

Study Proposal

Deer immunocontraception in the Village of Hastings-on-Hudson, New York
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Background

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 densely populated or intensively used areas that are 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 open spaces within these communities. To be practical for suburban and urban deer management, a contraceptive should induce long-acting effectiveness with a single treatment; be deliverable remotely; and be relatively inexpensive. Previous studies have shown that the porcine zona pellucida (PZP) vaccine is free of serious health side effects, dramatically reduces pregnancy rates in treated female white-tailed deer, and can stabilize and gradually reduce deer populations over limited areas ((Turner, Liu et al. 1992; Naugle, Rutberg et al. 2002; Rutberg, Naugle et al. 2004; Rutberg and Naugle 2008). However, these earlier PZP deer studies employed vaccines that require two initial shots and annual boosters to achieve full effectiveness, which increases stress on treated animals, poses technical and logistical challenges, and limits the scope of potential management applications.

Three technologies for single-shot effectiveness have emerged in recent years: Spay-Vac®, ImmunoVaccine Technologies, Halifax (Brown, Bowen et al. 1997; Fraker, Brown et al. 2002; Locke, Cook et al. 2007; Rutberg, Naugle et al. *in press*); GonaCon®, USDA National Wildlife Research Center, Fort Collins (Gionfriddo, Eisemann et al. 2009; Gionfriddo, Denicola et al. 2011); and the standard PZP/adjuvant emulsion supplemented with timed-release lactide-glycolide PZP pellets (Turner, Liu et al. 2007; Turner, Rutberg et al. 2008). One of these, GonaCon®, has been EPA-registered for use in deer; however, the EPA registration specifies hand-delivery, and efficacy in the field has been low compared to the other two approaches. The multiple-shot PZP/adjuvant emulsion vaccine has been EPA-registered (as ZonaStat-H®) for use in wild and feral horses and burros, but has not yet been registered in any form for deer.

Tests of controlled-release PZP vaccines on white-tailed deer began on Fripp Island in 2005 (Turner, Rutberg et al. 2008; Rutberg and Naugle 2012; Rutberg, Naugle et al. *in press*). In this study, captured deer were injected by hand in February-March with an emulsion of PZP in Adjuvac® (USDA, Fort Collins) or modified Freund's Complete Adjuvant (mFCA; Calbiochem) plus 3 controlled-release lactide-glycolide pellets containing PZP plus the QA-21 adjuvant (Antigenics, Lexington, MA), with the pellets engineered for peak release at 1, 3 and 12 months. Through 2010, pregnancy rates among treated deer were 4% in year one and 26% in year 2, vs 78% in all untreated does, although there was some inconsistency between years. For at least some individuals, effectiveness appears to last into a third year.

Boosters of PZP/Freund's Incomplete Adjuvant (FIA) emulsions with and without controlled release pellets delivered remotely in late summer 2.5+ years after initial treatments extended infertility for 1-2 years. However, sample sizes (N=4) for dart-delivered boosters of emulsions with controlled-release pellets were too small to draw firm conclusions.

In 2010, a small pilot trial was conducted in which a number of previously untreated does received remote vaccinations of the pellet treatment using modified Pneu-darts or Palmer darts; there was some evidence of effectiveness in years one and two, but sample sizes were again too small to be more than suggestive.

At the population level, estimates of deer densities at Fripp Island declined by 50% between 2005 and 2011, reflecting a more rapid decline than those observed at other study sites (Rutberg and Naugle 2008; Rutberg and Naugle 2012). (Deer population densities at an adjacent unmanaged control site, Hunting Island, showed no time trends during that period.) It is not clear whether this improvement in population-level effectiveness at Fripp Island is due to use of improved technology, to lower levels of immigration relative to the Fire Island and NIST sites, or to other demographic or environmental factors.

Study Purpose: Research Questions

Testing of dart-delivered pellet/emulsion boosters. The Fripp Island study gives us confidence that PZP/adjuvant emulsion plus lactide-glycolide controlled-release PZP pellets provides 1-3 years of effectiveness following a single hand administration in February-March (Rutberg, Naugle et al. *in press*). Because New York and other states require permanent tagging, and thus capture, of deer receiving contraceptives, hand-administration will continue to be appropriate for initial treatments. Once tagged, however, deer should receive boosters by dart. The specific experimental question we wish to pose is whether using a dart that delivers timed-release pellets in addition to the standard PZP/FIA emulsion extends the contraceptive effectiveness of the booster. This is important because, at least at the current stage of technology, the controlled-release pellets add approximately \$200 to the cost of the PZP emulsion booster.

