2008

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Human–Wildlife Conflicts 2(1):60–67, Spring 2008

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Abstract: Observations of extended estrus seasons in female white-tailed deer (*Odocoileus virginianus*) vaccinated with porcine zona pellucida (PZP) immunocontraceptives have led to speculation that management use of PZP would, perversely, increase the rate of deer–vehicle collisions. To test this hypothesis, we studied PZP-treated female deer at the National Institute of Standards and Technology, Gaithersburg, Maryland. PZP-treated female deer did not differ from untreated female deer in risk of death by vehicle collision, and the number of deer killed in vehicle collisions was uncorrelated with the number of deer treated with PZP, whether or not population size was controlled for. The reduction in deer population size observed during PZP applications was associated with a reduced frequency of deer–vehicle collisions. Our results suggest that any added collision risk caused by behavioral effects of PZP is unmeasurably small compared to the effects of population management and other mitigation actions.

Key words: deer–vehicle collision, human–wildlife conflict, immunocontraception, Maryland, *Odocoileus virginianus*, PZP, suburban deer, white-tailed deer

Wildlife biologists for more than 30 years have been exploring the possibility of controlling free-ranging deer populations using contraception (e.g., Harder and Peterle 1974, Bell and Peterle 1975, Matschke 1977, Roughton 1979). The pace of exploration has accelerated in the last 15 years, with the development and testing of several new technologies. Such technologies include immunocontraceptive vaccines using porcine zona pellucida (PZP) and GnRH antigens, as well as GnRH agonists, such as deslorelin and leuprolide (Turner et al. 1992, 1996; Miller et al. 2000, 2001; Herbert and Trigg 2005). The research has been driven both by technological advancements and by increased public interest in novel techniques for managing deer populations living in cities, suburbs, and other areas.

Small to moderate-sized populations of white-tailed deer (*Odocoileus virginianus*) have been stabilized and modestly reduced at 2 localized suburban sites (Naugle et al. 2002, Rutberg et al. 2004). These population-level effects were accomplished with contraceptive vaccines that require annual retreatments to remain effective. Recently, however, the development of effective, long-acting, single-treatment contraceptive vaccines, as well as other technical refinements, expands the range of potential management applications (Fraker et al. 2002, Miller et al. 2004, Turner et al. 2007).

The accumulation of safety and efficacy data together with pending changes in federal regulation of wildlife contraceptives also raise the likelihood that such contraceptives will become available to wildlife managers in the near future. The limited commercial prospects of contraceptives for deer and other wildlife offered little hope of recouping the costs of meeting the manufacturing, tracking, and testing standards imposed by the federal Food and Drug Administration for the marketing of new drugs. However, the assumption of authority over wildlife contraceptives by the Environmental Protection Agency (under the Federal Insecticide, Fungicide, and Rodenticide Act) brings regulatory standards more into line with the technology and its applications, and significantly raises the prospects for federal approval. EPA has already registered 1 wildlife contraceptive, OvoControl™G (Innolytics, Calif.; EPA reg. #085712), for use in controlling resident Canada goose populations.

If contraceptives are going to be useful and publicly acceptable for the management of urban and suburban deer populations, they will have to control deer numbers and help mitigate the conflicts between deer and people that cause public concerns about deer. One of the most important concerns raised by elevated numbers of deer in cities, towns, and suburbs (as well as rural areas) is the associated increase in the frequency of deer–vehicle collisions (DVCs) (Conover et al. 1995, Hussain et al. 2007, Storm et al. 2007). One should not overstate the significance of the problem; animal–vehicle collisions cause <0.5% of traffic fatalities of humans nationwide, and most of them are associated with high-risk behavior, e.g., failure to wear seat belts and riding motorcycles, especially while not wearing a helmet (Williams and Wells 2005, Nelson et al. 2006). However, no deer management technique that increases the chances of a DVC will be, or should be, publicly acceptable.

