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Field testing of single-administration porcine zona pellucida contraceptive vaccines in white-tailed deer (*Odocoileus virginianus*)

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Abstract

Context. Many contraceptive agents have demonstrated effectiveness in wild species, most notably immunocontraceptives such as GnRH conjugates and porcine zona pellucida (PZP). The major challenge in using these agents to control deer and other wildlife populations in the field now lies with safe, effective and efficient delivery to a large-enough proportion of the population to suppress growth.

Aims. Because deer and other wildlife are typically difficult to access for treatment, contraceptives that require multiple or repeated treatments will be of limited management value. To address this constraint, we conducted a field study of white-tailed deer (*Odocoileus virginianus*) on Fripp Island, SC, USA, to test two different technologies for achieving single-administration, multi-year efficacy in PZP vaccines.

Methods. Between 2005 and 2010, we captured, ear-tagged and blood-sampled a total of 245 individual adult and yearling female deer. Deer were hand-injected at capture with one of two preparations of SpayVac or a combination native PZP–adjuvant emulsion plus PZP–adjuvant incorporated into lactide–glycolide polymer pellets engineered to release at 1, 3 and 12 months post-treatment. Pregnancy was determined from serum assays of pregnancy-specific protein B sampled from captured deer.

Key results. Aqueous SpayVac, and the PZP–adjuvant-containing polymer pellets manufactured through a heat extrusion (H/X) method administered simultaneously with PZP–AdjuVac or modified Freund's complete adjuvant emulsions reduced pregnancy rates from control levels by 95–100% in the first year after treatment, and by 65–70% in the second year after treatment.

Conclusions. A single, hand-injected vaccination with SpayVac or PZP–adjuvant emulsion combined with H/X PZP pellets reduced fertility for multiple years.

Implications. Single-treatment, multi-year immunocontraceptive vaccines bring contraceptive management of wildlife populations one step closer. Future efforts should focus on improving handling and storage, developing technologies for remote delivery, and addressing remaining regulatory and management concerns.

Additional keywords: immunocontraception, PZP, SpayVac.

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Introduction

White-tailed deer (*Odocoileus virginianus*) have achieved very high densities in parts of eastern North America, especially in pockets of urban forest and in suburbs surrounding major metropolitan areas. These high deer densities are often associated with elevated numbers of deer–vehicle collisions, depredation of ornamental plantings and farm crops, depletion of forest understorey and associated wildlife species, and high levels of tick-borne infectious diseases (although the relationship between deer densities and disease prevalence is complex) (Conover *et al*. 1995; Côté *et al*. 2004; Bissonette *et al*. 2008; Ostfeld 2010). Deer–human conflicts are particularly difficult

to resolve in urban and suburban communities, where traditional population-reduction techniques such as public hunting are often unsafe, ineffective, illegal, inconsistent with other land uses, or at odds with public sensibilities. Hence, there is strong public interest in exploring new methods to mitigate deer conflicts and reduce deer populations.

Approaches to non-lethal methods of management of deer populations have focussed on surgical sterilisation, steroid contraception and immunocontraception. Surgical sterilisation of urban white-tailed deer has been conducted at several sites (e.g. Gilman *et al*. 2010). It is effective at the individual level, and several modelling exercises suggest that it can be effective as a population-control technique in populations with little or no immigration (Merrill *et al*. 2003, 2006). However, surgical sterilisation is expensive; Boulanger *et al*. (2012) cited costs of US$1000 or more per deer in three limited field studies. Moreover, surgical sterilisation requires the availability of specialised equipment and the direct participation of experienced veterinarians and other highly skilled personnel (MacLean *et al*. 2006; Boulanger *et al*. 2012). These constraints are likely to limit practical application.

Testing of steroid contraceptives on white-tailed deer by using a variety of delivery techniques has been carried out since the late 1960s, with some agents and delivery techniques effectively reducing fertility (Harder and Peterle 1974; Matschke 1977*a*, 1977*b*, 1980; Roughton 1979; Plotka and Seal 1989; Fagerstone *et al*. 2010). However, problems with steroid delivery, side effects, and the potential for consumption by non-target animals, including secondary consumers, have blocked significant progress on field applications (Turner and Kirkpatrick 1991).

