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

2009 PROGRESS REPORT
to the South Carolina Department of Natural Resources
and the Fripp Island Property Owners Association

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SUMMARY

In 2009, 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 fifth year of a field test of the safety, effectiveness, longevity, and population impacts of novel preparations of the porcine zona pellucida (PZP) contraceptive vaccine in white-tailed deer on Fripp Island, SC.

Both distance-sampling and mark-resight methods indicated a stabilizing of deer densities and continued decline in the number of fawns on Fripp Island between 2008 and 2009. From 2006-2009, deer numbers appear to have declined by about 30% on Fripp, and fawn:doe ratios have declined by about 84%. Population densities on the Hunting Island control site have remained stable during that period, and an apparent decline in fawn:doe ratios on Hunting Island appears to have been an artifact of a change in sampling procedure. Female:male ratios have been consistently but modestly lower on Fripp Island than on Hunting Island, but there has been no clear trend over time on either site. Annual survivorship of tagged females on Fripp Island averaged 85% between 2005 and 2009.

During February-March 2009, 14 new deer and 34 previously tagged deer were captured on Fripp Island using chemical immobilization, averaging approximately 6.8 person-hours/deer captured. All 14 of the new captures were ear-tagged, and 8 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. In August 2009, 23 deer that had been treated with one of a variety of PZP preparations in previous years were remotely darted with emulsions of PZP and mFCA or Freund's Incomplete Adjuvant, averaging approximately 2.2 person-hours/remote treatment. The additional costs of pellet-delivered vaccine, immobilizing drugs, and labor involved in capture drove the costs of capturing and treating deer with pellets to approximately 5X those of remote delivery, although these costs are offset in part by the multi-year effectiveness of the pellets.

Cumulative results from pregnancy tests on the blood serum of captured females showed that, with the exception of the 2007 treatment cohort (which we excluded because of vaccine handling problems), PZP/adjuvant primers delivered with heat-extruded (HE) controlled-release pellets produced a 0% pregnancy rate in the initial year (N=20), a 21% pregnancy rate in the second year (N=14), and a 0% pregnancy rate in the third year (N=8). Does that had previously given birth and were given remotely-delivered boosters showed a 13% pregnancy rate (N=16) in the first year after boosting, regardless of initial treatment.

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, poses technical and logistical challenges, 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 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 white-tailed deer, wild horses and other species (Gionfriddo et al. 2009; Killian et al. 2008; Fagerstone et al. 2008). As of September 2009, GonaCon® has been registered with the Environmental Protection Agency to control white-tailed deer populations (EPA Reg. No. 56228-40).

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), Wildlife Biometrics, and 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.

METHODS

Population studies. During the winters of 2005-2009 Frank Verret of Holterra Wildlife Management and Wildlife Biometrics 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 2009, 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. Most 2009 surveys were performed during nighttime (1800-0530 hr) hours. This year, however, some daytime transects were conducted to increase sample size for herd composition and doe/fawn ratios. 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. During these surveys, we also attempted to assess group composition to determine buck:doe ratios and fawn:doe ratios.

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.

To supplement the distance-sampling transect data, we carried out an inventory of tagged and untagged animals between 6-8 P.M. over a seven-day period in August, 2009, also noting the number of fawns and the sex of untagged animals.

Deer captures. Deer on Fripp Island were captured during February-March, 2009, 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 Model CO_2 PI or Model JM Standard CO_2 rifle. 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 additional darting was necessary to accomplish immobilization, we delivered the ketamine in either a 1 or 2cc standard Pneu-Dart darts with a 1" needle and single wire barb.

When the effects of the Telazol and/or Ketamine began to wear off (blinking, swallowing, tail twitching, etc.), Tolazoline was given intravenously and/or intramuscularly at approximately 4mg/kg to reverse the effects of xylazine.

Deer were either captured as we encountered them 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).

PZP treatments. After all standard procedures were completed, a sample of deer newly captured in 2009 was hand injected in the hip with 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 into the hip with a Dan-Inject Blo-jector® or Model JM Standard® CO_2 rifle. Each dart was then recovered and examined to confirm complete discharge. To test field applicability of novel equipment, and its potential for remote injection of pellets, a small number of deer were also darted with Palmer 1cc Aero-Inject® disposable darts with 1" plain needles and Palmer 1cc reusable air-inject darts with 1 1/8" needles with small nodules.