Several investigators working primarily with captive or pastured deer have shown that female deer treated with PZP show repeated estrous cycles when they fail to conceive. These repeated cycles effectively extend the mating season, which normally winds down in December, into February or even March (Turner et al. 1996, McShea et al. 1997, Thiele 1999, Miller et al. 2001). This extended breeding season has not been shown to be harmful to the treated deer themselves or to late-born fawns (McShea et al. 1997, Thiele 1999, Miller et al. 2001, Walter et al. 2003). However, it has been repeatedly suggested in peer-reviewed, nonpeer-reviewed, and popular literature that such an extended mating season could increase deer activity during winter months and, consequently, have the perverse effect of elevating the risk of DVCs (e.g., Winand 1999; Miller et al., 2000, 2004). However, there is no evidence that directly addresses the relationship between PZP use and frequency of deer–vehicle collisions. Here we present data on frequency of deer deaths caused by vehicle collisions from a long-term study of PZP contraception of white-tailed deer at a site in suburban Maryland, and we examine the relationship between the administration of PZP vaccine and the incidence of DVCs.

Study area

The study was carried out at the 233-ha campus of the National Institute of Standards and Technology (NIST), a division of the U.S. Department of Commerce, in Gaithersburg, Maryland, USA. The NIST campus comprised research buildings and parking lots, lawns, open fields, 2 ponds, and 2 small wooded lots with canopies dominated by and tulip trees (*Liriodendron tulipifera*) and oaks (*Quercus* spp.). Because of a long history of deer browsing, the wooded lots largely lacked understory vegetation. When observations began in 1993, NIST was bounded on >85% of its perimeter by shopping centers, high-density suburban housing, and a 12-lane interstate highway. An additional tract of adjoining undeveloped land was cleared for housing construction early in 1997, and major construction projects were undertaken on campus during 1998–2001 and 2002–2005. The campus was surrounded by a 2.5-m-high chain-link fence, but deer passed over and under the fence, and through gates open to vehicle and pedestrian access.

Deer began receiving contraceptive treatments at NIST in late autumn 1995 (too late to be effective that year). Population size grew from about 180 (77/km^2) in 1993 to nearly 300 (129/km^2) in summer 1997, then declined to just above 200 (86/km^2) in 2002. This year-to-year population decline was positively associated with the proportion of females treated with contraceptive vaccines (Rutberg et al. 2004).

Methods

Data reported here were collected between 1994 and 2004 as part of a long-term study of the efficacy, safety, and population-level effects of different preparations of the PZP vaccine at NIST (Rutberg et al. 2004, Rutberg 2005). Animal capture and treatment protocols were approved by the University of Maryland's Institutional Animal Care and Use Committee (#R96-32), and were also reviewed by the Maryland Department of Natural Resources and the U.S. Food and Drug Administration under Investigational New Animal Drug file #8840 held by The Humane Society of the United States.

Deer capture and tagging

We captured deer using solid-sided box traps, chemical immobilization with Telazol® or Ketaset® combined with Xylaject®, and hand-capture of fawns (as described in detail in Rutberg et al. 2004). All captured animals received a brightly-colored plastic livestock ear tag (Nasco Farm and Ranch, Wis.) bearing a unique identification number (3-cm high) and a metal ear tag with the same identifying number, "NIST", and a contact telephone number.

Contraceptive vaccine preparation and delivery

PZP was prepared and dissolved in phosphate-buffered saline (PBS) as described by Liu et al. (1989). We prepared doses in the field that consisted of PZP in 0.5-ml PBS emulsified with 0.5-ml adjuvant. Adult, yearling, and fawn females received 1 of 5 PZP preparations, which employed several different adjuvants and doses and which varied in effectiveness. Approximately 58% of the PZP-treated females received an initial vaccination of 65-µg PZP in Freund's Complete Adjuvant (Sigma, St. Louis, Mo.) followed in autumn by a booster of 65-µg PZP in Freund's Incomplete Adjuvant (FIA; Sigma, St. Louis, Mo.), and by subsequent annual boosters of PZP in FIA (after Kirkpatrick et al. 1990). Rutberg et al. (2004) and Rutberg (2005) provide detailed descriptions of other vaccine preparations tested.