Thus, most research on deer contraception conducted over the past two decades has focussed on immunocontraceptive vaccines. GonaCon immunocontraceptive vaccine (a GnRH-conjugate vaccine developed by the National Wildlife Research Center (NWRC), US Department of Agriculture, Fort Collins, CO, USA) has been shown to be effective for one or more years in captive and field trials in female deer and other wildlife, and has been registered with the US Environmental Protection Agency for use on adult female white-tailed deer (Miller *et al*. 2000*a*; Curtis *et al*. 2002, 2008; Gionfriddo *et al*. 2009, 2011*a*; Fagerstone *et al*. 2010). No adverse health effects have been detected, except for localised injection-site reactions in some vaccinated animals (Gionfriddo *et al*. 2009, 2011*b*). Behavioural effects of GonaCon on white-tailed deer have not been reported. The EPA registration currently requires GonaCon to be hand-injected, although remote delivery of GnRH vaccines has been carried out successfully in the field (Miller *et al*. 2000*b*; Curtis *et al*. 2002; Fagerstone *et al*. 2010).

Other research on deer immunocontraception has focussed on the porcine zona pellucida (PZP) vaccine. Multi-shot protocols of native PZP, involving hand-injection or remote (dart) delivery of a priming dose of PZP emulsified in Freund's complete adjuvant (FCA), followed by boosters of PZP emulsified in Freund's incomplete adjuvant (FIA) have proved highly effective in blocking pregnancy for 1 year in initial trials with penned deer, with annual boosters extending the period of effectiveness (Turner *et al*. 1992, 1996; Miller *et al*. 2000*b*). Two-injection, remote-darting also proved effective in initial field trials at Fire Island National Seashore, New York, and other locations, although with slightly reduced effectiveness (Kirkpatrick *et al*. 1997; Naugle *et al*. 2002; Walter *et al*. 2002; Rutberg 2005). Consistent with its mechanism of action, PZP did not cause abortions when administered to pregnant females, and health and behavioural effects were minimal (Miller *et al*. 2001; Naugle *et al*. 2002; Rutberg 2005). The only concerns that arose involved the potential extension of the mating season in treated females, although these have not been associated with either adverse health effects or elevated frequencies of deer–vehicle collisions (McShea *et al*. 1997; Miller *et al*. 2001; Curtis *et al*. 2002; Walter *et al*. 2003; Rutberg and Naugle 2008*a*). PZP contraception has also been associated with reductions in fawn : doe ratios and declining deer populations at several field sites, indicating a potential to suppress population growth (Naugle *et al*. 2002; Rutberg *et al*. 2004; Rutberg and Naugle 2008*b*). Since 2012, a PZP–adjuvant emulsion has been registered with EPA under the trade name ZonaStat-H (Science and Conservation Center, Billings, MT, USA), for use on wild and feral horses and burros, but the registration has not yet been extended to deer.

Nevertheless, the requirement for two separate treatments in the initial year, followed by annual boosters, seriously limited the potential for field application. Additionally, regulatory and safety concerns were raised about the use of FCA in deer (Rutberg 2005). In field tests of PZP–FCA emulsions, injection-site reactions were mild, and no serious health effects were detected (Naugle *et al*. 2002; Rutberg 2005). However, FCA may cause false positives in tests for detection of tuberculosis in deer, which is of great concern to deer managers (Lyda *et al*. 2005). Thus, research began in the late 1990s to develop single-treatment multi-year PZP and GnRH vaccines, and to explore alternatives to FCA.

Alternatives to the FCA–FIA adjuvant sequence have been identified (Walter *et al*. 2002; Rutberg 2005). AdjuVac was developed by the NWRC (Fort Collins, CO, USA) for use with GonaCon. AdjuVac is a modification of MycoPar (Fort Dodge Animal Health, Fort Dodge, IA, USA), a USDA-approved Johnes disease vaccine (Fagerstone *et al*. 2010). A second promising alternative was modified Freund's complete adjuvant (MFCA, Calbiochem, LaJolla, CA, USA), which had previously been proven effective with PZP in zoo animals and wild horses (Deigert *et al*. 2003; Lyda *et al*. 2005). Adjuvac and MFCA are both mineral oil-based vaccines, but because they substitute cell-wall fragments from *Mycobacterium avium* and *M. butyricum*, respectively, for the *M. tuberculosis* cell-wall fragments found in FCA, they do not produce false positive TB tests (Deigert *et al*. 2003; Fagerstone *et al*. 2010).