Observations of study animals. During one week in August 2009 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. Nineteen (19) sampling sessions were conducted during 11 February – 20 February 2009. 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 33.6 miles of roads on Fripp Island and systematically sampled 39.2 miles of road within Hunting Island State Park. The transect line added at Hunting Island in 2007 was retained (but see Discussion of population results below).

A total of 282 fawn, yearling, and adult deer in 103 clusters was detected on Fripp Island, and 87 fawn, yearling, and adult deer in 62 clusters were detected within Hunting Island State Park. Deer densities for Fripp Island and Hunting Island State Park were 130.1 deer/mi^2 (90%

C.I.:99.6-169.9 deer/mi^2) and 53.2 deer/mi^2 (90% CI: 37.4-76.5 deer/mi^2), respectively. Assuming areas of 2.32 mi^2 for Fripp Island and 3.14 mi^2 for Hunting Island leads to estimates of 302 deer on Fripp Island and 167 deer on Hunting Island. The estimated sex ratio was 1M:1.78F at Fripp Island and 1M:2.57F at Hunting Island State Park (standard errors of estimates of sex ratios <20%), with 0.23 fawns per doe observed at Fripp and 0.84 fawns per doe observed at Hunting Island.

Since 2005, estimated population density has declined by about 30% on Fripp Island, with most of that decline taking place between 2006 and 2008. Estimated population density has remained stable on Hunting Island (Fig. 1a). Since 2006, the number of fawns per female observed has declined by approximately 84% on Fripp Island, but has also declined by about 43% on Hunting Island (but see sampling issues raised in Discussion) (Fig. 1b). The number of females per male has been stable on Fripp Island, and the apparent decline on Hunting Island has been reversed, with Fripp Island continuing to show a slightly lower proportion of females in the population (Fig. 1c).

Figure 1. Deer population trends in distance samples conducted at Fripp Island and Hunting Island State Park, February-March 2005-2008. (b); (c).

