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Administering GonaCon™ to white-tailed deer via hand-injection versus syringe-dart

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Abstract: Immunocontraceptive vaccines have shown some promise for fertility control of white-tailed deer (*Odocoileus virginianus*) in urban and suburban habitats where traditional methods of population control may not be applicable. Currently, the only contraceptive vaccine approved by the U.S. Environmental Protection Agency for use in white-tailed deer is GonaCon™ Immunocontraceptive Vaccine, but it is registered for use via hand-injection only. It has been suggested that remote-delivery of immunocontraceptives would be more cost-effective than hand-injection, but there is the potential for incomplete injection from a syringe-dart. Therefore, the purpose of our research was to: (1) conduct a dart configuration assessment trial to determine the ideal syringe-dart configuration for remote-delivery of GonaCon to white-tailed deer and (2) use the determined syringe-dart configuration in a subsequent trial to evaluate the vaccine efficacy when administered to female white-tailed deer via hand-injection versus syringe-dart. We saw comparable results with regard to vaccine dispersal during the dart configuration assessment and the efficacy trial; syringe-dart injected deer presented vaccine deposits and reaction sites both subcutaneously and intramuscularly, whereas, hand-injected deer presented vaccine deposits and reaction sites only intramuscularly. One year after administration, 4 of 5 deer treated with syringe-darts were pregnant, compared to 3 of 6 deer that received hand-injections. Anti-GnRH titers were negatively related to pregnancy status. We did not observe a high level of vaccine efficacy with the syringe-dart delivery method we used. Therefore, we recommend further research of syringe-dart delivery of GonaCon with a larger sample size where the vaccine is deployed in a single bolus similar to a hand-injected presentation.

Key words: dart, GonaCon™, human–wildlife conflicts, immunocontraception, nonlethal, white-tailed deer

Dense populations of white-tailed deer (*Odocoileus virginianus*) coupled with urban and suburban expansion have led to increased human–deer interactions. When negative, these interactions often result in significant economic losses resulting from deer–vehicle collisions (DVCs), tick-borne pathogens, and landscape damage (DeNicola et al. 2000).

DeNicola and Williams (2008) demonstrated that reducing local deer densities can significantly reduce DVCs. Lowering deer densities may also reduce the incidence of Lyme disease, because there is a positive correlation between white-tailed deer and blacklegged tick (*Ixodes scapularis*) abundance (Stafford 2004, Kilpatrick et al. 2014). The recommended deer density for the reduction of blacklegged ticks and associated occurrences of Lyme disease is <8 deer/km^2 (Rand et al. 2003). Low deer densities may also reduce damage to personal property such as landscaping and gardens (DeNicola et al. 2000).

Historically, lethal removal methods (i.e., sharpshooting, hunting, or trap and euthanasia) have been the accepted action of management for deer population reduction (Williams et al. 2013). However, when suburban communities are faced with the need to lower deer densities, nonlethal control options are often preferred because of legality, safety concerns, and social attitudes related to lethal management (McCullough et al. 1997). The primary methods for nonlethal reduction of deer populations are translocation and fertility control. Translocated deer have low survival rates due to capture myopathy, DVCs, and losses to hunters (Óbryan

and McCullough 1985, Jones and Witham 1990). Even if survival rates were increased, relocating deer is usually not an option because of both cost and legal issues concerning the spread of pathogens (Conover 2002).

Among nonsurgical fertility methods of control, immunocontraceptive vaccines have shown the most promise (Massei and Cowan 2014). Treatments with porcine zona pellucida (PZP) vaccines have reduced deer densities (Rutberg et al. 2013*a*, *b*). However, PZP is not registered for use in deer, and it allows females to have repeated estrous cycles, which may cause more males to move into females' home ranges, potentially leading to increases in DVCs and damage to ornamental plantings (Boulanger et al. 2012, 2014). Deer may also suffer physiological stress as a result of the extended breeding season caused by PZP, (Killian and Miller 2000).

Figure 1. One year after the administering GonaCon™, deer were euthanized by sharpshooting.

GonaCon™ is the only immunocontraceptive vaccine registered by the U.S. Environmental Protection Agency (EPA) for use in deer, and its labeling requires administration by hand-injection (EPA Reg. No. 56228-40). Unlike PZP, GonaCon suppresses the secretion of reproductive hormones, thus, preventing female deer from ovulating or coming into estrus (Miller et al. 2004). GonaCon in single-shot form was shown to be 88% and 47% effective during the first and second years, respectively, in female white-tailed deer (Gionfriddo et al. 2009). Treatment of female deer with GonaCon via hand-injection causes no adverse effects on mobility or general health of deer. The only adverse reactions documented in female deer have been intramuscular injection-site lesions (Gionfriddo et al. 2011).

