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James P. Gionfriddo
USDA/APHIS/Wildlife Services' National Wildlife Research Center, james.p.gionfriddo@aphis.usda.gov

Anthony J. DeNicola
White Buffalo, Inc.

Lowell A. Miller
USDA/APHIS/Wildlife Services' National Wildlife Research Center, Fort Collins, CO, lowell.a.miller@aphis.usda.gov

Kathleen A. Fagerstone
National Wildlife Research Center, kathleen.a.fagerstone@aphis.usda.gov

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Original Article

Health Effects of GnRH Immunocontraception of Wild White-Tailed Deer in New Jersey

JAMES P. GIONFRIDDO,[1,2] *United States Department of Agriculture/Animal and Plant Health Inspection Service/Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA*

ANTHONY J. DENICOLA, *White Buffalo, Inc., 26 Davison Road, Moodus, CT 06469, USA*

LOWELL A. MILLER, *United States Department of Agriculture/Animal and Plant Health Inspection Service/Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA*

KATHLEEN A. FAGERSTONE, *United States Department of Agriculture/Animal and Plant Health Inspection Service/Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA*

ABSTRACT We evaluated the health effects of GonaCon™ Immunocontraceptive Vaccine in individual white-tailed deer (*Odocoileus virginianus*) on a fully fenced corporate office campus in suburban New Jersey, USA. We captured and vaccinated adult females, fawns of both sexes, and yearling and adult males, and evaluated their health status through field and necropsy observations, assessment of blood chemistry, and histopathological examination of selected tissues. One 1.0-mL intramuscular injection of vaccine was delivered by hand to the hind limb of each GonaCon-treated deer. Control deer received sham injections (ad F) or no injections (yearling and ad M). Mean body-condition scores of GonaCon-treated adult females and males were greater than those of corresponding control groups. No evidence of limping or impaired mobility was noted in study deer during the 2-year study. No adverse effects of vaccination were detected in major organs, organ systems, body condition, fat deposits, or blood chemistry. Injection-site lesions (granulomatous nodules and sterile abscesses) occurred in the deep hind-limb musculature of >85% of GonaCon-treated and sham-injected deer but were not detectable externally. Reactions at injection sites and in lymph nodes were typical responses to injection of vaccines formulated as water-in-oil emulsions, especially those, like GonaCon, that contain mycobacteria. The formation of injection-site lesions may be a necessary component of the immune response that causes infertility in treated animals. Natural resource managers who use GonaCon to manage deer in settings such as developed areas and public parks will ultimately determine its value and applicability. © 2011 The Wildlife Society.

KEY WORDS contraception, gonadotropin releasing hormone, granuloma, infertility, *Odocoileus virginianus*, overabundance.

Management of overabundant populations of white-tailed deer (*Odocoileus virginianus*) has become a major challenge for natural resource managers in many urban, suburban, and public park settings in the United States. Traditional management methods cannot be used in many such settings because of safety issues, conflicting views regarding the social acceptability of hunting, or laws prohibiting the discharge of firearms (DeNicola et al. 1997). Fertility control may offer a nonlethal management alternative, especially for deer subpopulations that are isolated by fencing or natural barriers. Immunocontraception and other nonlethal methods are increasingly advocated by the public (Stout et al. 1997, Lauber et al. 2007) and hold promise as a means of reducing reproduction in overabundant wildlife populations (Hobbs et al. 2000, Curtis et al. 2002, Fagerstone et al. 2010).

GonaCon™ Immunocontraceptive Vaccine (National Wildlife Research Center, Fort Collins, CO) is a gonadotropin releasing hormone- (GnRH-) based water-in-oil emulsion that has safely induced temporary infertility in many mammalian species, including free-ranging California ground squirrels (*Spermophilus beecheyi*; Nash et al. 2004), captive Norway rats (*Rattus norvegicus*; Miller et al. 1997), domestic cats (*Felis catus*; Levy et al. 2004), domestic and feral swine (*Sus scrofa*; Miller et al. 2003; Killian et al. 2006*c*), wild horses (*Equus caballus*; Killian et al. 2006*a*), bison (*Bison bison*; Miller et al. 2004*a*), elk (*Cervus elaphus*; Killian et al. 2009), and white-tailed deer (Miller et al. 2008*b*; Gionfriddo et al. 2009). It can be administered as one intramuscular injection that suppresses reproduction in treated animals of both sexes for multiple years (Miller et al. 2004*b*). GonaCon was registered by the United States Environmental Protection Agency in September 2009 as a restricted-use product for the contraception of adult, female, white-tailed deer via hand injection (Fagerstone et al. 2010).

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[1]*E-mail: james.p.gionfriddo@aphis.usda.gov*
[2]*Present address: National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA.*

We assessed the health effects of GonaCon treatment in white-tailed deer adults and fawns of both sexes. Adult females were the primary focus of the study because they will be the targets of operational applications of GonaCon vaccine by natural resource managers. Fawns were tested because a GnRH immunocontraceptive agent could potentially be delivered orally in the future, and treated bait might be consumed by deer of all ages. We also tested GonaCon in yearling and adult males. Although GonaCon is not registered or recommended for use in male deer (Killian et al. 2005, Curtis et al. 2008), antlerless males could inadvertently be treated during winter management activities because they can be mistaken for females. Inadvertent treatment of males could also occur if a new GnRH vaccine is registered for remote injection or oral delivery in bait.

This research was part of a larger investigation that also evaluated GonaCon's contraceptive efficacy, which is addressed separately in another manuscript (Gionfriddo et al. 2011). The objective of the research covered in this manuscript was to evaluate the health effects of GonaCon Immunocontraceptive Vaccine in wild white-tailed deer through field observations, necropsy examinations, laboratory assessment of blood chemistry, and histopathological analysis of selected reproductive and other tissues. We tested several hypotheses to determine whether mean body condition scores were higher in some groups of study deer than in others. In addition, we tested a series of hypotheses to determine whether mean values for blood-chemistry parameters differed between treatment groups (GonaCon-injected ad F vs. sham-injected control F) and between years (pretreatment vs. posttreatment). We also tested the hypotheses that the incidence of injection-site lesions was higher 1) in GonaCon-treated than in sham-injected control adult female deer, and 2) in GonaCon-treated adult female deer that became pregnant than in those that did not, for each year. Finally, we tested the hypothesis that the GonaCon-injected rear leg of each deer was greater in circumference near the injection-site location than the other rear leg.