a) Population density

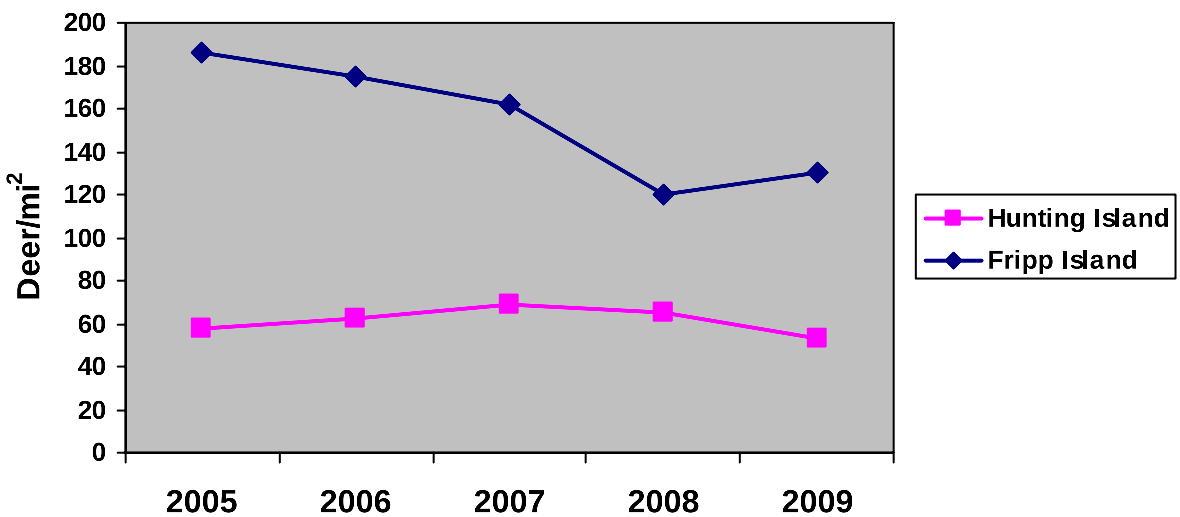
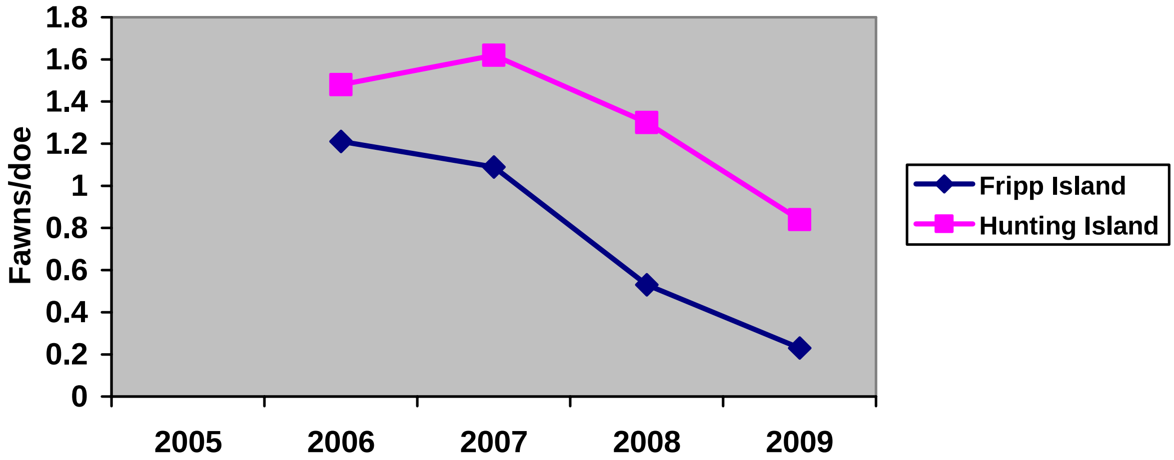
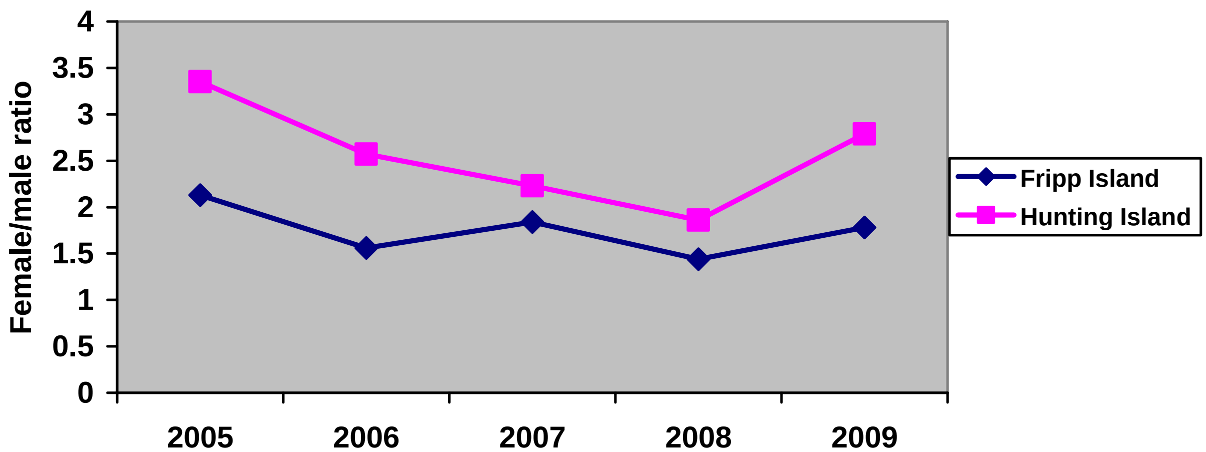


Figure 1 (cont'd)

b) Fawns/doe



c) Females/male



Summer mark-resight surveys also suggest a long-term 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 2009 suggest a 14% reduction in the number of does (and a similar decrease in overall population size) over that period. The approximately 66% reduction in fawn:doe ratios between 2006 and 2009 also parallels the decline observed in distance sampling.