The cost of reducing population densities through fertility control, with current delivery methods, is high (Massei and Cowan 2014). As currently approved, hand-injection of GonaCon requires the live capture of each deer prior to treatment with the vaccine. It has been suggested that an effective immunocontraceptive for urban and suburban deer management ideally should be deliverable remotely (Rutberg et al. 2013*b*). The purpose of our research was to: (1) conduct a dart configuration assessment trial to determine the ideal syringe-dart configuration for remote-delivery of GonaCon to white-tailed deer and (2) use the determined syringe-dart configuration in a subsequent trial to evaluate the vaccine efficacy when administered to female white-tailed deer via hand-injection versus syringe-dart.

Methods

Study area

This study was conducted on a 176-ha property in southern Connecticut consisting of upland deciduous forest with a thick understory, wetlands, and open fields. The property is enclosed by a 3-m-high chain-link fence with 5-cm mesh and surrounded by urban development. The deer on the site are privately-managed, not exposed to hunting, and habituated to human presence. There are no individual pens or confinement areas, deer on site are free to range within the fence, and no supplemental feeding regimes are present. Over the past 5 years pregnancy rates of untreated adult females have ranged from 92 to 95%, and the population has fluctuated between 30 and 50 individuals.

Dart configuration assessment

Two adult males and adult female deer were captured under a drop net (Conner et al. 1987) and anesthetized by injection of Telazol® (4.4 mg/kg) and xylazine hydrochloride (2.2 mg/kg). Each deer received an injection of GonaCon in both hamstrings and quadriceps (4 injections per deer). Ten of the 12 injections were administered remotely, with the deer perpendicular to the darter, from a distance of 5 m using a gas-based dart projector (Figure 1). The syringe-dart configurations consisted of 2-cc and 1.5-cc, rapid and slow-inject (1/3-sec) syringe-darts with 3.18-cm and 2.54-cm barbless, tri-port needles (Pneu-Dart Inc., Williamsport, Penn.). We included the larger volume syringe-darts in the trial to assess whether vaccine dose suitable for a larger mammal (e.g., horse [*Equus caballus*]) could be delivered remotely. Two of the 12 injections were administered via hand-injection with 1.5-cc syringes equipped with a 2.5-cm and 3.8-cm needle. While still under anesthesia, each deer was euthanized with a gunshot to the center of the brain. Hind limbs were then dissected to reveal the distribution of the vaccine deposits (GonaCon is a white and viscous vaccine, enabling visual examination of distribution).

Figure 2. Captured deer were randomly assigned to 1 of 2 treatment groups. One group was administered GonaCon™ via hand-injection, and the other group received GonaCon via syringe-dart delivery.

Vaccine efficacy trial

Capture. Adult (≥1.5 years) female white-tailed deer were captured using drop-nets during February and March 2013. Deer were anesthetized by injection of Telazol® (4.4 mg/kg) and xylazine hydrochloride (2.2 mg/kg) and aged using tooth replacement and wear (Severinghaus 1949). Each deer was fitted with ear tags (Destron Fearing, St. Paul, Minn.) and a VHF radio-collar (Advanced Telemetry Systems, Isanti, Minn.) to facilitate locating the individual for euthanasia 1 year after capture. All deer capture and handling was approved by the Institutional Animal Care and Use Committee and U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center, Quality Assurance Protocol-QA-2075.

Vaccination. Captured deer were randomly assigned to 1 of 2 treatment groups. One group was administered GonaCon (1.5 cc) via hand-injection with 1.5-cc syringes equipped with 3.81-cm needles administered intramuscularly to the upper hind limb via hand-injection (Figure 2). The other group while under anesthesia received the same dose administered by a 1.5 cc slow-inject syringe-dart with a 3.18-cm barbless, tri-port needle. Deer were darted with a gas-based dart projector from a distance of 5-m with the deer perpendicular to the darter. All darts were examined to determine if the entire volume of GonaCon was expelled from the dart upon impact. After treatment, the reversal agent tolazoline hydrochloride (2.0 mg/kg IV) was administered and the individuals were visually monitored for complications with recovery. One year later, deer were located and euthanized by sharpshooting techniques as described in DeNicola et al. (1997).

Anti-GnRH antibody titers. Serum concentrations of anti-GnRH antibodies can be used to estimate immunocontraceptive efficacy (Miller et al. 2008). Blood samples were collected via cardiac puncture within 1

Table 1. Trial data for vaccine deposit results in white-tailed deer with varied inject rates and needle lengths of syringe-darts with barbless, tri-port needles and hand-inject syringes.

