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Field testing of immunocontraception on white-tailed deer (*Odocoileus virginianus*) on Fire Island National Seashore, New York, USA

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Application of contraception for the control of suburban populations of white-tailed deer (*Odocoileus virginianus*) has been much debated, but few data are available on field applications and even fewer on population effects. Between 1993 and 1997, 74–164 individually known female deer living on Fire Island, New York, USA, were treated remotely with an initial shot of 65 μg porcine zona pellucida (PZP) in Freund's complete adjuvant followed by booster injections of 65 μg PZP in Freund's incomplete adjuvant. Starting in 1996, progressively increasing numbers of deer were treated with vaccinating/marking darts. Estimates of population density and composition, using distance sampling methods, began in 1995 in selected portions of the study area. Between 1993 and 1997, fawning rates among individually known, treated adult females decreased by 78.9% from pretreatment rates. Population density in the most heavily treated area increased by 11% per year from 1995 to March 1998 and then decreased at 23% per year to October 2000. In 1999–2000 surveys, fawns comprised 13–14% of the total population in the most heavily treated area, versus 16–33% in nearby untreated areas. These results show that PZP can be delivered effectively to sufficient deer to affect population density and composition in some environments, but that technical and logistical improvements are needed before contraception can be used widely to manage suburban deer populations.

Introduction

In the eastern United States, dense (> 50 per km^2) populations of white-tailed deer (*Odocoileus virginianus*) occupy many urban and suburban areas, where they raise public safety and health concerns, damage gardens, crops and ornamental plantings, and profoundly affect

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community structure in natural areas (Conover, 1997; Waller and Alverson, 1997). In these communities, traditional means of wildlife population control, such as hunting, may be illegal, ineffective or publicly unacceptable. The management challenges posed by dense deer populations in these environments have stimulated renewed interest in alternative approaches and the use of contraception to control urban and suburban deer populations in particular is being discussed widely (McAninch, 1995; Warren *et al.*, 1997; Rudolph *et al.*, 2000).

Controlling white-tailed deer populations by contraception is not a new idea. Oestrogen and progestin contraceptives delivered orally, by i.m. injection or by surgical implant have been tested on deer with mixed success since the late 1960s (Harder and Peterle, 1974; Matschke, 1977; Roughton, 1979; Plotka and Seal, 1989; White *et al.*, 1994). However, the large dosages required for effective steroid contraception have raised logistic, safety and ecological concerns that have so far proven insurmountable obstacles to practical field application (Turner and Kirkpatrick, 1991; Warren *et al.*, 1997).

Coinciding with the increased interest in deer contraception has been the adaptation of immunocontraceptive vaccines for use in field settings (Turner and Kirkpatrick, 1991). The small volumes required for immunocontraceptives improve the prospects for remote delivery, and the biodegradability of protein-based immunocontraceptive vaccines greatly reduces the risks involved in secondary consumption (Miller, 1997; Warren *et al.*, 1997). Among the most promising of the immunocontraceptive vaccines is porcine zona pellucida (PZP). PZP delivered remotely via dart to wild horses at Assateague Island National Seashore, Maryland, reduced fertility by at least 95%, and contraceptive effects were reversible after 3 or more consecutive years of treatment (Kirkpatrick *et al.*, 1990, 1995; Kirkpatrick, 1995). Soon afterwards, it was shown that PZP also significantly reduced fertility in white-tailed deer, with contraception being reversible after 2 consecutive years of treatment (Turner *et al.*, 1992, 1996; McShea *et al.*, 1997; Miller *et al.*, 1999). However, few data are available on the effects of immunocontraception on the density and composition of deer populations in the field.

The present study was conducted in two phases. The aims of the initial phase (1993–1997) were: (i) to determine whether it was possible to deliver PZP vaccine remotely to an ecologically significant number of females in an unconfined suburban population; (ii) to test the efficacy of the PZP vaccine in the field; and (iii) to explore techniques that could be used in population management applications of immunocontraceptive vaccines (preliminary results from this phase of the study were reported in Kirkpatrick *et al.*, 1997). In the second phase, the experience of the first phase was applied to examine the effects of contraception on population density and composition.