Three approaches to one-shot, multiple-year contraceptive vaccines have been pursued, one based on GnRH and two on PZP. In one field test, Gionfriddo *et al*. (2009) showed that a single dose of GonaCon (a GnRH conjugate adjuvanted with AdjuVac) hand-injected in July or August significantly reduced pregnancy rates in white-tailed deer over two breeding seasons. SpayVac (ImmunoVaccine Technologies, Halifax, NS, Canada), a proprietary formula that incorporates native PZP into cholesterol–lecithin liposomes, has shown single-dose, multi-year effectiveness in grey seals (*Halichoerus*

grypus), fallow deer (*Dama dama*), Columbian black-tailed deer (*O. hemionus columbianus*) and white-tailed deer (Brown *et al*. 1997; Fraker *et al*. 2002; Locke *et al*. 2007). The other approach simulated booster doses by embedding PZP and adjuvant in timed-release lactide–glycolide polymer pellets, and delivering them simultaneously with a priming dose consisting of a PZP–FCA emulsion. This approach reduced foaling rates in free-roaming wild horses by 90% in Year 1, 75% in Year 2, and 38% in Year 3 (Turner *et al*. 2007, 2008).

The goals of the present study were to field-test efficacy and longevity in a group of PZP-based one-treatment immunocontraceptive vaccines. We examined SpayVac and emulsion–pellet vaccines, each prepared with several different manufacturing processes; and two different emulsions, AdjuVac and MFCA. Data on the effects of PZP treatments on population dynamics are being published elsewhere (A. T. Rutberg, R. E. Naugle and F. Verret, unpubl. data).

Materials and methods

Study site

Fripp Island is a 9.1-km^2 resort island located on the Atlantic coast of South Carolina, USA, ~31 km east of the city of Beaufort. The closest land areas include Pritchards Island 0.4 km to the west and Hunting Island 0.8 km to the east. Elevation on Fripp Island ranges from sea level to 3 m. Fripp Island is accessible to vehicles only by means of a two-lane highway bridge from Hunting Island.

Approximately 25% of Fripp Island is residentially developed, with two golf courses encompassing another 20–25% of the island. The remainder is salt marsh and maritime forest. Pockets of unmanaged vegetation across the site are dense and include oaks (*Quercus* spp.), pines (*Pinus* spp.), palms and palmettos (*Palmarum* and *Sabal* spp.), wax myrtle (*Myrica cerifera*) and an abundance of ornamental plants. The island is a state-designated wildlife sanctuary and hunting is not permitted. Because of high security and limited island access, illegal hunting of deer is virtually non-existent. Speed limits are low and strictly enforced, and residents and visitors commonly travel by golf cart, so deer are rarely struck and killed by vehicles. Feeding of deer and other wildlife is discouraged, but does occur at scattered sites on the island.

Consistent with other communities experiencing serious conflicts with deer, deer densities on Fripp Island were high, ~70–75 deer km^{-2} at the beginning of the study, although declining ~44% between 2005 and 2010 in association with contraceptive treatments (A. T. Rutberg, R. E. Naugle and F. Verret, unpubl. data). Typical of island populations in the south-eastern USA, deer are small-bodied, with females typically weighing 30–40 kg. Fawning occurs late April to mid-July, with most fawns born in May. Site fidelity of adult females appears to be high, with home ranges of radio-collared females typically ranging from 15 to 30 ha (P. C. Gillikin, unpubl. data).

Study design

Free-roaming female white-tailed deer were captured in February–March 2005–2010, tagged with uniquely numbered, highly visible ear-tags, blood-sampled for pregnancy testing, and hand-injected with one of the following PZP treatments (described in detail below):

(1) aqueous SpayVac, containing 200 µg of PZP prepared at 85°C or 75°C and emulsified with AdjuVac,
(2) non-aqueous SpayVac, containing 200 µg of PZP prepared at 85°C or 75°C and mixed with AdjuVac,
(3) cold-evaporated (C/V) timed-release lactide–glycolide pellets, containing 550 µg of PZP and the 500 µg of QA-21 (Vrbac Corp, Brussels, Belgium) adjuvant, plus an aqueous solution of 100 µg of PZP emulsified with AdjuVac or MFCA, and
(4) heat-extruded (H/X) timed-release lactide–glycolide pellets, containing 550 µg of PZP and 500 µg of QA-21 adjuvant, plus an aqueous solution of 100 µg of PZP emulsified with AdjuVac or MFCA.