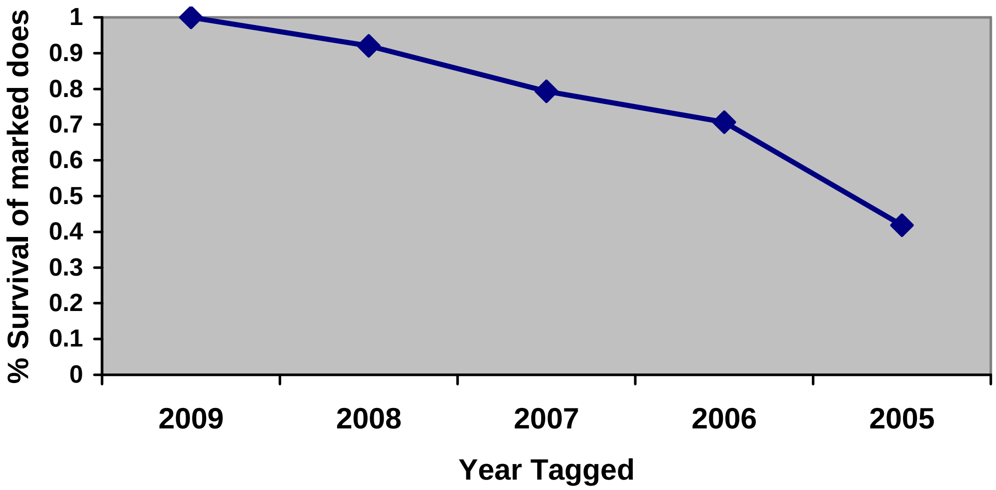
Table 1. Island-wide surveys on Fripp Island, 2006-2009. Surveys conducted in 2006 and 2008 were transect-based daytime surveys; the 2009 survey was conducted in early evening, and records the total number of individuals sighted. Total number of does is estimated using a simple Lincoln-Peterson index 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	Does /buck	Fawns /doe	Estimated # Does	Estimated Total Population
August 2006 (3)	76	56	147	2.18	0.32	200	357
November-December 2006 (5)	48	41	147	2.04	0.28	173	308
August 2008 (2)	53	50	151	1.64	0.20	158	285
August 2009 (7)	152	138	157[1]	1.59	0.11	173	299

[1] This number reflects marked females seen in February/March and/or August 2009, and therefore does not account for deaths or disappearances between February and August. This could lead to an overestimate of population numbers.

Female survivorship. During inventories of tagged females conducted during August 2009, we sighted 39 of 93 (41.9%) of females tagged in 2005, 58 of 83 (70.7%) of the females tagged in 2006, 23 of 29 (79.3%) of the females tagged in 2007, and 23 of 25 (92.0%) of females tagged in 2008 (Fig. 2). A log-linear regression over the five year period yields a crude annual survival rate among marked adult and yearling females of 84.5%. If the initial (2005) cohort is excluded, log-linear regression yields an annual survival rate of 89.3%.

Figure 2. Survival to August of cohorts of adult and yearling female deer eartagged in February-March since 2005.



Deer capture and treatment. Three people spent 22 days in February-March 2009 capturing 14 new deer and 34 previously tagged deer using chemical immobilization. A total of 325.5 person-hours was spent capturing and handling the deer, at an average of 14.8 person hours/day, 2.2 deer captured/day, and 6.8 person-hours/deer captured. Two deer were not captured after being darted and 1 deer was missed. All three darts were recovered.

Eight of the 14 new deer were ear-tagged and treated with 3 heat evaporated pellets + PZP emulsified in mFCA as described above. Six were ear-tagged but not treated. Blood samples were successfully obtained for 11 of the 14 new deer, and for 32 of the 34 recaptures.

Over a 7 day period in August, 2009, one person remotely treated 23 deer previously treated with either Spay-Vac®, Adjuvac® + pellets, or mFCA + PZP and pellets. All 23 females had been determined pregnant either visually (distended udder) or by P-SPB diagnosis. Of the 23, 21 received treatments of PZP/FIA and 2 received treatments of PZP/mFCA. In addition, we attempted to treat 4 deer with Palmer darts; none of the 4 received satisfactory injections (defined as 0.5 or more of the dart contents). A total of 54 person-hours was spent darting at an average of 3.6 deer/day and 2.2 person-hours/treatment.

An additional 66 person-hours were spent in 2009 practicing, preparing drugs, making darts, centrifuging blood, recording data, and carrying out other support functions in the field during capture efforts. During remote darting, approximately 14 person-hours were spent in practicing, preparing vaccine, loading darts, recording data, and carrying out other field support functions.

Reproduction and vaccine effectiveness. Pregnancy diagnoses using PSPB indicated that 6 of 11 (54.5%) new females captured in 2009 were pregnant, and 8 of 32 females (25%) recaptured in 2009 were pregnant. Overall, from 2005-2009, 183 of 231 females (79.2%) were pregnant at the time of initial capture, presumably reflecting the baseline pregnancy rate among untreated female deer at Fripp (Table 2). Across all years, pregnancy rates among recaptured does are significantly lower than pregnancy rates among first-time captures ($\chi2=82.01$, df=1, $P<0.0001$).