Population management. The effectiveness of contraception or any other localized population control method will be site specific, with movement of deer on and offsite being an important variable determining success. Of the three sites from which we have data on deer population changes associated with PZP immunocontraception efforts (Fire Island, Fripp Island, and NIST), only one (Fripp Island) appears to have been truly isolated; the Fire Island study sites included

only part of the 34 mile long island, and immigration rates at NIST have averaged about 8% per year. Immigration rates for deer at Hastings-on-Hudson are unknown, but the absence of clear borders poses a new and challenging environment for the application of contraception for management of deer populations.

In summary, this trial will focus on two questions.

1. Do remotely delivered boosters consisting of PZP/FIA emulsion plus PZP/QA-21 pellets provide longer-lasting contraceptive efficacy than do remotely-delivered boosters consisting of the PZP/FIA emulsion alone?
2. What will be the effects on fawn production and population growth of PZP contraceptive treatments in an open suburban population of white-tailed deer?

Study Site

The study will be carried out in the Village of Hastings on Hudson, in the Town of Greenburgh, Westchester County, NY.

At the outset of the study, all capture and darting of deer will be carried out in Hillside Park, adjoining open space, and other identified open spaces. Hillside Park is a heavily wooded, 100-acre park with an open understory (partly due to heavy deer browsing). Typical of Westchester County, Hillside Park is characterized by a series of north-south running ridges, with elevations ranging from approximately 170-430'. The site is bounded by the Saw Mill River Parkway on the east, by forest in the Village of Dobbs Ferry on the north, and by housing (mostly zoned for 0.5 acres) on the south and west. Other capture or darting sites may be identified during the course of the study; all sites within Hillside Park and elsewhere will be chosen in consultation with the NYSDEC, village law enforcement, and affected private property owners so as to assure safety and compliance with all relevant laws and regulations.

Study Design

During the winters of 2014, 2015, and if needed, 2016, up to 50 female deer will be captured via chemical immobilization, ear-tagged, blood-sampled for pregnancy testing, and administered an initial treatment of PZP/mFCA emulsion plus PZP/QA-21 in lactide-glycolide polymer pellets engineered to release at 1, 3, and 12 months. Tagged, treated deer will be monitored for fawns to determine whether observations are consistent with pregnancy test results, and monitoring procedures will be adjusted in subsequent years if there are significant inconsistencies. To measure vaccine effectiveness and longevity, fawning of treated deer will be monitored for two to three years after initial treatment.

Beginning in late summer 2016 or 2017, all tagged, treated deer that can be relocated will receive dart-delivered boosters of either PZP/FIA emulsion alone or PZP/FIA emulsion plus PZP/QA-21 in lactide-glycolide polymer pellets engineered to release at 1, 3, and 12 months. Fawning of boosted deer will be monitored for 2 to 3 additional years to determine whether the two treatments differ in effectiveness and longevity.

At the same time, we will use semi-annual transect surveys, monitoring by resident volunteers, and other methods to examine trends in population fawning rates, population size, and to the extent practical, mortality/disappearance rates.

Methods

Deer capture. Deer will be captured during February-March using a combination of Xylazine HCl (at approximately 2.2 mg/kg) and Telazol HCl (at approximately 4.4 mg/kg). The drug combination will be loaded into self-injecting 1 cc Pneu-Dart® transmitter darts with 1" needles and single wire barbs or Palmer Cap-Chur transmitter darts with 1" needles and single wire barbs. Darts will be delivered intramuscularly in the hip from a Dan-Inject Model CO₂ PI pistol or Model JM Standard CO₂ rifle or equivalent. Dart transmitters have a tracking range of approximately 1 kilometer and will be tracked with a Telonics TR-4 receiver and Yagi antenna.

Deer that fail to become fully sedated or are difficult to restrain may be given supplemental injections of Ketamine HCL (at approximately 5 mg/kg). When additional darting is necessary to accomplish immobilization, the ketamine will be delivered in either a 1 or 2cc standard Pneu-Dart dart with a 1" needle and single wire barb. Captured deer will be fitted with two plastic eartags marked with a unique identifying number. Tags will be labeled on the back with "EXPERIMENTAL ANIMAL, DO NOT CONSUME" and a telephone number where information could be obtained in the event an animal is killed or found.

PZP treatments will be administered as described above, and measurements taken on girth, hind-foot length, and body length. Where practical, each deer will be weighed, and age (adult, yearling, fawn) determined by examining tooth wear. Deer will be assessed for any prior injuries, and mammary glands were visually examined for signs of lactation.

