

**Study Proposal : 8-14-13 Draft**  
**Deer immunocontraception in the Village of Hastings-on-Hudson, New York**  
**NYSDEC License to Collect or Possess**

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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.

One approach that is being explored is surgical sterilization. Surgical sterilization of urban white-tailed deer has been conducted at several sites (Gilman, Mathews et al. 2010). It is effective at the individual level, and several modeling exercises suggest that it can be effective as a population control technique in relatively closed populations (Merrill, Cooch et al. 2003, Merrill, Cooch et al. 2006). However, surgical sterilization is expensive; Boulanger et al. (2012) cite costs of \$1,000 or more per deer in three limited field studies, and there is no clear route to improved cost-efficiency, given requirements for specialized equipment and the direct participation of experienced veterinarians and other highly skilled personnel (MacLean, Mathews et al. 2006, Boulanger, Curtis et al. 2012).

Immunocontraception has also 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. 2013); 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. 2013). 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 Objectives

**1. Experimental comparison of dart-delivered pellet/emulsion boosters with standard dart-delivered emulsion-only 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. 2013). 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 pose is whether and how much does using a dart that delivers timed-release pellets in addition to the standard PZP/FIA emulsion increase and extend the contraceptive effectiveness of the booster. Knowing the difference in effectiveness and longevity between the two types of boosters will be necessary to predict the relative costs of applying them in a management context.

**2. 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. The Village of Hastings-on-Hudson has proposed that reducing the deer population by 35-45% over five years may meet short-term deer population management objectives. This rate is slightly faster than that seen in the initial years of the NIST study and in the Fire Island study, but slightly slower than that observed at Fripp Island (Rutberg and Naugle 2008, Rutberg and Naugle 2012) The study will include the collection of metrics that will seek to determine whether any drop in population levels will have a concurrent impact on the negative impacts of the deer (see below under “Impact Studies”).

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. Although the village is bordered on to the west by the Hudson River, and parkways and other geographic boundaries may inhibit deer ingress from the east, immigration routes from the north and south may permit deer to enter and leave the site. Thus, the absence of clear borders poses a new and challenging environment for the application of contraception for management of deer populations.

**3. Impact studies.** In association with the deer population management study, the Village of Hastings on Hudson will examine the impacts of deer on native vegetation, ornamental plantings, and deer-vehicle collisions, and if feasible will develop methods for evaluating changes in public attitudes towards deer.

In summary, this trial will focus on three 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?
3. What changes in deer impacts on vegetation and vehicle safety are associated with the contraception project?

## **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 potential capture or darting sites are shown in Figure 1; which of those sites are ultimately used for capture of deer will depend on deer movements, safety and suitability of the sites, consistency of activities with NY State and local laws and regulations, and willingness of adjacent property owners to allow darting less than 500 feet from occupied residences or other occupied structures.

## **Study Design**

During the winters of 2014, 2015, and if needed, 2016, up to 60 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 located again 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 camera-trap surveys, supplemented and informed by observations compiled by resident volunteers, and other methods as developed to examine trends in population fawning rates, population size, and to the extent practical, mortality/disappearance rates.

## Methods

### Deer capture

***Chemical Immobilization by Remote Darting.*** In the first year of the study (winter 2014), all captures will take place using chemical immobilization by darting. Darting will only occur at sites which are either more than 500' from an occupied structure or for which permission to dart has been obtained from all landowners and occupants within 500' of the darting site. When identifying potential sites, and when taking shots at identified sites, the darting team will assess safety risks associated with both taking the shot and with post-darting movement of deer, including proximity of other deer, other animals, people, roads, potentially hazardous landscape features, and other human activities that may interfere with safe darting and the handling of immobilized deer. It is anticipated that most darting will occur at baited sites. Bait will consist of cracked corn, potentially augmented with apples or other attractants. Bait stations will be pre-baited for 2-4 weeks prior to commencement of darting, and baiting will be discontinued immediately when darting activities cease. Every effort will be made to minimize the amount of bait used, and the time during which it is made available. Bait sites will be regularly inspected for signs that non-target animals are visiting the site. If needed to reduce labor costs, bait stations will each have 1-2 automatic ground feeders that can dispense bait at preset times.

Deer will be captured by dart 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 CO<sub>2</sub> PI pistol or Model JM Standard CO<sub>2</sub> 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.