In addition, we maintained ~20 animals as sham- or untreated controls through 2010, replacing with new captures any controls that died or disappeared.

Pregnancy was diagnosed by testing serum for pregnancy-specific protein B (P-SPB, Bio-Tracking, Moscow, ID, USA) in a sample of treated deer recaptured in February–March in each subsequent year. The design allowed us to compare pregnancy rates in deer treated with aqueous versus non-aqueous SpayVac, in deer treated with C/V pellets versus H/X pellets, in deer treated with the two types of SpayVac versus the two types of controlled-release pellets and in deer treated with the two adjuvants.

To test the effectiveness and longevity of boosters remotely delivered via dart gun in August immediately before the mating season, a sample of treated deer that had been diagnosed as pregnant received a remote injection of native PZP emulsified in either MFCA (if it had previously received AdjuVac) or FIA (if it had previously received MFCA). Except for the 2005 SpayVac treatments (which failed – see below), no boosters were given in the year immediately following treatment, so results for the first 2 years of pregnancy depended only on the initial treatment.

Capture and tagging

Female white-tailed deer on Fripp Island were captured via chemical immobilisation during February–March 2005–2010, using a combination of xylazine HCl (at ~2.2 mg kg^{-1}) and telazol HCl (at ~4.4 mg kg^{-1}). The drug combination was loaded into self-injecting 1-cm^3 Pneu-Dart transmitter darts with 1-in needles and double-wire barbs (Pneu-Dart, Williamsport, PA, USA) or Palmer Cap-Chur transmitter darts with 1-in needles and single-wire barbs (Palmer Cap-Chur Equipment, Powder Springs, GA, USA). Darts were delivered intramuscularly in the hip from a Dan-Inject Model CO_2 PI or Model JM Standard CO_2 rifle rifle (Dan-Inject ApS, Børkop, Denmark). Dart transmitters had a tracking range of ~1 km and were tracked with a Telonics TR-4 receiver and Yagi antenna (Telonics, Inc., Mesa, AZ, USA). Deer that failed to become fully sedated or were difficult to restrain were given supplemental injections of ketamine HCl (at ~5 mg kg^{-1}). When additional darting was necessary to accomplish immobilisation, we delivered the ketamine in either a 1- or

2-mL standard darts (Pneu-Dart) with a 1-in needle and single wire barb. When the effects of the telazol and/or ketamine began to wear off, yohimbine or tolazoline were given intravenously and/or intramuscularly at ~4 mg kg^{-1} to reverse the effects of xylazine.

Deer were either captured as we encountered them along community streets while driving a vehicle or at bait stations set up on empty lots, with minimal exposure and risks to residents or visitors. Each bait station consisted of a single 114-L barrel feeder suspended from a tree branch. Each feeder had an automatic timer and electronic dispensing unit and was programmed to dispense ~1.4–2.3 kg of whole corn one or two times daily, depending on local deer activity.

On capture, each deer's eyes were treated with an ophthalmic ointment and the head and eyes covered with a cloth hood. Heads were elevated and vital signs were checked every 10 min. When possible, heart rate and respiration were monitored continuously using a hand-held pulse-oxymeter, with the sensor attached to the tongue or ear. Deer showing any signs of respiratory distress (SpO_2 of <90%) were given ~0.3–0.5 mg kg^{-1} of Dopram V (Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO, USA) at 20 mg mL^{-1} intravenously. Deer in severe respiratory distress (SpO_2 of <80%) were given Dopram V and/or the antagonists yohimbine or tolazoline.

After stabilisation, removal of darts and treatment of dart wounds with a topical triple-antibiotic ointment, deer were tagged with a highly visible, uniquely numbered plastic livestock tag in the right ear, and a small, round, yellow livestock tag with a corresponding number, in the left ear.