When the effects of the Telazol and/or Ketamine begin to wear off (as exhibited by blinking, swallowing, tail twitching, or limb movements), Tolazoline will be given intravenously and/or intramuscularly at approximately 4mg/kg to reverse the effects of xylazine.

PZP treatments. At the time of capture, deer will be 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 lactide-glycolide pellets engineered to release at 1 months, 3 months, and 12 months. These three pellets will be loaded into 14 gauge needles and injected along with the PZP emulsion using a trochar syringe supplied by Dr. John Turner of the University of Toledo, Ohio.

Females receiving boosters will be given either (1) 100µg PZP in 0.5 ml PBS emulsified in 0.5ml FIA delivered in 1 cc Pneu-Dart darts with 1" or ¾" barbless needles; or (2) 100µg PZP in 0.5 ml PBS emulsified in 0.5ml FIA plus 550µg PZP and 500µg QA-21 prepared in three heat-extruded lactide-glycolide pellets engineered to release at 1 months, 3 months, and 12 months delivered in modified 1 cc Pneu-Dart or Palmer darts with 1" or ¾" barbless needles modified to simultaneously inject emulsion and pellets. All darts will be directed toward the hip from a Dan-Inject Model CO₂ PI pistol or Model JM Standard CO₂ rifle or equivalent. Every effort will be made to recover the dart and examine it to confirm complete discharge.

PZP for primers will be produced at the Science and Conservation Center, Billings Montana; PZP for pellets will be produced by Dr. Irwin Liu in Davis, CA. The PZP/QA-21 heat extruded pellets will be prepared in the laboratory of Dr. Douglas Flanagan, College of Pharmacy, University of Iowa.

Data Collection

Pending field experience in Hastings, we can only outline our possible approaches to monitoring individual fawning and population reproductive rates, and population growth.

Monitoring of individual reproduction. Fawning by individual females will be monitored by a combination of blood pregnancy testing (see below), systematic observations of fawns and swollen udders in females by project personnel in April-June, and observations of fawns of ear-tagged females and other deer by citizen volunteers (as described in “Deer metrics proposal”).

Pregnancy testing via blood sampling will be conducted at the time of initial capture. Capture and blood sampling of eartagged deer subsequent to initial capture will only be carried out if pregnancy diagnoses based on blood sampling at initial capture are inconsistent with observations of fawns. Capture of previously ear-tagged deer also depends on their accessibility for darting, with the understanding that darting and capture of any individual deer become progressively more difficult with each successive attempt.

At the time of capture, 10 ml of blood will be collected from each deer. Blood will be collected from veins in the lower leg or the jugular vein in the neck using an 18 gauge needle. Blood will be cooled for a minimum of 30 minutes and centrifuged. After centrifugation the serum will be poured off, labeled, and immediately frozen. Samples will be 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).

Monitoring population changes. Population size and density, fawn/doe ratios, and male/female ratios will be estimated using multiple methods. Transects will be designed based on preliminary observations of deer distributions by citizen volunteers, and transect counts will be carried out by project personnel in early spring and early autumn. Mark/resight estimates will be attempted once a significant number of females has been eartagged; and observations of deer by citizen volunteers will be compiled and interpreted. The population may be small enough and females predictable enough in their movements to allow identification of some untagged females based on association patterns and geographic distribution. Reports of deer deaths (including ear-tagged deer) from police logs and other sources will be used to provide minimum estimates of deer mortality.

Required Approvals

Project authorization will be required from the New York State Department of Environmental Conservation (NYSDEC), the Tufts University Institutional Animal Care and Use Committee,

and the federal Environmental Protection Agency. Consent will also be required from local jurisdictions and any private landowners whose property is used for study activities.

Timeline

2014	February-March	Initial capture, ear-tagging, and initial PZP treatments
	April – June	Fawn observations, spring population survey
	October	Fall population survey
2015	February-March	Initial capture, ear-tagging, and PZP treatments
	April – June	Fawn observations, spring population survey
	October	Fall population survey
2016	February-March	Initial capture, ear-tagging, and PZP treatments (if needed)
	April – June	Fawn observations, spring population survey
	August – September	Booster darting of deer tagged in 2014
	October	Fall population survey
2017	April – June	Fawn observations, spring population survey
	August – September	Booster darting of deer tagged in 2015
	October	Fall population survey
2018	April – June	Fawn observations, spring population survey
	October	Fall population survey
2019	April – June	Fawn observations, spring population survey

Designated Agents

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