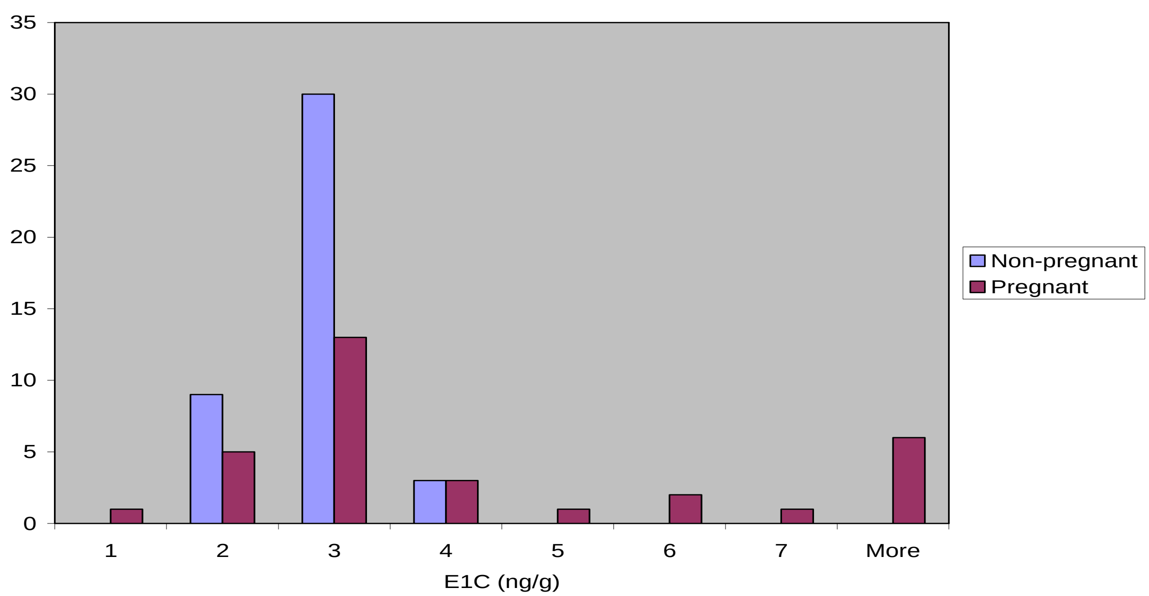


Figure 4. Distribution of E1C levels in pregnant and non-pregnant females captured on Fripp Island in February-March 2008. Pregnancy was diagnosed using serum P-SPB.



Miscellaneous. An additional 130 person-hours were spent in 2008 preparing drugs and darts to capture deer, collecting and labeling blood serum samples, recording data, and on other aspects of fieldwork.

Costs. The cost for vaccine, supplies, equipment, travel, and other related expenses in 2008 was approximately $39,048.88 (Table 1). Not included in that cost is the time of salaried HSUS, Tufts, and FIPOA employees, which was donated to the project.

Table 6. Summary of expenditures on Fripp Island deer research.

Description	Cost
Equipment and Supplies	$8348.15
Vaccine and Adjuvant	6,134.00
Blood Analysis	1975.00
Fecal sample analysis	1520.00
Travel	4664.52
Population Survey - Holterra	7807.21
Consultant labor – HSUS	8600.00
Total	**$39,048.88**

DISCUSSION

Distance sampling and mark-resight data, as well as informal accounts from Fripp Island residents, indicate a significant and visible reduction in fawning rates and deer numbers at Fripp in 2008. Deer densities on Fripp remain about twice as high as at the Hunting Island control site, but the gap has narrowed considerably since 2005-2006. Population reductions are occurring on Fripp more rapidly than observed at other contraception sites, perhaps because of (a) limited migration (suggested by the high proportion of tagged females in surveys); (b) annual mortality/disappearance rate of 18% among adult females and (c) higher contraceptive effectiveness.

The observation that 50 of 53 females observed during summer 2008 surveys carried ear tags suggests that the overwhelming majority of adult females on the island have now been incorporated into the study. Thus, future tagging efforts will probably focus on new female recruits in the population, and the occasional immigrant.

Visual and photographic evidence from winter 2008 also suggests an improvement in body and coat condition of deer on Fripp, although further analysis and additional years' data would be helpful to confirm this.

A growing sample size also indicates that single administrations of both SpayVac® (the 2006 preparations) and PZP in controlled-release pellets are highly effective in reducing pregnancy rates for two or more years. Remotely-delivered boosters also proved effective at providing one additional year's contraception; further data are required to determine whether some boosters produce contraception of longer duration.

The results of the analysis of i-PdG and E1C from fecal samples were both tantalizing and frustrating. Although there was a marked statistical difference in the levels of both of these steroids in pregnant and non-pregnant females, there was no clear boundary in either that would allow reliable pregnancy diagnosis. Additional analysis will be undertaken to discover whether some combination of the two values, or consideration of the time of sampling, will help produce a reliable index of pregnancy.

RESEARCH PLAN FOR 2009

Monitoring of Population Density. Distance sampling in Hunting Island State Park and on Fripp Island will be repeated in late early February 2009, following the 2005-2008 methodology. Likewise, island-wide capture-resight surveys will be conducted in August 2009.

Vaccination Procedures. Observations of female deer on Fripp Island during April-August 2008 suggest there may only be about 15 untagged adult females plus 10-15 untagged yearling females remaining. Vaccinations conducted in 2009 will consist of PZP in heat-treated pellets. For this year, new controls will be recruited in sufficient numbers to replace those that died in 2008. We project that vaccine efficacy trials will be complete after 2009; to better achieve population objectives, we will probably begin treating control females with contraceptives to 2010.

Beginning in February, we will capture up to 30 adult and yearling female deer using darting and handling procedures described above. These will be assigned to one of two treatment groups:

1) Untreated controls, or

2) 100µg PZP in 0.5 ml PBS emulsified with 0.5ml mFCA, plus 550µg PZP and 500µg QA-21 prepared in three "heat-extruded" (H-E) lactide-glycolide pellets.

In addition, during the summer of 2009 treated deer that have become pregnant after treatment will receive remote inoculations of PZP and mFCA (if they have not already been treated with mFCA) or Freund's Incomplete Adjuvant (FIA) (if they have previously been treated with FIA).

Pregnancy and fawning assessments. Using capture methods described above, we will recapture as many as possible of the surviving females (up to 70) that were treated and tagged in the winters of 2005-2008. Priority for capture will be placed on deer treated in 2006-2007 with PZP/H-E pellets, deer treated in 2008 with PZP/H-E pellets, and deer treated in 2007-2008 with remote boosters. Blood samples will be taken, and sent to BioTracking (Moscow, Idaho) for pregnancy testing using Pregnancy-Specific Protein B. Blood samples will also be taken from females captured for the first time in 2008 and submitted for pregnancy diagnosis.

Between May and September, 2009, attempts will be made to relocate all tagged deer to maintain an inventory of surviving deer, and to observe tagged deer for signs of lactation (extended udder), the repeated presence or absence of associated fawns, and nursing behavior by fawns.

Tissue collection. Reproductive tracts will be removed opportunistically from study subjects that are killed by cars or suffer other mortality incidental to the study. These tissues will be preservation for histological examination. All personnel will wear disposable rubber gloves and take other appropriate precautions while collecting and handling tissues from dead deer.

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