STUDY AREA

The study was conducted on a fully fenced (2.4-m-tall chain-link), 123-ha corporate office campus near Madison in New Jersey, USA. Habitat types included hardwood forest (about 20%), old fields reverted to grasslands and savannah (about 20%), lawns (about 40%), and landscaped areas around buildings. Deer were easily observed from paved and gravel roads located throughout the study area. During the 2-year study, a few deer emigrated or immigrated by running through security gates opened briefly by guards to allow ingress and egress of motor vehicles.

Northern New Jersey experienced a continental type of climate, with cold, damp winters and hot, humid summers. At Morris Plains, about 8.5 km northwest of the study area, mean monthly temperatures (1945–1990) ranged from −2.4° C (January) to 22.4° C (July). Mean annual precipitation (1941–1990) was 128.8 cm, with an annual growing season of about 142 days (Office of the New Jersey State Climatologist 2011; U.S. Department of Agriculture Natural Resources Conservation Service, National Water and Climate Center 2011).

METHODS

Capture and Treatments

All study deer.—We captured deer at night with tranquilizer darts fired from a Pneu-Dart rifle (Model 171; Pneu-Dart, Inc., Williamsport, PA). We used a mixture of Telazol® (4.5 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine hydrochloride (2.5 mg/kg; Bayer, Leverkusen, North Rhine-Westphalia, Germany) to immobilize deer. One of us (AJD) estimated deer body masses in the field by handling deer and by palpating body contours strongly influenced by fat deposits (Riney 1960, Robinson 1960, Audigé et al. 1998). We fitted deer with numbered ear tags and radiotelemetry collars equipped with mortality sensors (Model M2520; Advanced Telemetry Systems, Isanti, MN). We delivered a 1.0-mL injection (GonaCon vaccine or a sham material; see below) by hand to the deep musculature of the upper hind limb of each deer. If a deer had been darted in a hind limb we hand-injected the opposite hind limb. We administered tolazoline hydrochloride (3.0 mg/kg; Lloyd, Inc., Shenandoah, IA) intravenously to reverse anesthesia and hasten recovery of deer before we released them at their capture sites.

Adult females.—During July and early August 2005, we captured all adult females present on the study area ($n = 47$) and collected a blood sample from the jugular or femoral vein of each animal to measure pretreatment blood-chemistry parameters. We injected each female with GonaCon Immunocontraceptive Vaccine ($n = 32$; each 1.0-mL dose of vaccine contained 1,000 μg of conjugated GnRH–mollusk protein) or with a sham material ($n = 15$). The sham material consisted of AdjuVac™ adjuvant (National Wildlife Research Center, Fort Collins, CO) and mollusk stabilizing buffer (i.e., GonaCon vaccine without GnRH and without the mollusk protein that usually serves as the carrier molecule to which GnRH is conjugated).

Fawns and males.—We captured and vaccinated 8-month-old female fawns ($n = 4$) in February 2006 to determine the health effects of vaccination with GonaCon vaccine on young deer treated during their first winter. To evaluate the health effects of treating young fawns with GonaCon, we captured and vaccinated 3- to 4-month-old fawns ($n = 16$) during September–October 2006. We captured and vaccinated yearling and adult males ($n = 10$) during March 2007 to assess the health effects of wintertime treatment of male deer with GonaCon. To provide baseline data on untreated (control) males, we killed (via gunshot to head or neck) and necropsied previously uncaptured males (yearlings and ad; $n = 13$) during October 2007.

Evaluation of Health Effects of Vaccination on Treated Deer

We used field observations, necropsy examinations, laboratory assessment of blood chemistry, and histopathological evaluation of selected reproductive and other tissues to

identify potential health and safety problems associated with GonaCon treatment. We observed all study animals during spring(s) and summer(s) between their capture and October 2007. During field observations we looked for evidence of limping, impaired mobility, reduced range of motion of limbs, other abnormal behavior, and externally visible injection-site reactions. In October 2007, at the conclusion of field activities, we collected 62 deer (including representatives of all of the above treatment and control groups) after shooting them in the head or neck with a high-powered rifle. We shot deer as they foraged or rested, without a chase. We collected blood from each deer via cardiac puncture within 1 min of the animal's death, and we stored blood samples in a cooler or refrigerator before we isolated the serum via centrifugation and shipped it to a laboratory for analysis of blood chemistry. For the original group of adult females (the only experimental deer from which we collected blood at the time of initial capture), we used analysis of variance (ANOVA; mixed model) to compare mean values of blood-chemistry parameters of vaccinated versus control deer. Treatment group and year were treated as fixed effects and individual deer were treated as a random effect in the mixed-model analysis.

An experienced wildlife veterinarian performed standard necropsy examinations (Munson 2010) in the field immediately after deer were killed and blood samples were collected. We fixed and preserved tissue samples in formalin before we shipped them to Colorado, USA, for histopathological evaluation (Colorado Histo-Prep, Fort Collins, CO). Samples collected included ovaries, fallopian tubes, uteri, hypothalami-anterior pituitary glands, mammary glands, and the injection-site tissues and their draining (popliteal and iliac) lymph nodes. We also submitted other apparently abnormal tissues, if present, for histopathological analysis. We used Fisher's exact tests to determine whether the incidence of injection-site lesions was greater 1) in GonaCon-treated than in sham-injected control adult female deer, and 2) in pregnant than in nonpregnant GonaCon-treated adult female deer for each year.