We delivered initial vaccinations from January to October by hand-held syringes at the time of capture or remotely via 1.0-ml self-injecting darts fired from a Pneu-Dart® .22-cartridge-powered dart rifle. We administered all boosters from August to November via 1.0-ml darts fired from the dart rifle. For calculation of individual and population birth rates, we categorized females as treated if they had received >2 PZP treatments, including a booster in the late summer or autumn immediately prior to the mating season.

Estimating population size and DVC frequency

We used 3 methods of direct, complete counting to estimate population size (Davis and Winstead 1980). (1) For vehicle counts, 1 to 3 observers drove a vehicle along a prescribed route that covered the entire campus, counted all deer observed, and categorized them by age and sex. (2) For drive counts, after posting volunteer observers outside the largest (16-ha) wooded lot to monitor egress and ingress of deer, 1 observer drove a vehicle along a prescribed route throughout the entire campus, excluding only the large wooded lot, and counted all deer observed. When this count was concluded, a line of volunteer observers 30 m to 50 m apart walked parallel transects through the wooded lot, counting all animals observed. We also made video recordings along the wooded lot boundary to help corroborate real-time counts. (3) In tag counts, beginning in 2000, 1 observer drove a prescribed route throughout the entire NIST campus on 3 different days within a 2-week period. The observer recorded the tag number of every deer observed and the number and location of untagged deer. Because the population was relatively small and >90% of the deer on campus carried ear tags during that period, it was possible to individually identify all untagged and tagged deer. We also used vehicle counts and tag inventories to estimate the number of adult and yearling females present. Although precision probably increased in later years of the study due to our greater familiarity with the site and the high proportion of tagged animals, estimates produced by the 3 methods were highly consistent (Rutberg et al. 2004).

Individual and population birth rates were estimated from fawn sightings based on intensive observation. The campus was searched for fawns by vehicle and on foot 2 to 3 times per week in May and June, and 1 to 2 times per week in July, August, and September. Nonetheless, some stillbirths and fawns suffering neonatal mortality were probably missed, so all birth estimates represent minimums.

We drew data on DVCs from several sources. The NIST police and the NIST wildlife coordinator observed or received reports of DVCs on and adjacent to the NIST campus. These reports were supplied by those involved in the accident, observers, and local authorities. Deer carcasses with NIST ear tags found off campus were also reported to NIST police or the NIST wildlife coordinator, with descriptions of the circumstances under which they were found. When possible, we recovered the carcasses and performed gross necropsies to determine consistency with an inference of DVC. Deer found dead on or adjacent to the NIST campus were subject to gross necropsy whenever possible; some of the necropsies indicated death by collision with a vehicle even in the absence of any collision report. We also included these in the data. We cross-checked all reports to avoid duplication.

Data analysis

To determine whether treated and untreated females differed in the seasonal distribution of deaths of deer from DVCs, we lumped monthly totals from necropsy reports into 4 quarters: winter (January–March), spring (April–June), summer (July–September) and autumn (October–December). We carried out Fisher's Exact Probability Tests to examine differences between seasons. We estimated the annual proportion of treated and untreated female deer killed in a DVC by dividing the total number of females of each category killed by vehicles by the sum of yearly estimates of the number of females in the population in each category (i.e., number of collision deaths divided by deer-years of exposure), and we compared them with a z-test for equal proportions.