Materials and Methods

Study site

Fire Island is a 55 km long barrier island running approximately east–west off the southern coast of Long Island, New York (40° 41′ N, 73° 00′ W). The island varies in width from approximately 0.2 km at its central portion to approximately 1.0 km at its eastern and western ends. Approximately 48 km lies within Fire Island National Seashore (FIIS), a unit of the National Park Service (NPS). The western half of FIIS is a mosaic of 19 heavily developed residential communities with several stretches of undeveloped or lightly developed NPS land lying between them. Topographically, Fire Island is a typical Atlantic coastal barrier island, supporting saltmarsh, meadow, interdune, maritime forest and dune vegetation communities in less developed and undeveloped areas (O'Connell and Sayre, 1989). A diverse mix of non-native ornamental plants dominates the island in and near the communities.

The initial phase of the study (1993–1997) was performed in 14 Fire Island communities located between Kismet and Fire Island Pines. The most intensive vaccination efforts were focused on the five communities located between Kismet and Lonelyville (K–L). Starting in 1998, darting efforts expanded into NPS lands, so that vaccinations were carried out along a near-continuous, 13 km stretch from the western boundary of FIIS to Talisman. Population monitoring was initiated in 1995 in the K–L study areas for the purpose of estimating density and herd composition. Additional monitoring efforts included mid-island communities collectively referred to as Fire Island Pines (FIP), the east-end community of Davis Park (DP) and the Otis-Pike Wilderness area. Since 1999, most of the significant land areas of Fire Island, including Robert Moses State Park (RMSP), the Lighthouse Tract (LHT) (between RMSP and K–L) and another cluster of mid-island communities, referred to here as Ocean Bay Park (OBP), have been surveyed for deer population density and herd composition. This report focuses on data associated with the K–L communities.

Animals and identification methods

All of the deer treated were healthy adult or yearling females. Deer were not captured or handled during the study. For the efficacy studies, deer that received treatment were identified by volunteer monitors, who were year-round residents of the communities in which the deer were treated. Monitors maintained detailed records of individual deer, which they identified using body markings, nicks and scars unique to each animal. Nine monitors participated in the study, of which five participated throughout 1993–1997. These five monitors accounted for 86% of the individually recognized deer included in the efficacy study. In 1996, progressively increasing numbers of female deer were darted with darts that simultaneously vaccinated and placed a dye spot on the coats of treated deer. These darts allowed darters to distinguish between treated and untreated animals, but did not allow identification the following spring.

Vaccine preparation and delivery

The PZP vaccine was prepared from frozen–thawed pig ovaries as described by Liu *et al.* (1989) in 65 µg doses in 0.5 ml phosphate-buffered saline (PBS) and was stored frozen at –20°C until used in the field. Initial inoculations of vaccine consisted of 65 µg PZP in 0.5 ml PBS emulsified with 0.5 ml Freund's complete adjuvant (FCA). Booster inoculations consisted of 65 µg PZP in 0.5 ml PBS emulsified with 0.5 ml Freund's incomplete adjuvant (FIA), as described by Kirkpatrick *et al.* (1990).

Vaccine was delivered to deer in 1.0 cc darts with 19 mm or 25 mm barbless needles, or in 1 cc/2 cc injection-and-marking darts. Darts were fired from a blowgun, CO_2 pistol or a .22-cartridge powered dart rifle (all equipment from Pneu-Dart®, Inc., Williamsport, PA, except for the Dan-Inject®CO_2 pistol; Wildlife Pharmaceuticals, Fort Collins, CO). The dye used in 1996 was supplied by the American Coding and Marking Ink Company (Plainfield, NJ) and the dye used from 1997 onwards was red Sharpmark® Liquid Livestock Marker Concentrate (Nasco, Fort Atkinson, WI). Inoculations were given i.m. exclusively in the hip/gluteal regions of the deer, at ranges of 5–25 m. All darts were recovered and examined immediately after delivery to verify complete injection. On the few occasions when inspection indicated incomplete delivery, vaccination was repeated.