Fat stores and general body condition were evaluated for each deer at necropsy to provide information on individual and general herd health, which may have influenced vaccine efficacy. We measured (to the nearest 0.5 mm) the thickness of the thickest fat layer at the base of the heart and on the ventral surfaces of the kidneys; of the 6 indicator sites used by Kistner et al. (1980), these are the most predictive of body composition (Cook et al. 2001). Based on thickness, we assigned a value of 1–5 to each heart or kidney fat deposit (0.0–2.0 mm = 1, 2.5–4.5 mm = 2, 5.0–7.0 mm = 3, 7.5–9.5 mm = 4, $\geq$10.0 mm = 5). For each deer, we categorized general fat deposits as "little," "little to moderate," "moderate," "moderate to abundant," or "abundant," and we assigned values of 1–5 for these categories ("little" = 1, "abundant" = 5). In addition, the necropsy veterinarian classified each animal's general body condition on a scale of 1 through 4, based upon body size (relative to age), muscularity, and externally perceptible fat stores (1 = poor poor condition, with ribs, spine, and all bony prominences starkly visible, and with no fat cover in the concave rump; 2 = thin condition, with the spine, ribs and bony prominences clearly but not starkly visible, with no muscle development, and with little palpable fat in the flat rump; 3 = fair condition, with the spine visible, the ribs and bony prominences palpable but only slightly visible, and with little muscle development; 4 = good condition, with spine, ribs, and prominences not visible, and with at least moderate muscularity, but not a "fat" animal). None of the deer on this study site was considered "fat" or in "excellent" body condition. For each deer, the 4 values for fat stores and body condition were summed to produce a body condition score that took into account body size, musculature, and both externally apparent and deep internal fat stores. If one of the values was missing, we replaced it with the mean of the other 3 values, rounded to the nearest integer. We used these body condition scores, which ranged from 4 to 16, to assign each deer to a body condition class (4–5 = "poor," 6–8 = "thin," 9–11 = "fair," 12–16 = "good"). Body condition scores usually vary among males, females, and fawns due to differences in their ability to store fat and in their need to metabolize fat stores as the autumn breeding season proceeds (Kistner et al. 1980). Accordingly, we calculated a mean body-condition score for each of the following groups: GonaCon-treated adult females, control adult females, GonaCon-treated adult males, control adult males, male fawns vaccinated at 3–4 months of age, female fawns vaccinated at 3–4 months of age, and female fawns vaccinated at 8 months of age (Table 1). Modified Kistner scores have been used successfully to evaluate body condition in many cervids (e.g., Cook et al. 2001, 2007; Stephenson et al. 2002; Peterson and Messmer 2007). Although our body condition scores may not provide an absolute measure of fat deposits or body condition, they do provide standardized relative measures of these variables that permit valid comparisons of treatment–demographic groups within our study.

To determine whether injections had caused a swelling of hind limbs, or whether limb disuse had caused muscular atrophy, we used a standard vinyl tape measure to determine the circumference of each thigh of each deer at necropsy. We held the end of the tape at the top edge of the patella at the insertion of the quadriceps muscle group and wrapped the tape around the thigh at the narrowest point caudal to the stifle. Although this point was very slightly distal to the injection site, it provided a practical, standardized location for comparative measurements that would detect differences in girth at the injection sites. We used a paired t-test to compare the left and right thigh circumferences of each deer. For all statistical analyses, we used $\alpha = 0.05$. The Institutional Animal Care and Use Committee of the National Wildlife Research Center approved all aspects of this research.

RESULTS

All Study Deer

With one exception, no overt signs of limping, restricted range of motion of limbs, or impaired mobility were observed

Table 1. Body condition scores* of GonaCon-treated and control white-tailed deer in New Jersey, USA, 2005–2007.

Treatment group	Body condition				*N*	Group mean	SEM
	Poor	Thin	Fair	Good			
Ad F: GonaCon	2	7	4	3	16	8.8	0.8
Ad F: control[a]	3	5	2	0	10	6.8	0.6
3- to 4-month-old F fawns: GonaCon	1	1	2	1	5	8.8	1.4
3- to 4-month-old M fawns: GonaCon	1	3	2	0	6	7.7	1.1
8-month-old F fawns: GonaCon	0	1	1	0	2	8.0	1.0
Ad M: GonaCon	0	0	1	3	4	12.0	1.2
Ad M: control[b]	0	3	2	0	5	8.4	0.9
Totals	7	20	14	7	48		

* Body condition scores were based on evaluation of body size (relative to age), musculature, and both externally apparent and deep internal fat stores.
[a] Each control ad F received a sham injection of AdjuVac adjuvant and mollusk stabilizing buffer (i.e., GonaCon vaccine without the GnRH and mollusk hemocyanin).
[b] Control ad M received no injections.

in any vaccinated or control deer in this study. No externally visible evidence of injection-site reactions was observed in live deer in the field or in dead deer prior to dissection of the musculature at injection sites during necropsies. Hind limb circumferences measured at the injection sites did not differ ($P > 0.05$) in any animal in this study.

Major organs and organ systems were normal in most study deer, although several abnormalities (apparently unrelated to capture, handling, and treatment) were noted at necropsy. Livers of 5 (4 GonaCon-treated and 1 control) deer and spleens of 2 (1 GonaCon-treated and 1 control) deer showed evidence of current or previous parasitic infection. Several small nodules were found in the lungs of 2 (1 GonaCon-treated and 1 control) deer and in the parenchyma of the pancreas of 1 (control) deer.

Field estimates of deer body weights at initial capture ranged from 32 kg to 57 kg. Skin was normal on all examined deer, with either no external parasites or a few ticks present. Ovaries and uteri in many GonaCon-treated females (ad and fawns) were small and inactive. Ovarian and uterine quiescence is a normal response to GonaCon because the vaccine disrupts the synthesis of reproductive hormones.

Dissection of the deep musculature (semimembranosus, semitendinosus, and biceps femoris muscles) at injection sites revealed lesions of 2 types within muscles and in fascia between muscles: hard nodules and abscesses. Nodules were partially or fully encapsulated, yellowish-white globular masses that occurred singly or in groups; most were 3–6 mm in diameter. Histopathologic examination confirmed that these nodules were chronic inflammatory granulomas of long duration, characterized by macrophages, multinucleated giant cells, vacuoles, and mineralized and/or necrotic centers. Most abscesses were small (0.5–4 cm^3; $n = 6$) or moderate (30–100 cm^3; $n = 5$) in size, but one was very large (about 1,000 cm^3). During histopathological examinations, abscesses were identified as granulomas that had progressed to sterile abscesses.