Darted 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. Vital signs (heart rate, respiration, and body temperature) will be monitored throughout (see below).

***Drop Netting.*** Depending on the proportion of female deer captured in the first year using chemical immobilization, deer may be captured in the second and subsequent years of the study using small free-standing tension release drop nets (approximately 25' X 25') (Lopez, Silvy et al. 1998, D'Eon, Pavan et al. 2003, Peterson, Lopez et al. 2003) . Drop nets will be employed in preference to Clover traps because of their greater target selectivity, and a desire to minimize the trapping and handling of non-target animals. To improve security and safety, drop nets will be set on private property only. Sightings by volunteers and research team members will be used to guide site selection. Nets will be set up and pre-baited for 1-3 weeks, and monitored directly by volunteers or with IR-triggered cameras for evidence of use by target animals. When use by target animals has been established, the net will be set, and the area monitored for target animal use by members of the research team or by volunteers who will summon the research team.. No

more than two deer will be captured at a time; there must be at least two trained research team members and one helper present when the net is dropped.

Upon capture and restraint each deer will immediately be hand-injected in the hip with a combination of Xylazine at 100mg/ml and Telazol® at 200mg/ml loaded in a 3cc syringe with a 1", 18-20 gauge needle. To avoid injuries and keep deer from becoming too tangled in nets each deer will be restrained until it becomes fully sedated. Deer responding poorly (still active after 15-20 minutes of restraint) to the Xylazine/Telazol mixture may be given additional Xylazine and Telazol in increments of 0.2-0.5ml, but not to exceed 1.5ml of the drugs. Deer responding well to the Xylazine/Telazol mixture but still alert after 20 minutes may be given a supplemental dose of Ketamine HCl at 100mg/ml by hand injection. Each drug's dosage will be dependent on the size of the deer and its physical condition (deer in poor condition will not be captured). Deer not responding to any of the aforementioned drugs will be given the appropriate amount of the Xylazine antagonist Tolazoline at 100mg/ml and released.

Telazol, which comes lyophilized, will be reconstituted with 2.5ml Xylazine to produce a dose of 200mg/ml. To assure their effectiveness once mixed the Xylazine/Telazol mixture will be kept warm until darts are placed in guns (just prior to shots) or syringes will be used.

**Handling Immobilized Deer.** Upon appropriate sedation and immobilization each deer's vital signs (heart rate, temperature, and respiration) will be checked. Netted deer will then be freed from the net and processed as described above. Processing after capture in net will include a thorough check for cuts, abrasions, and broken bones as a result of the capture and restraint. Injuries will be cleaned and treated according to the project veterinarian's instructions.

Captured deer will be blindfolded and then fitted with two plastic ear tags 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 below, 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.

Captured deer showing signs of respiratory distress ( $O_2$  saturations <85-90%) will be given Respiram™ (Dopram V) at 20mg/ml intravenously and/or intramuscularly. In cases of severe respiratory distress ( $O_2$  saturations <80%) deer will be given the Xylazine antagonist Tolazoline at 100mg/ml intravenously in addition to or in place of Respiram. Deer with lower than normal body temperatures will be wrapped in wool blankets and placed on plastic sheets. Deer with extremely low temperatures (<37 C) will be given the Tolazoline immediately and stimulated until they are able to walk or run.

In all captured deer, once the Telazol effects begin to wear off (swallowing, ear twitching, alertness, etc.) and depending on the amount of drugs injected deer will be injected intravenously and/or intramuscularly with 1.5-2.5 ml of Tolazoline to reverse the effects of the Xylazine and restore normal body functions. Tolazoline is typically injected 0.5- 0.75% of dose intravenously

and 0.25-0.5% of dose intramuscularly. To insure subdued recoveries the antagonist is typically injected into the veins in the hind leg and the hip.

**PZP treatments.** At the time of capture, deer will be hand injected in the hip with 100 $\mu$ g PZP in 0.5 ml PBS emulsified with 0.5ml mFCA plus 550 $\mu$ g PZP and 500 $\mu$ g 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) 100 $\mu$ g PZP in 0.5 ml PBS emulsified in 0.5ml FIA delivered in 1 cc Pneu-Dart darts with 1" or  $\frac{3}{4}$ " barbless needles; or (2) 100 $\mu$ g PZP in 0.5 ml PBS emulsified in 0.5ml FIA plus 550 $\mu$ g PZP and 500 $\mu$ g 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  $\frac{3}{4}$ " barbless needles modified to simultaneously inject emulsion and pellets. All darts will be directed toward the hip from a Dan-Inject Model CO<sub>2</sub> PI pistol or Model JM Standard CO<sub>2</sub> 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.