To examine the relationship among deer deaths from DVCs, population size, and the administration of contraceptives, we calculated Pearson Correlation Coefficients between the frequency of deer deaths from DVCs in a given year, the size of the population that year, and the number of females treated the previous fall, under the assumption that behavioral and reproductive changes associated with fall PZP vaccinations would show themselves primarily in the subsequent year. To separate the effects of population size and number of females treated on the number of DVCs, we also calculated partial correlations for both, in each case controlling for the other variable, and carried out multiple regression using population size and number of females treated as independent variables and number of DVCs as the dependent variable. Except as otherwise specified, all statistical tests were carried out on SPSS 13.0 for Windows.

Results

Between March 1994 and December 2004, we captured and ear-tagged 691 deer (329 males and 362 females). Of these, 421 (229 males and 192 females) were fawns captured by hand, 208 (76 males and 132 females) were captured by chemical immobilization, and 62 (24 males and 38 females) were box-trapped. Over that period, population sex ratio (adult and yearlings only) ranged from approximately 1 male:1.3 females to 1 male:2.2 females, averaging approximately 1 male:1.8 females. There was no change in population sex ratios over time.

Between November 1995 and November 2003, we administered 1,042 PZP treatments to 276 does. The proportion of treated does rose from 39% for the 1997 birth season to 83% for the 2000 birth season, then rose gradually to 97% prior to the 2004 birth season (Figure 1). From the 1997 through the 2004 birth seasons, PZP-treated females were observed to give birth to an average of 0.23 fawns per female per year; untreated females were observed to give birth to 0.63 fawns per year, for a total observed population birth rate of 0.38 fawns per female per year (Figure 1). The low fertility in untreated females in 2004 and earlier is due largely to small sample size and the inclusion in this category of some female deer that had been treated in previous years with PZP but had been removed from treatment as part of a study of vaccine reversibility.

One-hundred sixty-nine NIST deer were reported killed in DVCs between 1994 and 2004. Of these, we necropsied 129 (76%). Deer road-kill reports were distributed throughout the year (Figure 2). The seasonal distribution of road-kill reports involving PZP-treated females did not differ from that of collisions involving untreated females ($P = 0.56$). Although DVCs in February and March were reported for PZP-treated female deer only, PZP-treated females were no more likely to be involved in DVCs in winter relative to other seasons than untreated females ($P = 0.40$). Between 1996 and 2004, 32 PZP-treated and 13 untreated female deer were reported killed by vehicles, for an average of 0.040 collisions per deer per year for PZP-treated females and 0.043 collisions per deer per year for untreated female deer ($z = 0.16$, $P = 0.87$). The number of DVCs involving adult (>1.5 years) male deer peaked in autumn and then declined sharply during the winter (Figure 2).

Following the population peak of just under 300 deer in March 1998, the deer population declined 7.9% per year through 2002 and remained roughly stable through 2004 (Figure 3). The number of reported DVCs also peaked in 1998 and declined in subsequent years. Between 1994 and 2004, the annual number of DVCs was strongly correlated with population size ($r = +0.65$, $n = 11$, $P = 0.03$) and uncorrelated with the number of females treated with PZP ($r = +0.19$, $n = 11$, $P = 0.57$). We found no relationship between the number of DVCs and the number of female deer treated with PZP, even when population size was controlled for through partial