Adult Females

Of the 30 adult female deer (18 GonaCon-treated and 12 control) that were collected in October 2007, 26 were assigned body condition scores based on necropsy evaluation (Table 1). None of the 10 control females and only 3 of the 16 GonaCon-treated females were in "good" condition. The mean body condition score for all GonaCon-treated adult females (regardless of pregnancy outcomes) was higher than that of controls ($t_{24} = 1.92$, $P = 0.034$; Table 1). The mean body condition score for GonaCon-treated adult females that became pregnant during the study (1 or both yr) did not differ from that of control females ($t_{16} = 0.47$, $P = 0.323$).

Fat reserves in necropsied study deer generally were meager. Ten of 16 GonaCon-treated and 6 of 8 control females had "little" general body fat and 3 others (1 GonaCon-treated and 2 control) had "little-to-moderate" fat. Only 3 deer (all GonaCon-treated) had "moderate" or "moderate-to-abundant" fat, and none had "abundant" fat. Fat reserves on the heart and kidneys ranged from 0 mm to 10 mm in thickness; most deer had 2–4 mm of fat.

None of the 15 blood-chemistry parameters we measured differed ($P > 0.05$) between GonaCon-treated and control adult female deer at the time of initial capture in July–August 2005 or when deer were collected in October 2007 (Table 2). Although 2005 and 2007 mean values of some blood-chemistry parameters differed, all values were within the normal ranges for adult white-tailed deer (Seal et al. 1981, Kie et al. 1983) except creatinine phosphokinase (CPK), potassium, and phosphorus values for 2007, which were abnormally high.

Injection-site lesions (hard nodules, or hard nodules and abscesses) were found at necropsy in 17 (94%) of 18 adult female deer that had been vaccinated with GonaCon, and in 9 (82%) of 11 adult female deer that had received sham injections (Table 3). The incidence of lesions was not higher in GonaCon-treated females ($P = 0.316$) than in controls. Of the 17 GonaCon-treated females with hard nodules at injection sites, 8 (47%) also had abscesses (2 small, 5 moderate, 1 large). Two (22%) of the 9 sham-injected control females with hard nodules also had abscesses (both small). Among GonaCon-treated adult females, the incidence of lesions was not higher in pregnant ($n_{2006} = 6$; $n_{2007} = 8$) than in nonpregnant ($n_{2006} = 12$; $n_{2007} = 13$) animals for either year ($P_{2006} = 0.333$; $P_{2007} = 0.619$). Injection-site tissue samples from 12 adult females (7 GonaCon-treated

Table 2. Means and standard errors of the means for blood chemical parameters of adult, female, white-tailed deer in GonaCon-treated groups and control groups in New Jersey, USA, 2005–2007. Values marked with different letters (A and B) within a row differed significantly from each other. Note that some values are marked with both A and B. Values for 2005 are pretreatment values. ALK, alkaline phosphatase; CPK, creatinine phosphokinase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.

	2005				2007					
	Control[a]		GonaCon		Control		GonaCon			
Parameter	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Group, P^b	Yr, P^c
Sodium (mEq/L)[d]	142.5 A	3.1	142.0 A	2.2	148.6 A,B	3.5	157.5 B	2.9	0.174	<0.001
Potassium (mEq/L)[e]	4.8 A	0.6	4.9 A	0.4	11.5 B	0.7	10.7 B	0.6	0.562	<0.001
Calcium (mg/dL)	8.7 A	0.3	8.6 A	0.2	9.7 B	0.3	10.3 B	0.3	0.399	<0.001
Phosphorus (mg/dL)	5.7 A	0.6	5.3 A	0.4	11.6 A	0.7	11.6 A	0.6	0.796	<0.001
ALK (U/L)[e]	32.6 A	30.3	26.2 A	20.8	205.5 B	33.9	215.7 B	27.7	0.947	<0.001
Glucose (mg/dL)[d]	142.8 A	25.2	150.0 A	17.3	189.0 A	28.2	183.5 A	23.0	0.972	0.098
Total protein (g/dL)	6.6 A	0.2	6.7 A	0.15	6.7 A,B	0.3	7.3 B	0.2	0.111	0.052
Albumin (g/dL)	2.7 A	0.1	2.62 A	0.1	2.6 A	0.1	2.8 A	0.1	0.576	0.793
Globulin (g/dL)	3.9 A	0.2	4.0 A	0.1	4.1 A,B	0.2	4.6 B	0.2	0.080	0.012
CPK (U/LD)[e]	345.7 A	1,667.4	241.2 A	1,141.6	3,388.3 B	1,864.2	6,332.9 B	1,522.1	0.369	0.005
Total bilirubin (mg/dL)	0.1 A	0.0	0.10 A	0.0	0.3 B	0.0	0.4 B	0.0	0.817	<0.001
ALT (U/L)[d]	26.8 A	5.5	23.5 A	3.8	59.5 B	6.2	45.7 B	5.0	0.104	<0.001
Creatinine (mg/dL)	1.0 A	0.1	1.2 A	0.1	1.4 B	0.1	1.6 B	0.1	0.059	<0.001
BUN (mg/dL)	17.3 A	2.4	18.1 A	1.7	22.0 A	2.7	21.1 A	2.2	0.986	0.094
Amylase (U/L)	106.8 A	10.2	115.9 B	7.0	133.6 A,B	11.3	140.8 B	9.2	0.426	0.006

[a] Means are from the PROC MIXED procedure in SAS.
[b] Between-groups comparison.
[c] Between-yr comparison.
[d] The inverse ($1/x$) was used for ANOVA. The *P*-value reported in the table is the value for the ANOVA of the inverse values.
[e] The natural log (ln) was used for ANOVA. The *P*-value reported in the table is the value for the ANOVA of the natural logs.

and 5 control) were submitted for histopathological examination. Chronic granulomatous inflammation was detected in 11 of these 12 samples, and the remaining sample had a chronic abscess. Necrosis of muscle fibers at injection sites was common.