With the exception of the 2007 cohort of mFCA/Heat-extruded (HE) controlled-release pellet-treated deer, the primer/HE pellet combination showed consistently high effectiveness for up to three years (Table 3; see Discussion). In 2009, none of the 11 recaptured does treated in 2008 with the HE pellets and PZP/mFCA primer was diagnosed as pregnant; however, 6 of the 8 does treated with PZP/mFCA and pellets in 2007 were pregnant. None of the 6 recaptured does treated in 2006 with HE pellets and PZP/Adjuvac® primer was pregnant.

No additional data were collected in 2009 on unboosted females initially treated in 2006 with cold-evaporated pellets. Only 3 unboosted Spay-Vac® treated deer (2005-2006) were captured in 2009; one was pregnant.

Data on the effectiveness of booster treatments continue to indicate at least one year of additional contraception. Between 2007 and 2009, 2 of 16 deer treated with boosters (12.5%) became pregnant in the year after boosting; 1 of 2 became pregnant two years after boosting. There were insufficient data to determine whether different initial treatments responded differently to boosters (although both of the deer that became pregnant after one year had been treated with non-aqueous Spay-Vac®).

Table 2. Proportion of captured does pregnant, as indicated by Pregnancy-Specific Protein B (P-SPB) in blood samples, 2005-2009.

Proportion Pregnant	2005	2006	2007	2008	2009	Total
Newly captured does	79/92 (85.9%)	61/76 (80.3%)	19/27 (70.4%)	18/25 (72%)	6/11 (54.5%)	**183/231 (79.2%)**
Recaptured does[1]	--	21/38 (55.3%)	12/48 (25%)	18/53 (34%)	8/32 (25%)	**59/171 (34.5%)**

[1] Includes untreated and sham-treated controls

Table 3. Pregnancy rates of deer treated with a priming dose of PZP/mFCA or PZP/Adjuvac® plus heat extruded pellets in 2005-2009

	Initial Capture	**Year 1**	**Year 2**	**Year 3**
PZP/mFCA + HE pellets (2007)	19/27 (70.4%)	4/11 (36.4%)	6/8 (75%)	-
PZP/mFCA + HE pellets (2005, 2008)	24/31 (77.4%)	0/13 (0%)	1/2 (50%)	0/2 (0%)
PZP/Adjuvac® + HE pellets (2005-2006)	19/25 (76.0%)	0/7 (0%)	2/12 (16.7%)	0/6 (0%)
Total	62/83 (74.7%)	4/31 (12.9%)	9/22 (40.9%)	0/8 (0%)

2009 Costs. The actual cost for vaccine, supplies, equipment, travel, consultants and other related expenses in 2009 was approximately $24,240 (Table 4). Incorporating estimated salary costs for the PI and field director for all project-related expenses (including project design, permit and protocol preparation, data summaries, correspondence, and other activities) adds $16,250 to the cost, for a total estimated project expenditure of $40,940.

Estimated Direct Costs of 2009 Treatments. To make cost estimates more useful to wildlife managers, we used effort and cost estimates above to derive per deer direct costs of capture and PZP treatment (Table 5). Initial capture and vaccination with PZP/primer + HE pellets amounted to an estimated $513 per deer (with labor and vaccine costs accounting for approximately 73% of the total). Delivering remote injections with a standard PZP/mFCA or PZP/FIA booster cost approximately $103 per deer (with labor and vaccine costs accounting for approximately 65% of the total). These estimates assume a uniform labor cost of $15/hour, and include

- all time spent in the field (by paid and unpaid participants) as well as the 80 hours spent in supporting activities
- onsite travel and expenses and travel to and from the site
- all vaccine and adjuvant costs
- all field supplies such as immobilizing drugs, syringes, darts, and transmitters for darts

The estimates do not include costs of labor (such as salaries) for offsite, research-related activities, blood sampling for pregnancy diagnosis, or population surveys, and they are not comparable to expenditures summarized in Table 4. These estimates also do not include start-up equipment costs such as dart rifles and radiotelemetry monitoring equipment.

Table 4. Summary of 2009 research expenditures on Fripp Island deer research.