Treatment was administered between 15 September and 30 October in 1993–1995 and between 1 September and 30 September in 1996–2000, usually, but not exclusively, at pre-selected feeding sites baited with cracked corn or commercial deer feed. Five year-cohorts of deer (1993–1997) were treated in the efficacy study. We attempted to give all subjects two

initial injections in the autumn of their first year of treatment and a single annual booster in each of the following years. As animals sometimes could not be relocated and darted on schedule, 10.2% of females included in the efficacy study failed to receive the PZP–FIA booster in the initial year of treatment, and 11.5% missed a subsequent booster treatment.

Measuring vaccine efficacy

The effectiveness of the vaccine was assessed by examining the fawning rates in treated and untreated females. Separate control females were not used, but one year of pretreatment fawning data was available for females first treated in 1993–1995, and these pretreatment data were pooled to establish a baseline for comparison. Fawn counts for individually identified deer were carried out by monitors between 1 July and 31 October. Does were considered to have fawned if fawn(s) were observed suckling from the doe or if visibly lactating does (does with a distended udder) were often observed to be close to a particular fawn or fawns. Sustained suckling of fawns other than their own offspring is extremely rare among female white-tailed deer (Marchinton and Hirth, 1984; Thiele, 1999).

Efficacy data were analysed on SYSTAT 6.0 for Windows, using the statistical tests available in CROSSTABS and LOGLIN. All 2 × 2 chi-squared tables use the Yates continuity correction. The level of significance was $P \leqslant 0.05$.

Density estimation

Distance sampling methods (Burnham *et al.*, 1980; Buckland *et al.*, 1993) were used to estimate deer density in each of the communities in which deer were treated with immunocontraceptives. Conceptually, distance sampling is straightforward. From a sample of perpendicular distances from the objects of interest to a line transect, a mathematical function is generated which describes how detection of objects changes with increasing distance from the transect. From this function, the area from which objects are counted can be computed.

Density is computed as the number of deer encountered divided by the effective area sampled. Distance sampling theory allows for the fact that some of the deer within the study area, except for those directly on the survey route or transect, will not be detected, thus alleviating the need to correct for missed animals. A series of robust estimators have been formulated to calculate animal density efficiently from a modest sample of perpendicular distances. The software program DISTANCE version 2.0–3.5 (Laake *et al.*, 1993) was used to analyse the data.

The name and length of each transect segment was stored in a database from which samples were drawn randomly and without replacement for a given survey. The total number of segments selected was based on a minimum length of transect required to achieve a desired level of precision. Using the same sample selection criteria, surveys were replicated on 2–4 consecutive days depending on the number of groups sampled. Therefore, a given boardwalk segment could have been sampled on consecutive days, although never twice on the same day.

A nearest neighbour criterion (LaGory, 1986) and behavioural or social cues were used to aid in identification of a deer group. On detecting a deer group (⩾ 1 deer), the perpendicular distance from the observer was recorded using a handheld laser-rangefinder (Yardage Pro™ 400; Bushnell, Overland Park, KS) or a handheld prismatic rangefinder (Ranging™ Optimeter Models 60 and 120; American Visionware, San Antonio, TX). In situations in which the perpendicular distance could not be measured directly, a radial distance and angle were recorded using a pocket transit and the perpendicular distance was computed later in program DISTANCE. For a given area and time period, multiple surveys were most often treated as

replicate counts, or sometimes pooled into a single count, and a density estimate and associated 90%, log-based confidence intervals were computed (Buckland *et al.*, 1993).