Injection sites of tranquilizer darts used at initial capture (summer 2005) were found in 7 (24%) of 29 adult female deer (GonaCon-treated and controls) at necropsy in October 2007. A tissue sample from the tranquilizer-dart injection site of an adult female was examined histopathologically and found to have chronic granulomatous inflammation.

At necropsy, the popliteal lymph node in the hind leg that was hand-injected was enlarged or hard in 11 (61%) of 18 GonaCon-treated adult female deer and in 6 (50%) of 12 sham-injected control females. The iliac lymph node associated with the injected hind leg was enlarged or hard in 6 (35%) of 17 GonaCon-treated females and in 6 (50%) of 12 sham-injected females.

Twenty-five popliteal or iliac lymph nodes from 14 (10 GonaCon-treated and 4 control) adult study females were submitted for histopathological examination. Chronic granulomatous inflammation was found in 4 unidentified (popliteal or iliac) lymph nodes from 3 (2 GonaCon-treated and 1 control) deer. Chronic hyperplasia was detected in 2 popliteal lymph nodes (from 2 GonaCon-treated deer) and 1 unidentified lymph node (from a control deer). Acute hemorrhage was noted in 2 unidentified lymph nodes (from 1 GonaCon-treated and 1 control deer). Foreign material was found in one unidentified lymph node (from a GonaCon-treated deer). No significant findings were reported for 5 popliteal, 4 iliac, and 7 unidentified lymph nodes.

In several lymph nodes in our study deer (both sexes), vacuoles were found among the macrophages and multinucleated giant cells or within their cytoplasm. These vacuoles were probably mineral oil droplets from the AdjuVac in GonaCon.

Table 3. Occurrence of injection-site lesions in wild white-tailed deer injected with GonaCon Immunocontraceptive Vaccine or a sham material in New Jersey, USA, 2005–2007.[*,**]

Treatment group[a]	Deer examined	Deer with injection-site lesions	Deer without injection-site lesions
Ad F: GonaCon	18	17 (94%)	1 (6%)
Ad F: sham	11	9 (82%)	2 (18%)
3- to 4-month-old fawns: GonaCon	12	8 (67%)	4 (33%)
8-month-old fawns: GonaCon	2	2 (100%)	0 (0%)
Ad M: GonaCon	6	6 (100%)	0 (0%)
Totals	49	42 (86%)	7 (14%)

[*] Sham material consisted of AdjuVac adjuvant and mollusk stabilizing buffer (i.e., GonaCon vaccine without the GnRH and mollusk hemocyanin).
[**] No injection-site lesions were found in 13 ad M control deer that received no injections.
[a] Ages in this column are ages at time of injection.

Fawns (F, 8 Months Old)

Two of the 4 fawns vaccinated in February 2006 were collected for necropsy in October 2007. Deer number 76 was "thin" based upon body condition score, with "little" general body fat. Deer number 78 was in "fair" body condition, with "little-to-moderate" general body fat (Table 1). Heart and kidney fat deposits were 3–5 mm thick in these 2 deer.

Lesions were found at vaccine injection sites in both deer at necropsy (Table 3). An intramuscular scar caused by a tranquilizer dart also was found in the hind leg of deer number 78. The popliteal and iliac lymph nodes were hard in deer number 78; only the popliteal lymph node was hard in deer number 76.

Fawns (M and F, 3–4 Months Old)

Twelve of the 16 (8 M and 8 F) fawns we captured and vaccinated in September–October 2006 were collected and necropsied in October 2007. Although body condition varied substantially, half of these deer were in "poor" or "thin" condition, and only one was in "good" condition (Table 1). General fat deposits were "little" in half of these deer, and no deer had more than "moderate" fat stores. Heart and kidney fat stores were $\leq$2 mm in thickness in 4 of the 12 necropsied deer, and from 3 mm to 10 mm in the remaining deer ($n = 7$) that we evaluated.

Vaccine injection-site lesions (hard nodules, no abscesses) were found in 8 (67%; 5 [100%] of 5 F and 3 [43%] of 7 M; Table 3), and tranquilizer dart scars were found in 7 (58%) of the 12 GonaCon-treated deer for which these data were recorded. Histopathological examination of tissue samples taken from the vaccine injection sites of 2 deer detected chronic granulomatous inflammation in both cases. One of these 2 deer also had chronic granulomatous inflammation in its other hind limb from the tranquilizer dart injection.

Enlargement of the popliteal lymph node was observed at necropsy in 3 (27%) of 11 examined deer for which data were recorded, and enlargement of the iliac lymph node was detected in 1 of these 3 deer. The popliteal and iliac lymph nodes from 2 (1 M and 1 F) of these 3 deer were examined histopathologically. One (unidentified) lymph node from the male had chronic granulomatous inflammation, and acute hemorrhage was found in one lymph node from the female. The remaining lymph node from each animal showed no signs of pathology.

Adult Males

Of the 10 adult males that were captured and vaccinated with GonaCon during March 2007, 2 died of unknown causes and 2 left the study site before October 2007. Body condition scores were determined for 4 of the 6 remaining males; the mean was higher than that for 5 noninjected control males ($t_5 = 2.31$, $P = 0.032$; Table 1). Heart and kidney fat deposits were 4–8 mm thick in 3 males that had "moderate" general fat deposits, and they were 2–4 mm thick in a male that had "little-to-moderate" general fat deposits.

Vaccine injection-site lesions were observed in all 6 males at necropsy (Table 3), but no tranquilizer dart wounds or scars were found. Two (33%) of these 6 males had small abscesses in addition to hard nodules. Popliteal lymph node reactions were evident at necropsy in 5 (83%) of these males. Enlargement of the iliac lymph node was seen in 2 (33%) of the males. Relatively small testes and delayed or abnormal antler development were observed in 2 of the adult males and in males that had been vaccinated as 3- to 4-month-old fawns (J. P. Gionfriddo, unpublished work).