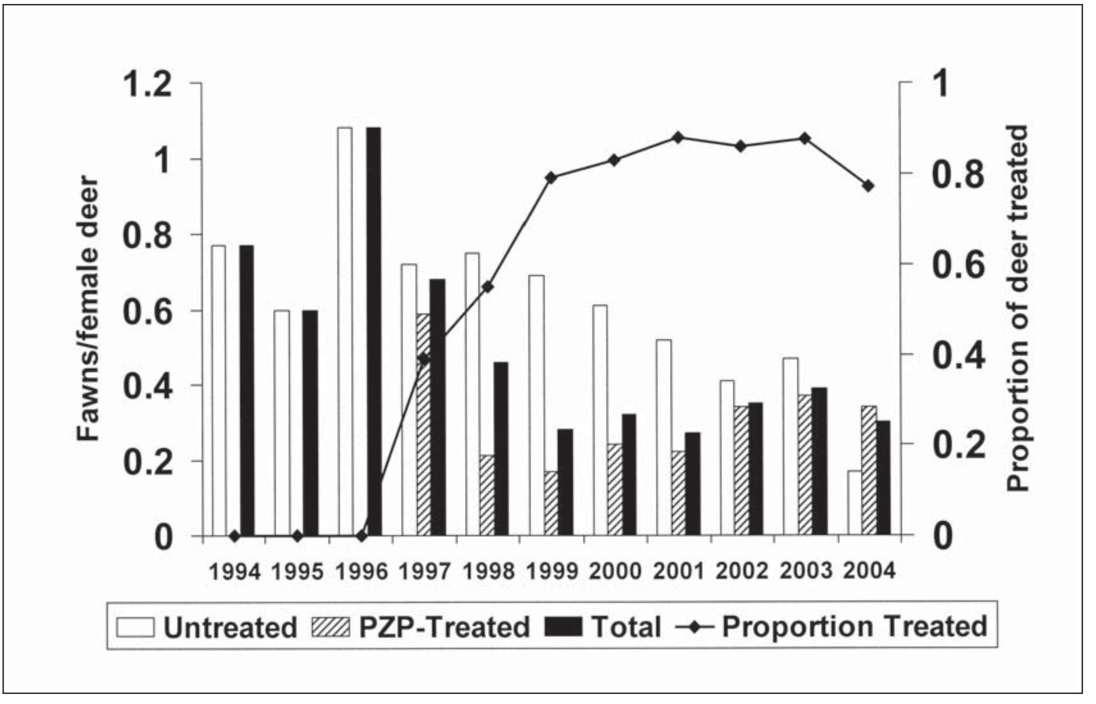

Figure 1. Proportion of females treated with PZP immunocontraceptive vaccines and average fertility among PZP-treated and untreated deer, and all female white-tailed deer at the National Institute of Standards and Technology (NIST) campus, Gaithersburg, Md., 1994–2004.