### **Deer Monitoring**

Methods will be refined as we proceed, but in general individual fawning, population reproductive rates, and changes in population size will be monitored by a combination of observations by resident volunteers and spring and fall camera trapping surveys.

***Monitoring of individual reproduction.*** Fawning by individual females will be monitored by a combination of blood pregnancy testing (see below), observations of fawns of ear-tagged females and other deer by citizen volunteers (as described below), and associations of fawns with ear-tagged deer recorded at camera traps.

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 inferences from observations of fawns by residents and at camera traps. Because darting and capture of any individual deer become progressively more difficult with each successive attempt, re-captures will only be attempted if absolutely necessary.

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).

***Resident volunteer observations.*** Residents of 40-50 households in Hastings will participate in a deer monitoring program. The Village will set up an online system on which residents will report their observations of deer, with time and date, location, numbers of animals sighted by age-sex class, and (once individuals have been eartagged) tag numbers and association of fawns with tagged females. (A prototype system has already been created.) Recorded sightings will be summarized on GoogleMaps to provide a village-wide picture of deer movements and activity patterns. Volunteer observations will initially be used to guide the location of capture sites and camera traps. It is also expected that useful supplementary information on fawning and the home range of individual marked females will also emerge from volunteer observations.

Reports of deer deaths (including ear-tagged deer) from police logs, volunteer observers, and other sources will be used to provide minimum estimates of deer mortality.

***Camera trapping surveys.*** Tentatively, we plan to conduct camera trapping surveys twice a year for the five years of the study. Guided by volunteer observations, approximately 12-15 Reconyx™ or similar IR-triggered cameras (approximately 1/33 ha potential deer habitat, following (Curtis, Boldgiv et al. 2009)) will be placed at secure locations along paths known or likely to be travelled by deer. The primary population survey will be carried out in late September/early October, and last approximately two weeks. Population estimates will be made in two ways: Weckel et al.'s (2011) modification of Jacobson's individual branch-antler male method, and mark-resight estimates using individually tagged adult females, using a Poisson log normal estimator or similar model (McClintock et al. 2008). These approaches should yield estimates of population numbers plus the number of adult males, spike bucks, adult females, and surviving young of the year. A second survey, to be carried out in June-July, will focus on counting young of the year and associating them with marked adult females. Because antlers will be relatively undeveloped, only estimation methods using eartagged and untagged females will be effective. The 2014 June-July survey will also be used to refine the placement of cameras, establish the duration of surveys, and work out other logistical details in preparation for the autumn survey. Camera trap locations will be pre-baited with cracked corn for 7-10 days before formal surveys begin, and baiting will continue during the survey period, with the primary goal of providing the most reliable identification of individuals visiting traps.

## **Measuring Impacts**

***Sentinel Oaks.*** Sentinel oak plots will be established in natural areas on private land at approximately 4 locations, subject to resources and availability. Two sets of sentinel oak studies will be conducted, once in 2014-2015, reflecting initial conditions, and once in 2018-19, reflecting conditions at the end of the study period. Each of the (four) locations will support two plots of 20 plants each. Half of the seedlings will be protected by 1 m tall mesh cages. The plots should be separated by 30-50 m; each should receive at least partial (>25%) direct sun during the day. As nearly as possible, oak seedlings in each plot should be laid out in a 4 X 5 grid,

alternating protected and unprotected seedlings in a checkerboard pattern, with plants separated by ~ 1 m.

Planting and data collection procedures will generally follow Blossey (B. Blossey, “The Cornell Sentinel Project”). In brief, 300-350 acorns will be collected in autumn 2013 and stored refrigerated for 3-4 months. In February or March, acorns will be removed from storage and germinated in potting soil in individual Conetainers (<http://www.greenhousemegastore.com/product/cone-tainers>). Seedlings will be transplanted to the experimental plots in May-June, at approximate heights of 12-30 cm, and individually marked. Seedling height and number of leaves will be recorded at time of transplant. Protected and unprotected seedlings should be roughly matched in size. At 7-10 days, new plantings will be inspected for transplant mortality, and any dead or moribund seedlings replaced. Thereafter, we will visit plants monthly and record survival/general appearance, plant height, number of leaves (old and new), and % leaf area removed by deer. Observations will continue through October, and resume in May through October of the following year (2015). At that time, we will decide whether to continue monitoring of the 2014 cohort in subsequent years, or end the trial pending repetition in 2018-19.