Control Males

Thirteen previously untreated males (12 of which had never been captured) were collected for necropsy during October 2007 for comparison with GonaCon-treated males. Heart and kidney fat deposits were $\leq$5 mm, and general fat deposits were "little" to "moderate" in 5 control males we evaluated at necropsy. Necropsy observations and photographs of the other 8 control males indicated that they also were generally in "thin" to "fair" body condition (J. P. Gionfriddo, unpublished work).

DISCUSSION

Treatment with GonaCon Immunocontraceptive Vaccine caused no serious adverse effects on deer mobility, major organs and organ systems, or general health and welfare. Intramuscular injection-site lesions were the only adverse effect of vaccination that we detected via field observations, necropsy examinations, laboratory assessment of blood chemistry, and histopathological evaluation of selected reproductive and other tissues collected from study deer. These results are consistent with those of earlier studies of GonaCon vaccination of white-tailed deer (Killian et al. 2006*b*, Gionfriddo et al. 2009). In New York, USA, Curtis et al. (2008) detected no adverse health effects (except injection-site lesions) associated with hand- and remote-vaccination of female white-tailed deer with 2 early formulations of GonaCon. Instead of AdjuVac, Freund's complete adjuvant (FCA) was used in the primary dose and Freund's incomplete adjuvant (FIA) (Freund 1956) was used in the booster injection.

Although transitory limping can be common among animals that receive intramuscular vaccinations that produce injection-site inflammation and granulomas (Macy 1997), we saw no limping or decreased mobility in injected deer in this study. Although histopathological evaluation found chronic granulomas in both rear legs of one of the adult males (from the tranquilizer dart and a GonaCon hand injection), the animal was never seen limping during many field observations in 2007.

Body Condition and Fat Reserves

Prior to the start of our field research, deer had not been actively managed on the study site for many years, and the herd had increased to about 95 animals by early spring 2005 (A. J. DeNicola, White Buffalo, Inc., unpublished data). At capture in July 2005, most deer were noticeably thin, as reflected in their low body masses. Sustained and severe overbrowsing by deer was evident in the condition of native and ornamental vegetation throughout the study site. Thus it was not surprising that fat reserves of study deer at necropsy generally were meager.

GonaCon-treated adult females, especially those that had not been pregnant during the field study, were in better body condition at necropsy than control females. By relieving females of the annual energetic burdens of gestation and lactation, immunocontraception may have enabled treated deer to maintain better body condition than control females that produced and raised fawns (McShea et al. 1997). Body condition was not evaluated in previous GonaCon studies. The effects of porcine zona pellucida (PZP) immunocontraception on body condition have been equivocal. Adult female white-tailed deer contracepted with a PZP vaccine in Connecticut, USA were not in better condition than untreated controls after a 3-year field study (Walter et al. 2003). Among captive white-tailed deer in Pennsylvania, USA, no differences in annual autumn body weights were detected between PZP-treated and control females during a 4-year vaccine trial (Miller et al. 2001). On the other hand, female deer successfully contracepted with PZP in Virginia, USA were heavier 1 year posttreatment than untreated control females that had become pregnant (Kirkpatrick et al. 1997). Many deer treated with PZP undergo repeated estrous cycling that is energetically costly and may adversely affect body condition (Miller et al. 2009). In addition, fertilization during later estrous cycles can produce fawns whose late birthdates could reduce their survivorship during their first winter (McShea et al. 1997). GonaCon does not cause repeated estrous cycling.

The better condition of GonaCon-treated males when compared to control males may be related to the rut and to the relatively short interval between vaccination and necropsy. Control males may have lost body condition through seasonal rut-associated behavior such as fasting, sparring, and chasing. Although GonaCon-treated males generally do not grow as large as untreated males (Killian et al. 2005), males in our study were injected with GonaCon in March 2007 and killed in October 2007, perhaps before some effects of the vaccine (such as reduced muscle development) were manifested.

Blood Chemistry

Blood-chemistry parameters may be influenced by many factors, including sex, age (Johnson et al. 1968), season, reproductive status (Klinger et al. 1986), nutrition (Sams et al. 1998), and capture-related factors such as capture and handling methods (Mautz et al. 1980, Kock et al. 1987*b*, DelGiudice et al. 1990), including the use of immobilizing agents (Marco and Lavín 1999, Poljičak-Milas et al. 2006, Vengušt et al. 2006). Several aspects of our blood-sampling procedures may have affected blood parameters and could have contributed to differences between treatment groups or years. At initial capture in 2005, blood was drawn from the jugular veins of live deer, whereas cardiac puncture of lethally gunshot deer was used at the end of the field study in 2007. The latter collection method may have affected the poststudy (2007) values of some blood parameters (White and Cook 1974). For example, cardiac puncture of a dead animal tends to yield higher values for CPK, potassium, and phosphorus than venipuncture of a live animal, and trauma associated with lethal shooting also can elevate these parameters (Savignano et al. 1969, Seal et al. 1981, Latimer et al. 2003). Creatinine phosphokinase, potassium, and phosphorus were the only blood-chemistry parameters in our study with values outside the normal ranges for adult white-tailed deer. A second potential capture-related source of variation in blood-chemistry results was our use of immobilizing agents in 2005 but not in 2007. These compounds may affect blood parameters and could have influenced our results (Seal and Bush 1987). Finally, observed differences in the values of some blood parameters could be due to seasonal (July vs. October) variation in nutrition and physiology (Seal et al. 1981, Waid and Warren 1984).

Despite the above limitations, our data show that GonaCon caused no serious adverse health effects that were reflected in blood parameters. Other researchers have reported similar results. Curtis et al. (2008) measured 28 standard blood parameters in white-tailed deer in New York and found no differences between GnRH-treated and control animals. Killian et al. (2006*b*) used standardized methodology under carefully controlled conditions to handle captive, GonaCon-treated, white-tailed deer and to sample their blood. Their comparison of the blood chemistry and hematology of 19 captive adult females detected no statistically significant treatment effects for any of the 33 blood parameters tested. As expected, however, seasonal changes in some variables were detected via repeated sampling over a 20-week period (Killian et al. 2006*b*).