Individuals within each deer group were classified according to sex (male, female or unknown) and age at the time of sampling (fawns: < 1 year old; yearlings: > 1 year and < 2 years old; adults: > 2 years old; or unknown). Other than from the presence of conspicuous antlers, sex and age were determined from physical and morphological criteria developed from thousands of observations of deer. The analysis of herd composition was restricted to the months between July and December in each year owing to the increased difficulty of distinguishing sex and age during the other months. Herd composition was summarized as the proportion of males, females and fawns in the total sample. Only observations in which all group members were classified according to sex and age were used in this analysis. Standard errors for the proportions were calculated using a binomial approximation, which treats the group as the sampling unit (Bowden *et al.*, 1984).

Results

Vaccine treatments and efficacy

A total of 353 individually known deer was treated with PZP vaccine between 1993 and 1998. The number of known individuals treated annually increased from 74 in 1993 to 164 in 1995, as additional monitors were recruited and the study was expanded (Fig. 1a). Studies initially focused on K–L and, to a lesser extent, FIP; treatments in OBP grew slowly in number and have been somewhat erratic due, in part, to a lower level of community co-operation (Fig. 1b). The number of individually known deer treated decreased in 1996, as increasing numbers of deer were treated with vaccinating/marking darts. Expansion of the geographic area covered plus an increase in the intensity of effort led to a peak in the total number of individuals treated in 1998. Overall (1993–1998), 1.0 h was spent per treatment and 1.4 h was spent per deer treated in a season, as, in a given season, the deer being treated for the first time required two vaccinations. Treating unidentified deer with marking darts (0.5 h per deer; 1996–1998) was more efficient than treating individually recognized deer (1.4 h per deer; 1993–1997). The direct costs of the project between 1993 and 1997, exclusive of bait and labour, averaged $34 per treatment. Bait costs could not be tracked because volunteers supplied most of the bait between 1993 and 1997. When the costs of bait and automatic bait stations were incorporated in 1999–2000, costs averaged $64 per treatment (again excluding labour).

Of the 353 known adult and yearling deer that received i.m. injections of PZP in FCA by dart in the hip, approximately 60% developed sterile swellings 2–8 cm in diameter at the injection site and the swellings remained visible for 1–4 weeks. Two deer developed abscesses at the injection site a few days after vaccination; both of the abscesses drained and healed in 5–14 days with no evident long-term effects. NPS personnel and resident deer monitors reported no other adverse health effects specific to treated females and noted no unusual mortality among fawns of treated females.

Among individually known deer, the proportion of treated adult does that had fawns decreased by 62.2% after a single year of PZP treatment, from 83.5% before treatment to 31.6% after treatment (chi-squared = 47.08, 1 df, $P < 0.0001$; Fig. 2a). Overall, the proportion of treated adult does producing fawns was 17.6%, with vaccine efficacy increasing significantly with successive years of treatment (Cochran's linear trend test for treatment years 1–4, chi-squared = 11.96, 1 df, $P = 0.001$). Of females treated for the first time as yearlings, 13 of 62 (21.0%) fawned in the year after treatment. However, the absence of data on fertility rates among untreated yearlings at FIIS prevents interpretation of this value.