Injection	Volume (cc)	Needle (cm)	Dart retention	Vaccine deposit[a]
Slow-inject dart	2.0	3.18	Retained	VM, VWC
Slow-inject dart	2.0	3.18	Retained	VM, VWC
Slow-inject dart	2.0	3.18	Retained	VM, VWC
Slow-inject dart	2.0	3.18	Bounced	VM, VWC, VG
Slow-inject dart	1.5	3.18	Bounced	VM, VWC, VG
Slow-inject dart	1.5	3.18	Retained	VM, VWC
Slow-inject dart	1.5	2.54	Bounced	VM, VWC, VG
Slow-inject dart	1.5	2.54	Bounced	VM, VWC, VG
Rapid-inject dart	2.0	3.18	Bounced	VM, VWC, VG
Rapid-inject dart	2.0	3.18	Retained	VM, VWC
Hand-inject syringe	1.5	2.54	N/A	VM
Hand-inject syringe	1.5	3.81	N/A	VM

[a] VM = vaccine in muscle depth. VWC = vaccine in wound channel; VG = substantial vaccine on ground.

minute after death. Serum was isolated via centrifugation and then frozen and shipped to the National Wildlife Research Center (NWRC) in Fort Collins, Colorado. Antibody titers were measured using enzyme-linked immunosorbent assays as previously described (Miller et al. 2008).

Necropsy. Hind limbs containing the injection site were examined externally for abscesses. We then necropsied each deer to permit internal examination. Presence or absence of granulomatous nodules and sterile abscesses at the injection site were noted. Presence or absence of fetuses were recorded to assess pregnancy rates and forehead-rump lengths (FRL) were measured on fetuses to determine fetal age in days as previously described (Hamilton et al. 1985).

Statistical analysis. All statistical analyses were performed using R version 3.1.2 (R Core Team, Vienna, Austria, 2015) and significance level was set at $\alpha = 0.05$. We used Fisher's exact test to assess differences between the 2 treatment groups in the proportion of deer pregnant. We then used the Wilcoxon signed-rank test to assess if there was a significant difference in titer levels between the 2 treatment groups to assess any difference in titer levels between pregnant and nonpregnant deer, regardless of treatment group.

Results

Dart configuration assessment

The syringe-darts with 3.18-cm needles were retained in the deer frequently, whereas the 2.54-cm needles bounced out upon impact (Table 1). However, the 3.18-cm needles did not retain on 3 occasions. With each dart-injection site, there was a substantial amount of vaccine that travelled back up the wound channel depositing subcutaneously; vaccine left in the musculature was dispersed in fascia between muscles. Syringe-darts that were not retained in the deer deposited most of the vaccine on the ground with only a minimal amount deposited in the muscle. Hand-injection deposited the vaccine in a single bolus in the muscle at the depth of the needle.

Vaccine efficacy trial

Thirteen female deer were captured under drop nets from February 22 through March 3, 2013, and treated with the GonaCon vaccine. Six females received the vaccine via syringe-dart (all darts were retained in the deer) and seven via hand-injection (Table 2). Eleven of these 13 females were euthanized during February 2014. We were unable to locate 1 female due to transmitter failure, and the skeletal remains of the other were found during telemetry-assisted searches.

Table 2. Treatment data for white-tailed deer in the hand-inject versus dart-delivery GonaCon™ trial, treated February 22 - March 3, 2013 and euthanized for necropsy February 2014.

Treatment group	Tag number	Age (years) 2013	Titer level	Lactating 2013	Pregnant 2014[a]	Injection site reaction[a]
Hand	34R/9L	2	1:128,000	Yes	1 fetus	Concentrated IM
Hand	181/181	7	1:128,000	Yes	NP	Slightly dispersed IM
Hand	8R/7L	7	1:128,000	Yes	NP	Concentrated IM
Hand	47R/48L	4	1:64,000	Yes	NP	Concentrated IM
Hand	42R/37L	5	1:2,000	Yes	1 fetus	Slightly dispersed IM
Hand	43R/44L	9	0	Yes	1 fetus	Concentrated IM
Hand	55R/20L	7	–	–	TF	–
Dart	41R/40L	6	1:128,000	Yes	NP	Subcutaneous—dispersed IM
Dart	19R/21L	8	1:8,000	Yes	1 fetus	Small subcutaneous—concentrated IM
Dart	32R/33L	2	0	Yes	1 fetus	Small subcutaneous—dispersed IM
Dart	35R/36L	6	0	Yes	2 fetuses	Subcutaneous—small IM
Dart	18R/17L	2	0	Yes	2 fetuses	Small subcutaneous—dispersed IM
Dart	50R/52L	8	–	–	MS	–

[a] IM = intramuscular; NP = not pregnant; TF = transmitter failure; MS = mortality signal.