Posttreatment values for CPK, potassium, and phosphorus were abnormally high in our study deer (Table 2). Elevated CPK values usually reflect stress or trauma to heart, brain, or skeletal muscle. Lethal gunshot wounds to the head or neck, and cardiac puncture for blood collection immediately after death could have caused CPK to increase dramatically. Stress and tissue damage associated with chronic inflammation at injection sites and in lymph nodes (reactions to injection) also could have contributed to the elevation of CPK. Evaluation of CPK values may not be useful due to the extreme fluctuations that commonly occur. For example, 200-fold increases in serum CPK were observed in penned white-tailed deer 24 hr after deer were physically restrained (Seal et al. 1972).

Elevated concentrations of potassium and phosphorus, like CPK, often are associated with excess destruction or necrosis of cells, and may reflect the extent of tissue (especially muscle) damage (Kock et al. 1987*a*, Latimer et al. 2003). Wilber and Robinson (1958) collected Maryland, USA white-tailed deer via lethal gunshots to neck vertebrae and then immediately sampled blood via heart puncture. They found consistently high concentrations of plasma potassium (similar to our values) and phosphorus, which they attributed to "shock." Using similar methods in Oklahoma, USA, Sams et al. (1998) found very high serum phosphorus concentrations in white-tailed deer. Elevated potassium and phosphorus concentrations in our study deer were likely due to muscle necrosis associated with collection of deer and blood samples.

Mean posttreatment alkaline phosphatase (ALK) values showed a 6-fold increase from pretreatment values in our

study, but they remained within the ranges of values reported for white-tailed deer (e.g., Sams et al. 1998, Curtis et al. 2008) and for our sample of noninjected control male deer ($\bar{x} = 221.3$, SE = 48.5, $n = 13$). A published range of normal values for white-tailed deer could not be found for this variable, but the range is very broad for large mammals and, therefore, ALK is not a sensitive indicator (Latimer et al. 2003).

Injection-Site Lesions

The occurrence of intramuscular injection-site lesions in 5 (29%) of 17 GonaCon-treated deer in a previous field study (Gionfriddo et al. 2009) alerted us to the possibility that deer in the current study might develop such reactions. Necropsy observations and histopathological evaluation revealed that >85% of the GonaCon-treated and sham-injected deer in New Jersey had injection-site granulomas or sterile abscesses in the deep musculature of the hind limbs in October 2007. The similarity in the incidence of injection-site lesions in GonaCon-treated and sham-injected adult female deer strongly suggests that the causative agent(s) was present in both inocula.

Adjuvants and vaccines often cause localized reactions at injection sites, including pain, local inflammation, swelling, necrosis, granulomas, ulcers, and sterile abscesses (Aguilar and Rodríguez 2007). For example, adjuvants that include alum (Gupta et al. 1995, Valtulini et al. 2005, Reed et al. 2008) or *Mycobacterium* spp. (Broderson 1989) often cause reactions at injection sites. Larger amounts of mycobacteria cause proportionately more inflammation and necrosis (Broderson 1989). Water-in-oil emulsions, especially those containing mineral oil, also induce local inflammation and granuloma formation at injection sites (Humphrey 1982, Straw et al. 1985, Yamanaka et al. 1992, Macy 1997, Petrovsky and Aguilar 2004). Injection-site granulomas and other lesions are routinely encountered in livestock, from which they are excised at slaughter (George et al. 1995, Van Donkersgoed et al. 1997, Valtulini et al. 2005). They also commonly occur in humans (Hay 1995, Bordet et al. 2001, Marsee et al. 2008).

GonaCon Immunocontraceptive Vaccine contains AdjuVac, a mineral-oil-based adjuvant developed from a USDA-approved Johnes disease vaccine called Mycopar™ (Fort Dodge Animal Health). AdjuVac contains killed *Mycobacterium avium*, which is needed for GonaCon vaccine to induce a rapid, strong (Miller et al. 2008*a*), and sustained (Perry et al. 2008) contraceptive effect. *Mycobacterium* spp. are commonly used in adjuvants to strengthen the immune response (Gupta et al. 1993, Aguilar and Rodríguez 2007). Like Freund's complete adjuvant (which contains *Mycobacterium paratuberculosis* and has been used extensively in laboratory animals), AdjuVac's combination of water-in-mineral-oil emulsion and killed mycobacteria results in a highly potent adjuvant that stimulates both humoral and cellular immunity (Warren et al. 1986). Freund's complete adjuvant, however, cannot be used in human or veterinary vaccines because it may cause severe pain, fever, abscess formation, and the possibility of permanent organ damage (Edelman 1980). Leenaars et al. (1998), for example, found extensive intramuscular lesions in rabbits (*Oryctolagus cuniculus*) and mice (*Mus musculus*) after injection with FCA and FIA (as prime and boost, respectively), although palpation had detected no swelling or nodules. Wild horses injected with an early formulation of a PZP immunocontraceptive vaccine that contained FCA developed externally visible nodules, swelling, and abscesses at injection sites (Roelle and Ransom 2009). Porcine zona pellucida and GnRH vaccines (early formulations of GonaCon that contained FCA or FIA instead of AdjuVac) also caused granulomas at hand-injection (PZP) and dart-injection (PZP and GnRH) sites in nearly all treated white-tailed deer in New York (Curtis et al. 2007, 2008). The intramuscular injection of large doses of FCA or FIA can cause diffuse necrosis and extensive atrophy of functional muscle groups, impairing function (Broderson 1989). Such severe reactions have not been observed in animals injected with GonaCon (containing AdjuVac), possibly because of the relatively small amount of mycobacteria it contains (Miller et al. 2008*a*), or because GonaCon uses a different mycobacterial species.