Category	Cost
Equipment and Supplies	$4,237
Vaccine and Adjuvant	3,286
Blood Analysis	1,075
Travel (Naugle and Gilllikin)	3228
Population Survey - (Verret)	8714
Paid Consultant labor – Tufts	3700
SUBTOTAL DIRECT EXPENSES	**$24,240**
HSUS salary & benefits for field and support work (Naugle) (20% FTE)	9,000
Tufts salary & benefits for scientific support, permit & report preparation (Rutberg) (8% FTE)	7,250
SUBTOTAL, SALARIES	**$16,250**
GRAND TOTAL 2009 COST	**$40,490**

Table 5. Summary of estimated direct per-deer costs of capture, treatment, and boosting of deer on Fripp Island in 2009.

Deer Capture and Treatment	Cost per Deer
Labor (8.2 person-hours @ $15/hr)	$123
Vaccine (PZP/mFCA primer + HE pellets)	$252
Drugs and supplies	$77
Travel (onsite and to and from field site)	$61
TOTAL FOR CAPTURE AND TREATMENT	**$513**
Remote Booster Delivery	
Labor (3.0 person-hours @ $15/hr)	$45
Vaccine (PZP/mFCA or FIA booster)	$22
Supplies	$23
Travel (onsite and to and from field site)	$13
TOTAL FOR REMOTE BOOSTER DELIVERY	**$103**

DISCUSSION

Population Trends. Distance sampling and systematic observations on tagged and untagged animals confirm that the deer population on Fripp Island is about 30% smaller than it was at the beginning of the study, whereas the deer population on the Hunting Island control site has remained stable. Population numbers at Fripp in 2009 appeared similar to those of 2008; however, both distance sampling and observations indicate that the proportion of does with fawns on Fripp continued to drop in 2009, which is encouraging with respect to future population trends.

Curiously, the trend with respect to fawn:doe ratios on the Hunting Island control site appears to parallel the decline observed on Fripp Island (Fig. 1b). One hypothesis to explain the Hunting Island decline is that PZP-treated does are moving from Fripp to Hunting Island. However, Mr. Verret and his associates report never having seen an eartagged doe (or one with a torn ear that would indicate a lost tag) during transect surveys on Hunting Island, suggesting that the Hunting Island fawning trend is unrelated to activities on Fripp. A better-supported hypothesis is that the trend is an artifact of a change in sampling methods. In 2007, a transect along U.S. 21 was added to the sampling protocol (and its influence increased in subsequent years); virtually no fawns were observed on this transect, probably because of deer behavioral responses to heavy vehicle traffic and the dense vegetation barrier that lines the road (F. Verret, pers. comm.). Excluding the U.S 21 transect from the 2008 and 2009 samples raises both fawn:doe ratio estimates considerably (from 1.3 to 1.43 and 0.83 to 1.29, respectively), and nearly eliminates the apparent decline.

Re-sighting of ear-tagged females that previously had been described as missing also raised estimates of annual adult survival on Fripp Island. Annual survival of ear-tagged females on Fripp since 2006 is approaching 90%, which suggests that future declines in the population will be slow until the current cohort begins to die of age-related causes. Similar trends have been seen at the deer immunocontraception site at the National Institute of Standards and Technology (NIST) in Maryland; future data analysis at both sites will focus on survivorship of PZP-treated females and the impacts of changes in survivorship on population dynamics.

Vaccine Effectiveness and Duration. The growing sample size continues to indicate that single administrations of both SpayVac® (the 2006 preparations) and PZP in heat extruded (HE) controlled-release pellets are highly effective in reducing pregnancy rates for two or more years. Both Adjuvac® and modified Freund's Complete Adjuvant (mFCA) both appear to be highly effective. Examination of records from the 2007 PZP/mFCA treatments strongly suggest that the reduced effectiveness of vaccine delivered that year was due to prolonged accidental thawing of the PZP vaccine, rather than the adjuvant used; other years in which mFCA has been used so far indicate very high efficacy.

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.

Cost and Effort. Total direct cost of the project declined significantly this year, principally due to reduction in the number of animals captured and treated with initial shots of PZP/mFCA and HE pellets.

This year we developed new per-deer estimates of field costs for prospective management purposes. What stands out is that the per-deer cost of capturing and hand-treating deer with primer and controlled release pellets is approximately five times (5X) the cost of remote delivery of a simple PZP/adjuvant emulsion. This cost differential is partly offset by the multi-year effectiveness of the controlled-release pellet; whether it would be fully offset would depend on (1) the duration of effectiveness of the controlled-release pellet; (2) increased deer wariness due to repeated dartings, which could drive up the labor cost of repeated boosting; and (3) potential reductions in the cost of the controlled-release pellets through increased manufacturing efficiency. Capacity to remotely deliver controlled-release pellets would also save considerably on costs, for both initial treatments and boosters.