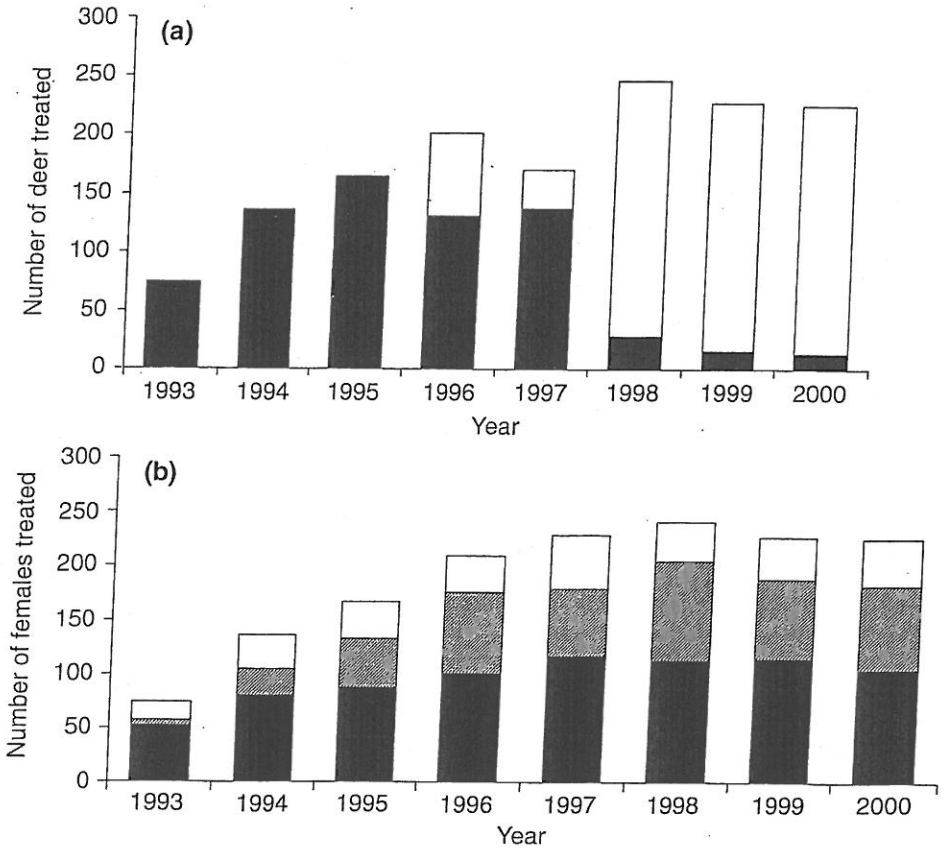

Fig. 1. Number of white-tailed deer (*Odocoileus virginianus*) treated annually with the porcine zona pellucida (PZP) immunocontraceptive vaccine on Fire Island National Seashore, New York, USA, from 1993 to 2000. (a) The number of individually recognized deer treated with 1 cc darts (■) is differentiated from the number of deer treated with vaccinating/marking darts that were not individually recognized (□). (b) The number of deer treated is sorted by community. Earliest efforts focused on the western communities of Kismet–Lonelyville (K–L; ■) and the eastern community of Fire Island Pines (FIP; ▨), with efforts in the mid-island communities designated Ocean Bay Park (OBP; □) beginning later and remaining inconsistent.

There is some indication that efficacy of the vaccine after a single year of treatment increased from the 1993 through to the 1996 treatment cohorts (Fig. 2b; Cochran's linear trend test by cohort, adults only, chi-squared = 4.52, 1 df, $P = 0.034$). However, the 1996 sample is small ($n = 5$) and eliminating the 1996 data also eliminates statistical significance (Cochran's chi-squared analysis = 2.12, 1 df, $P = 0.145$).

Population effects

In autumn 1999 and 2000, surveys of the intensively treated K–L area indicated that the proportion of fawns in the population was 0.14 ± 0.03 and 0.13 ± 0.03%, respectively, and 0.20 and 0.21 fawns per yearling and adult female, respectively (Fig. 3). The estimated proportion of fawns in the populations of other areas in which treatments were carried out varied between 0.11% and 0.24% in 1999, and between 0.10% and 0.20% in 2000, with the number of fawns per yearling and adult female varying between 0.18–0.37 and 0.19–0.27. In