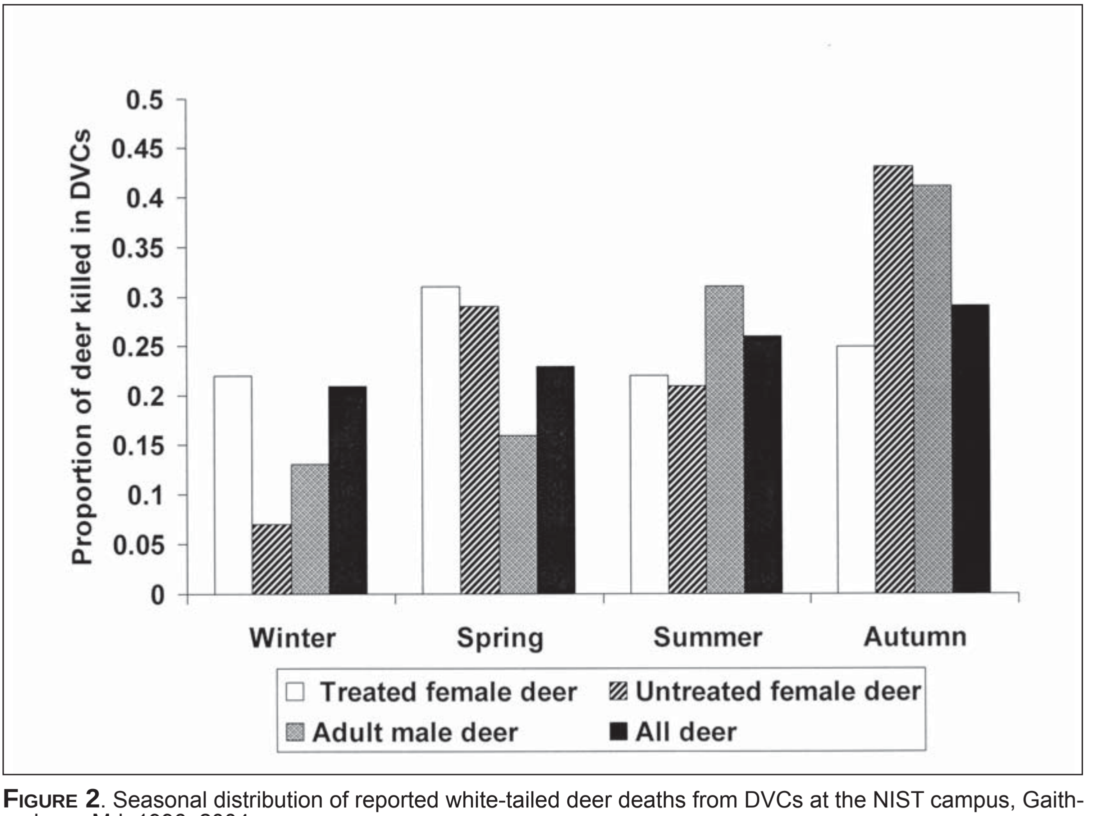

Figure 2. Seasonal distribution of reported white-tailed deer deaths from DVCs at the NIST campus, Gaithersburg, Md.,1996–2004.

correlation (r_8 = +0.15, P = 0.67). Multiple regression analysis produced similar results. In a simple forced entry regression model, population size was a positive predictor of DVC deaths (t_8 = 2.36, P = 0.05), but the number of PZP-treated females was not (t_8 = 0.44, P = 0.67; overall model summary, R^2 = 0.43, $F_{2,8}$ = 3.05, P = 0.10). Likewise, in stepwise regression, population size significantly predicted the number of DVC deaths (R^2 = 0.42, $F_{1,9}$ = 6.49, P = 0.03), but the number of PZP-treated females failed to meet the model inclusion requirements (P < 0.05).

Discussion

This study found no support for the hypothesis that PZP treatments increased the risk of DVCs. The risk of deer mortality due to DVCs was the same for PZP-treated and untreated female deer; there was no significant difference between PZP-treated and untreated female deer in the distribution of reports of collision-related deaths during the year; and there was no relationship between the number of females treated with PZP in a year and the number of DVCs reported the following year, even when variability in population size was taken into account. That all 5 DVCs involving does during February and March involved PZP-treated animals is consistent with the interpretation that the extended estrus cycling associated with PZP treatments may raise the risk of DVCs, but this occurrence was not statistically distinguishable from background variability. Moreover, there was no evidence of elevated risk of DVCs in adult males during the winter; male DVCs peaked during the normal mating season and declined rapidly during winter.

In contrast, our data indicated that deer population size was a strong predictor of the frequency of DVCs, explaining approximately 42% of the variance. Because contraceptive treatments were directly associated with a decline in deer population size (Rutberg et al. 2004), our data further suggest that the contraceptive-based deer population management program at NIST helped reduce the frequency of DVCs at NIST.