RESEARCH PLAN FOR 2010

Monitoring of Population Density. Distance sampling in Hunting Island State Park and on Fripp Island will be repeated in late early February 2010, following the 2005-2009 methodology. Likewise, capture-resight surveys and inventories of tagged and untagged deer will be conducted on Fripp Island in March and August 2010.

Pregnancy and fawning assessments. Using capture methods described above, we will recapture up to 70 surviving females that were treated and tagged in the winters of 2005-2009. 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 2009 and submitted for pregnancy diagnosis.

Contraceptive effectiveness in three groups of previously treated deer is of particular interest.

1) Deer treated with PZP/mFCA primers and heat-extruded (HE) PZP pellets in 2008 and 2009. Evidence from the females treated with PZP/mFCA and HE pellets in 2008 suggests a very high level of effectiveness for the first year, and small numbers of surviving deer treated in 2005 and 2006 hint at longer term effectiveness. Because of the poor results obtained from the 2007 PZP/mFCA and HE pellets, we want to confirm that PZP/mFCA+HE pellets are highly effective for one and possibly two years.
2) Significant numbers of females treated in 2006 with PZP/Adjuvac® primers and HE pellets are still alive, and pregnancy rates have been very low in this group. We are interested in determining pregnancy rates in this group to determine how effective this preparation is in its 4^{th} year.
3) Deer treated with a variety of initial preparations in 2005 and 2006 received boosters in 2008. Evaluating pregnancies in this group will allow us to estimate second-year effectiveness of remote boosters. Secondarily, we will also look at pregnancy rates among females treated with remote boosters in 2009.

Vaccination Tests. As noted in the discussion above, reducing the costs of the controlled-release vaccine, the need to capture deer, and the frequency of re-boosting deer are the keys to making immunocontraception a more cost-effective management tool. Dr. John W. Turner, Jr., at University of Toledo, Ohio, has developed a pressure-molding (PM) technique for manufacturing pellets that reduces PZP wastage (and costs) by 1/3 or more. Preliminary tests have been promising. I*n vitro* tests of vaccine release rates in PM pellets indicate that they are similar to those of HE pellets. Anti-PZP antibody titers of one- and three-month PM pellets closely matched those of HE pellets in captive wild horses over a ten-month period. Adding to potential savings, reducing the amount of PZP in the one- and three-month pellets by 50% had no impact on horse antibody titers. At the same time, one of us (RN) has been working with Palmer and Pneu-dart® to develop and test remote darts for remote delivery of pellets. Beginning with 2010 treatments, we will test half-dose PM pellets and remote delivery at Fripp, and follow up with monitoring for two more years.

Observations of female deer on Fripp Island in 2009 suggest that there are about 15 untagged adult (2+ year-old) females. In addition, there are approximately 20 previously ear-tagged but untreated females. Beginning in February, we will capture and/or remotely treat up to 35 adult female deer using darting and handling procedures described above.

To carry out the tests described above these deer will be assigned in equal numbers to one of three treatment groups:

1. Hand injection of PZP/mFCA primers plus three half-dose PM pellets engineered to release at 1, 3, and 12 months.
2. Remote injection of PZP/mFCA primers plus three full-dose HE pellets engineered to release at 1, 3, and 12 months.
3. Remote injection of PZP/mFCA primers plus three half-dose DC pellets engineered to release at 1, 3, and 12 months.

This design will allow us to compare (1) the effectiveness of HE pellets when delivered remotely to existing data on effectiveness of HE pellets when hand-injected; (2) the effectiveness of half-dose PM pellets when hand-injected to existing data on the effectiveness of HE pellets when hand injected; and (3) the effectiveness of half-dose PM pellets when delivered remotely to the effectiveness of DC pellets when hand-injected.

In addition, during the summer of 2010, up to 30 previously treated deer will receive remote inoculations of PZP and mFCA or FIA with HE or PM pellets, and up to 10 previously treated deer will receive remote inoculations of PZP with mFCA or FIA only. This will allow a comparison of effectiveness and longevity of the two kinds of pellets with the standard PZP/FIA emulsion booster and with each other.

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.

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