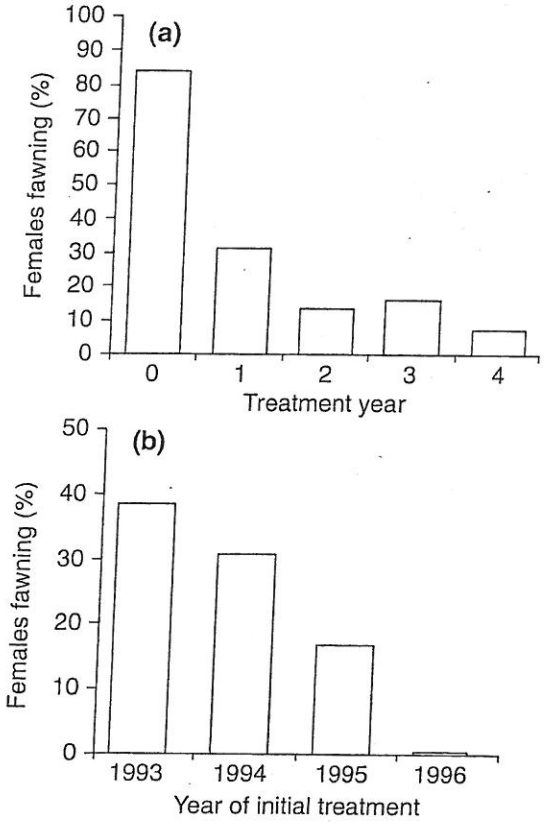

Fig. 2. (a) Percentage of adult female white-tailed deer (*Odocoileus virginianus*) reproducing on Fire Island National Seashore, New York, USA, 1993–1997 (n = 85, 95, 126, 92 and 53 deer in each year, respectively). Percentage reproducing is shown as a function of the number of successive years of treatment with the porcine zona pellucida (PZP) vaccine, with year 0 representing the pretreatment (control) year. (b) Percentage of deer reproducing in successive calendar years, 1993–1996 (n = 52, 26, 12 and 5 deer in each year, respectively) after a single year of PZP treatment (successive treatment cohorts).

two adjacent areas in which deer were not treated (RMSP and DP), the proportion of fawns in the population ranged from 0.16% to 0.33%, with the number of fawns per female varying between 0.26 and 0.67.

Estimated deer density in K–L initially increased 11.4% per year, from 77 per km^2 in March 1995 to 93 per km^2 in March 1998, and then decreased by 22.8% per year to 48 per km^2 in October 2000 (Fig. 4; see also Underwood and Verret, 1998).

Discussion

In the first phase of the present study it was demonstrated that annual PZP vaccinations can be delivered to large numbers of unconfined white-tailed deer without capturing animals and