Four of 5 deer treated with syringe-darts were pregnant at the time of euthanasia, compared to 3 of 6 deer that received hand-injections (Table 2). There was no significant difference between treatment groups in proportion pregnant (P = 0.54). Two of the females that were treated with syringe-darts had twins. The remaining pregnant females contained a single fetus. Conception was estimated to have occurred during the typical November peak breeding season, with the exception of 1 female in the syringe-dart treatment group that conceived mid-January.

Injection-site reactions ranged from concentrated intramuscular granulomas to dispersed intramuscular and subcutaneous granulomas. Only vaccines administered via syringe-dart caused subcutaneous injection site reactions; hand-injection caused intramuscular reactions only (Table 2). Hand-injection reactions resulted in concentrated granulomas and abscess masses, with the exception of two, which resulted in dispersed intramuscular granulomas.

There was no difference in titer levels between treatment groups (P = 0.21). However, titer levels were strongly correlated with pregnancy status. There was a significant difference in titer levels between pregnant and nonpregnant deer regardless of treatment group (P = 0.02). One individual (female #34/9) with an elevated titer contained a single fetus (Table 2). All other individuals with titers ≥1:64,000 were not pregnant; deer with titers <1:10,000 all were pregnant.

Discussion

We found that the efficacy of GonaCon 1-year post-administration was less than reported by Miller et al. (2008), Gionfriddo et al. (2009), and Gionfriddo et al. (2011). This may be related to the timing of vaccine delivery. In the aforementioned trials, deer were treated in July and August (2 to 4 months prior to the breeding season), whereas females in our trial were treated in February and March (8 months prior to the breeding season), which may have resulted in reduced titer levels at the time of the breeding season.

We saw comparable results with regards to vaccine dispersal during the initial dart configuration assessment and the efficacy trial.

In both trials, the syringe-dart resulted in a substantial amount of vaccine traveling back up the wound channel while hand-injections resulted in the vaccine deposited in a single bolus. In general, the hand-injection reaction sites had a larger concentrated granuloma and abscess confined within the muscle, whereas the syringe-dart injection sites displayed a more dispersed granuloma in the muscle plus some subcutaneous reaction areas. Gionfriddo et al. (2011) suggested that the formation of granulomas at injection sites may be an essential aspect of a strong immune response that induces infertility in GonaCon-treated animals. From our observations, the body can better compartmentalize the dispersed vaccine resulting in small granulomas and may reduce immune response. The larger bolus deposit of the hand-injected vaccine resulted in localized granulomas and abscesses that likely extend the exposure of the vaccine to the immune system, thus, increasing the efficacy. The significant difference in antibody titer between pregnant and nonpregnant animals supports the findings by Miller et al. (2000) of a titer level ≥1:64,000 is sufficient to suppress fertility in white-tailed deer.

Management implications

Given the performance of the vaccine coupled with remote-injection, we question whether syringe-dart delivery of GonaCon is an appropriate management tool at this time. The lack of a statistically significant difference in contraceptive efficacy between the 2 treatments may be due to a small sample size. We recommend further research on the development of this technique with a larger sample size. GonaCon may perform better when hand-injected as a single bolus, limiting the dispersion of the vaccine and amount exiting the wound channel. Research should include the use of different needle barbing and porting techniques, such as various configurations of gelatin-barbs and 3.8-cm, tri-port needles. The gelatin-barb and longer needle will more likely ensure complete delivery of the vaccine into the deep musculature of the individual similar to hand-injection. A gelatin-barb, smaller than the conventional size, may be adequate for single-bolus injection without compromising dart recovery. We recommend using 2.0-cc darts so that an additional 0.5 cc per required dose may be administered to compensate for the vaccine that may travel out the wound channel. This design will need to be accompanied by a slow injection rate (e.g., 1 second) for a more controlled vaccine deposit, similar to a hand-injection. Future trials should be conducted with similar timing to our trial (January through March) to further test the vaccine efficacy when administered during optimal months for capture and darting (i.e., when bait leverage is high and there is no foliage on trees to compromise darting opportunities).

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Robert J. Warren has been a professor in the Warnell School of Forestry and Natural Resources at the University of Georgia since 1983. From 1979 to 1983, he was on the faculty at Texas Tech University. He obtained his B.S. degree from Oklahoma State University and his M.S. and Ph.D. degrees from Virginia Polytechnic Institute and State University. His research interests include management of wildlife populations in parks and urban and suburban areas, predator ecology and management, and wildlife physiology.