However, other circumstances and manage-

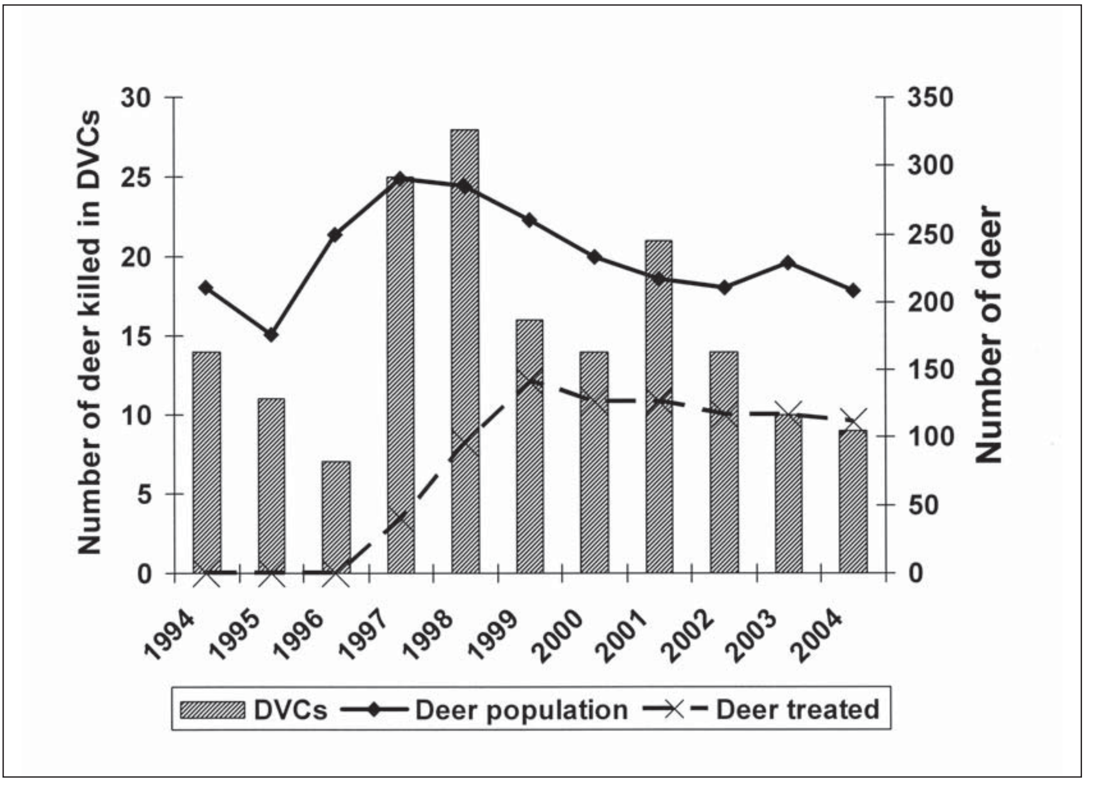

FIGURE 3. Population size, contraceptive treatments, and DVCs at the NIST campus, Gaithersburg, Md., 1994–2004.

ment actions also plausibly contributed to the decline in the number of DVCs in and around NIST following 1998. After September 11, 2001, security measures on site increased significantly. Peripheral gates were closed, and all traffic was directed through a single main gate. On-campus police patrols increased, perhaps improving compliance with posted speed limits. In 2002, NIST management also imposed a series of DVC prevention actions, including an aggressive campaign to educate campus employees and contractors and the placement of speed bumps and roadside warning signs. Thus, our study affirms that contraception and other wildlife population control methods can reduce DVCs when carried out as part of a comprehensive program.

Anecdotal observations of extended estrus cycling for some PZP-treated deer at NIST are corroborated by 1998–1999 data, demonstrating an extended birth season among PZP-treated NIST deer (Thiele 1999). Nevertheless, our data show that no increased risk associated with PZP-related behavioral changes could be detected in our field application. If such risks were present at all, they were completely masked by the observed reduction in deer population size and by other factors influencing deer and vehicle movements on the NIST campus.

There are still open issues concerning the potential for managing suburban deer populations with PZP immunocontraception, including cost, efficiency, the presence or absence of harmful long-term population and behavior effects, public acceptance, and the rate and magnitude of deer population reduction. However, conjectures that DVCs are increased by use of PZP are unsupported, and pending contradictory evidence from other studies, such conjectures should not stand in the way of potential applications of PZP to suburban deer populations.

Acknowledgments

We thank R. Hurt and the NIST police for their assistance in reporting DVCs and drawing our attention to deer carcasses on campus. We also thank R. Hurt, P. McElroy, L. Thiele, A. Pederson, J. Conaway, and the many others who have assisted with field work. J. Kirkpatrick and several anonymous reviewers commented helpfully on the manuscript. The study was supported by the National Institute of Standards and Technology and The Humane Society of the United States.

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