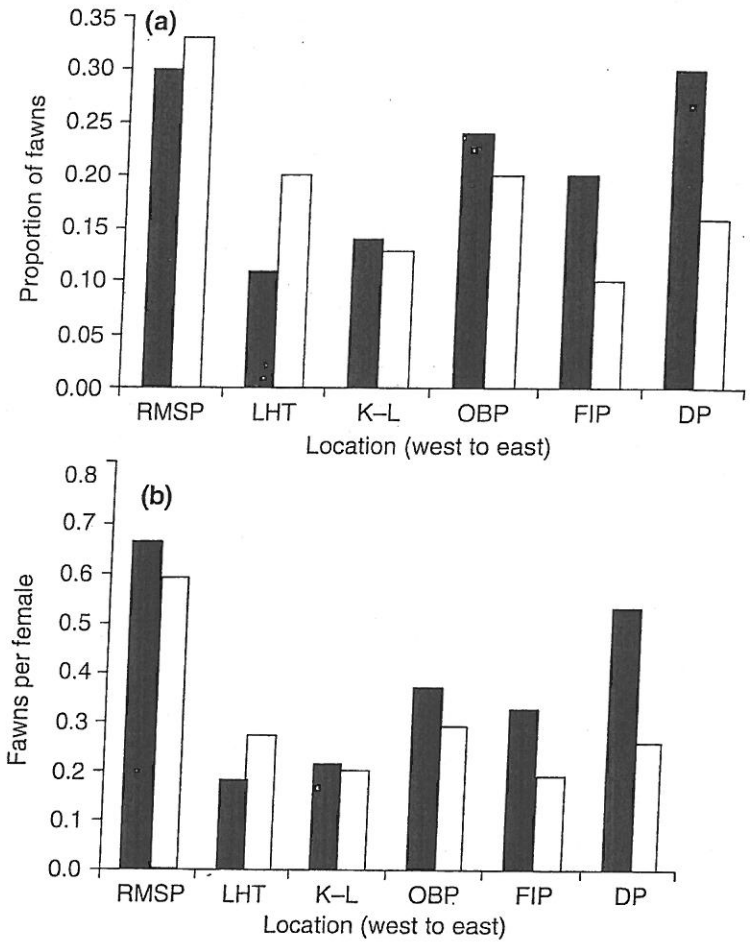

Fig. 3. Proportion of fawns (a) in groups of white-tailed deer (*Odocoileus virginianus*) and (b) fawns per adult-and-yearling females in sections of the study site with different histories of treatment with the porcine zona pellucida (PZP) immunocontraceptive vaccine, Fire Island National Seashore, New York, USA, 1999–2000. Proportions are derived from distance sampling. Locations are presented west to east along the island. Robert Moses State Park (RMSP) and Davis Park (DP) are untreated control areas. Most intensive treatments occurred in Kismet–Lonelyville (K–L), with Ocean Bay Park (OBP) and Fire Island Pines (FIP) receiving less intensive effort. Deer using K–L also used the Lighthouse Tract (LHT). Results from 1999 (■) and 2000 (□) are shown.

that such remotely delivered vaccinations significantly and markedly reduce the production of fawns among treated animals. However, vaccine efficacy in this study was conspicuously lower than in previous white-tailed deer studies, especially after the first year of treatment (fawning rates of 30% versus ≤ 10%; Turner *et al.*, 1992; McShea *et al.*, 1997). This reduction might be attributed to non-response to the vaccine (although the observed non-responders were too few to account for differences with previous studies) or to failures to identify individual deer correctly, thereby causing deviation from the standard vaccination protocol and misassignment of fawns (although likely predictors of identification accuracy, such as