## 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

**(Deer observations by residents may be conducted year-round.)**

2014	February-March	Initial capture, eartagging, and initial PZP treatments
	June-July	Fawn observations, initial camera-trapping population survey
	Sept. – Oct.	Fall population camera-trapping survey
2015	February-March	Initial capture, eartagging, and PZP treatments
	June-July	Fawn observations, spring camera-trapping population survey
	Sept. – Oct.	Fall population camera-trapping survey
2016	February-March	Initial capture, eartagging, and PZP treatments (if needed)

	June-July	Fawn observations, spring camera-trapping population survey
	August – September	Booster darting of deer tagged in 2014
	Sept. – Oct.	Fall camera-trapping population survey
2017	June-July	Fawn observations, spring camera-trapping population survey
	August – September	Booster darting of deer tagged in 2015
	Sept. – Oct.	Fall camera-trapping population survey
2018	June-July	Fawn observations, spring camera-trapping population survey
	Sept. – Oct.	Fall camera-trapping population survey
2019	April – June	Fawn observations, spring camera-trapping population survey

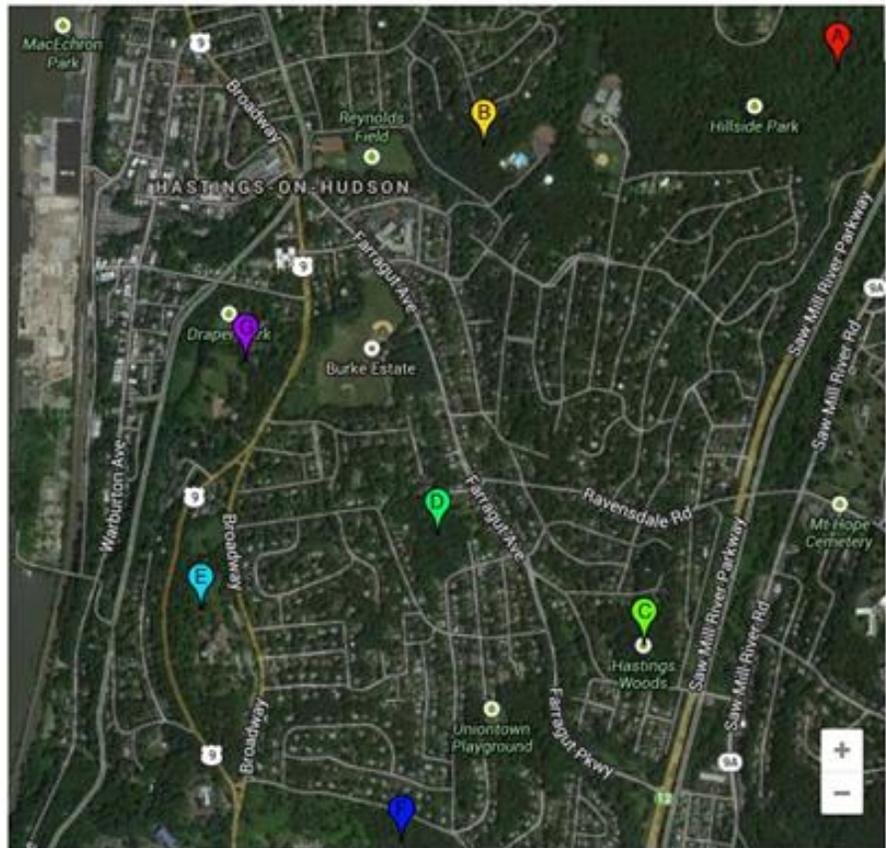
### **Designated Agents**

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Figure 1. Prospective sites for deer capture and treatment in Hastings-on-Hudson, New York.

**Immunocontraception Sites**

-  Hillside Park
-  Hillside Woods Location #2
-  Hastings Woods
-  Pulver Woods
-  Andrus Old Age Home
-  Andrus Children's Home
-  Lehrner household



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