Necrosis of muscle fibers at injection sites was observed in many of our study deer in New Jersey and in Maryland (Gionfriddo et al. 2009), and in captive white-tailed deer (Killian et al. 2006*b*) and rabbits (Powers et al. 2007) treated with GonaCon. Exposure to necrotic cells may be necessary for the maturation of dendritic cells and the initiation of an immune response (Gallucci et al. 1999, Sauter et al. 2000). Moreover, the strength of the immune response may be proportional to the extent of local tissue damage caused by the inoculum at the injection site (Schijns 2000). Thus the necrosis of muscle fibers at the injection site by adjuvants containing alum (Walls 1977), mineral oil (Straw et al. 1985, Yamanaka et al. 1992), or mycobacteria (Broderson 1989) may yield important immunological benefits.

Vaccines that contain alum or mycobacteria, or that are formulated as water-in-oil emulsions, also may induce strong immune responses because they form a repository ("depot") at the injection site (Glenny et al. 1931, White 1967, Fukanoki et al. 2000). The vaccine depot slowly releases antigen, which is carried by maturing, migratory dendritic cells via afferent lymph vessels to the paracortical regions of the local draining lymph node, where it is presented to resting, antigen-specific T cells that are activated by the now-mature dendritic cells (HogenEsch 2002). Antigen has been detected in lymph nodes as early as 3 hr after peripheral injection (Dupuis et al. 1998). Antigen may be protected and retained for months or even years on follicular dendritic cells in draining lymph nodes where antibody formation continues (Greene et al. 1975, Tew and Mandel 1979, Burton et al. 1994). Subsequent release of antigen from the depot acts as a secondary stimulus (similar to a booster injection) to the sensitizing action of the antigen released previously.

In response to the presence of the vaccine depot, a granuloma develops as the immune system attempts to wall off the irritating foreign material by enclosing it in a fibrous capsule of thick connective tissue. Granulomas that develop

at injection sites contribute substantially to antibody synthesis because they harbor large numbers of antibody-producing plasma cells (White 1967, World Health Organization 1976). The release rate of antigen from an injection-site granuloma decreases as the granuloma develops and becomes encapsulated, and eventually it becomes insufficient to maintain an optimal antibody response (Herbert 1968, Lascelles et al. 1989). After the granuloma is completely walled off and antigen release stops, excision of the granuloma does not affect antibody formation in the draining lymph node because antigen is already present there (Lascelles et al. 1989, Schijns 2000). Active but unused antigen remains trapped inside the encapsulated granuloma, as demonstrated by researchers who excised material from injection-site granulomas and injected it into the same or different animals, thereby inducing immune responses in the recipients (Harrison 1935, Holt 1950, Herbert 1968).

Lymph Nodes

Popliteal and iliac lymph nodes are the draining and regional lymph nodes for the hind limb area where hand injections were delivered to deer in our study. Enlargement of local lymph nodes is a normal response to infection or other types of antigenic stimulation, and also can be caused by injection of oil emulsions (Yamanaka et al. 1992). Stronger antigens and adjuvants induce greater inflammation of draining lymph nodes (Taub et al. 1970).

In our study, popliteal and iliac lymph nodes of noninjected control male deer were normal, as expected. Many of the lymph nodes of GonaCon-treated and sham-injected deer, however, were enlarged, hard, or otherwise reactive, indicating the involvement of these nodes in the drainage of the intramuscular injection site. These results demonstrate that the material that caused reactions in lymph nodes was present in both GonaCon vaccine and in the sham material (which contained AdjuVac adjuvant and mollusk stabilizing buffer). The mineral oil (Aucouturier et al. 2001) or the *M. avium* (Herbert 1968, Lind 1968) in AdjuVac could have caused the draining lymph nodes to react. Enlargement of draining lymph nodes also was reported in captive rabbits (Powers et al. 2007) and in captive (Killian et al. 2006*b*) and wild (Gionfriddo et al. 2009) white-tailed deer treated with GonaCon vaccine.

In $\geq$6 of our study deer, secondary granulomatous inflammation and hyperplasia occurred in draining lymph nodes to which the irritant at the injection site had been transported by lymph. Similarly, one of 13 captive adult female white-tailed deer vaccinated with GonaCon in Pennsylvania developed granulomatous lymphadenitis in a draining lymph node (Killian et al. 2006*b*). Development of granulomas in draining lymph nodes is common when alum-absorbed adjuvants (Humphrey 1982), FCA (Yamanaka et al. 1992), or FIA (Gaafar and Turk 1970) are used. In extreme cases, granulomatous lesions may replace the majority of the tissue in lymph nodes (Yamanaka et al. 1992). Unanue and Benacerraf (1973) demonstrated that granulomatous reactions may be passively transferred between animals by intravenously injecting lymph node cells from a granulomatous lymph node in one animal into a second animal.

The oil droplets detected in the lymph nodes of several of our study deer probably originated in the inoculum and were transported via lymphatic vessels to the draining lymph nodes. Previous studies have documented the presence of paraffin or mineral oil (used as an adjuvant) in lymph nodes after subcutaneous or intramuscular injection (White et al. 1955, Freund 1956, Broderson 1989). Long-term (probably lifelong) retention of oil in body tissues may result from the use of mineral oil adjuvants (World Health Organization 1976).

MANAGEMENT IMPLICATIONS

GonaCon Immunocontraceptive Vaccine provides a safe and humane way to reduce reproduction in individual adult female white-tailed deer, especially in demographically closed (e.g., fenced or otherwise spatially isolated) populations. The only adverse reactions to vaccination in our field study were normal inflammatory responses at injection sites and in associated draining lymph nodes. These responses were natural and essential expressions of the strong immune response that is required to induce temporary infertility. Safety issues should not impede acceptance of GonaCon as a deer management tool. Operational use of GonaCon Immunocontraceptive Vaccine and other wildlife contraceptives will likely be limited by efficacy, practicality, and politics to settings where traditional management methods cannot be applied. The widespread occurrence of such settings suggests that the potential contribution of contraceptive agents to deer management in developed landscapes is substantial. In addition to its contraceptive effects, GonaCon may improve deer body condition without the PZP-associated disadvantages of repeated estrous cycling and delayed birth of fawns to vaccine nonresponders. Although public demand for safe, humane contraceptive agents like GonaCon will continue to drive their research and development, wildlife management agencies and personnel will ultimately determine the value and applicability of these products.

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