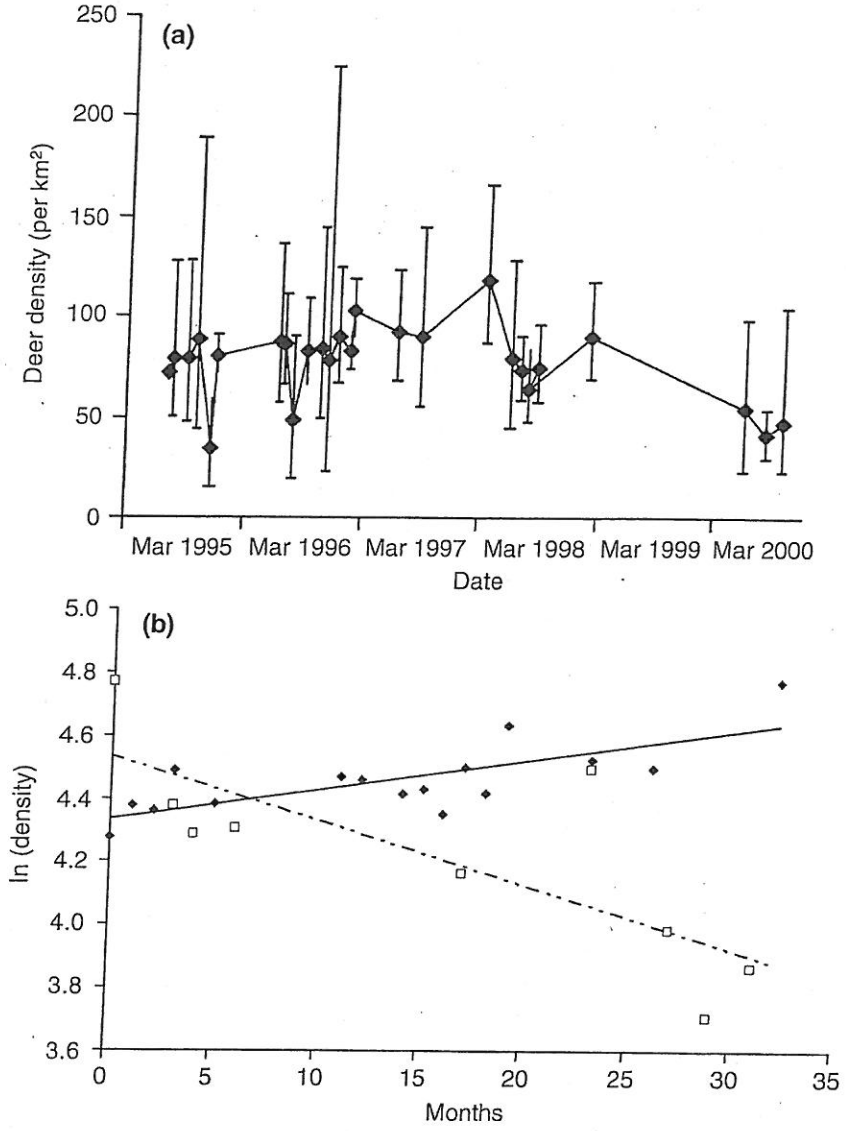

Fig. 4. Estimates of population densities of white-tailed deer (*Odocoileus virginianus*) in the Kismet–Lonelyville (K–L) section of Fire Island National Seashore, New York, USA, 1995–2000. Density estimates are obtained from distance sampling. Error bars represent 90% confidence intervals. Estimates are displayed (a) on a time line from 1995–2000, and (b) separated into trend lines from March 1995 to March 1998 (◆ symbols connected by a solid line) and from March 1998 to November 2000 (□ symbols connected by a broken line). For 1995–1998, $\ln(D) = 4.335 + 0.0093T$, and $\lambda = 1.114$ per year; for 1998–2000, $\ln(D) = 4.566 - 0.0216T$, and $\lambda = 0.772$ per year; where D = density in deer per km², T = time in months, and λ = annual rate of growth.

duration of involvement of deer monitors, were not associated with differences in observed fawning rates). It is probable that lower contraceptive efficacy was primarily a result of incomplete or poorly placed vaccinations, especially for the first treatment. Improvements in procedure (checking darts for discharge, using longer needles for full-sized adults) probably

contributed to improved first-year efficacy. Thiele (1999) reported that contraceptive efficacy in the first year after treatment was greater when the initial PZP shot was administered to deer by hand rather than by dart.

At Fire Island, the intensity of treatments was inversely related to the proportion of fawns in the population and after a period of gradual increase population density decreased in the most intensely treated area. However, at other prospective sites, population effects of immunocontraceptive treatment will vary, depending on the proportion of females that can be darted, the fertility of untreated animals, population mortality and movement of animals on and off the site. Although deer did leave the treatment areas for weeks or months at a time, delivery of immunocontraceptive treatments at Fire Island was relatively efficient. This was because many of the island's deer are highly habituated, and past feeding of deer by residents and visitors helped to attract deer to bait stations for darting and observation. Mortality of deer at Fire Island has not yet been estimated. Harsh physical conditions and limited forage options are offset by the absence of hunting, predators and high-speed vehicular traffic, and by recreational feeding. Seasonal changes in recreational feeding probably also influence deer movements and local density.

Although the time required to dart deer is estimated in the present study, the estimates of cost presented do not include these or other labour costs, which are typically the most significant cost of an immunocontraception programme (Rudolph *et al.*, 2000; Walter, 2000). Labour costs will vary widely from site to site. Models that include labour costs yield total estimates of cost ranging from $220 per deer treated per year (with a team of three darters and moderate darting efficiency) to $1055 per deer for 2 years of treatment if initial capture is required (Salmon, 1999; Walter, 2000).

Improvements in vaccine efficacy, longevity and delivery are critical for widespread management application. One-shot PZP preparations have been effective in grey seals (*Halichoerus grypus*) (Brown *et al.*, 1997) and feral horses (Turner *et al.*, 2001), so these improvements may be within reach. The use of vaccinating and marking darts markedly reduced the labour involved in identifying individuals and increased the efficiency of vaccination. However, the dyes tested were not completely satisfactory (R. Naugle, unpublished). In addition, regulatory concerns over treating untagged deer that may be subject to human consumption must be addressed before vaccinating/marking darts can be applied widely.

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