Vaccine preparation and delivery

We tested two classes of PZP vaccine preparation, SpayVac, and controlled-release PZP–adjuvant pellets administered simultaneously with a priming dose of native PZP–adjuvant emulsion.

SpayVac was prepared in the laboratories of R. G. Brown of ImmunoVaccineTechnologies (IVT, Halifax, NS, Canada), following Brown *et al.* (1997). Briefly, 200 µg of PZP was encapsulated in liposomes made of soybean L-α-lecithin and cholesterol in a ratio of 9 : 1. For one preparation of SpayVac ('aqueous'), the liposomes were mixed with 0.5 mL of phosphate-buffered saline, emulsified with 0.5 mL of AdjuVac, pre-loaded into syringes, frozen for storage and transport, and defrosted just before use in the field. For a second preparation ('non-aqueous'), SpayVac liposomes were mixed in 0.5 mL of mineral oil and 0.5 mL of AdjuVac, pre-loaded into syringes, and refrigerated at 5°C until use in the field. As part of a test to reduce the potential for contamination, the PZP used in the SpayVac batch administered in 2005 was extracted from oocytes at 85°C before incorporation into liposomes; consistent with other SpayVac preparations, PZP used in the 2006 batch was extracted at 75°C, before incorporation into liposomes.

Porcine zona pellucida (PZP) for emulsions were prepared at the Science and Conservation Center, ZooMontana, Billings, MT, USA, following the methods of Dunbar *et al.* (1980). PZP for controlled-release pellet preparations was produced in the laboratory of Irwin K. M. Liu (University of California, Davis, CA, USA), following methods described in Liu *et al.* (1989). PZP emulsions were prepared in the field by mixing 100 µg of PZP dissolved in 0.5 mL of PBS with either Adjuvac, MFCA, Calbiochem, LaJolla, CA, USA), or FIA (following Kirkpatrick *et al.*1990).

Porcine zona pellucida (PZP) and the water-soluble saponin adjuvant QA-21 were incorporated into synthetic biodegradable polymers (poly(lactide-co-glycolide); Birmingham Polymers, Birmingham, AL, USA; now Lactel/Durect, Pelham, AL, USA). The polymers were engineered to release PZP and QA-21 at ~1, 3 and 12 months post-injection by altering lactide : glycolide ratios. Pellets contained ~100 µg of PZP + 200 µg of QA-21 adjuvant in the 1- and 3-month pellets, and 250 µg of PZP + 500 µg of QA-21 for 12-month pellets. The pellets were prepared using one of two different methods, namely H/X or C/V. Polymer ratios and manufacturing methods are described in detail in Turner *et al.* (2008).

Initial vaccinations were delivered intramuscularly in the hip at the time of capture with in-hand syringe or (in the case of PZP–QA-21 polymer pellets) trochar with a 14-gauge needle. Booster injections of native PZP–MFCA or PZP–FIA were delivered remotely via 1-mL dart (Pneu-Dart) with a 0.75′ or 1′ needle fired from a Dan-Inject Model CO_2 PI pistol (range ≤15 m), Dan-Inject Model JM Standard CO_2 rifle or a Pneu-Dart Model 193 0.22-cartridge powered capture gun (range 15–35 m).

Pregnancy testing

At the time of capture, we collected 10 mL of blood from nearly all deer for determination of pregnancy. Blood was cooled for a minimum of 30 min and centrifuged. After centrifugation, the serum was poured off, labelled and immediately frozen. Samples were sent to Biotracking (Moscow, ID, USA) for pregnancy determination using ELISA tests for the presence of P-SPB.

Results

Between 2005 and 2010, a total of 258 female deer (42 yearlings and 216 adults) was captured and ear-tagged. Of the 258 females captured, 245 were successfully bled at the time of the initial capture and tested for pregnancy using P-SPB. Pregnancy rates among newly captured deer averaged 78.4% (Table 1). Between 2005 and 2009, 211 female deer received initial injections of SpayVac or PZP emulsion–pellet combinations; an additional 32 captured females were either left untreated (6) or received sham injections of saline and AdjuVac (26) (Table 2). Of the 258 females originally tagged, there were 197 recaptures, including 22 recaptures of control deer. Mortality risk per capture was ~1.5% (7 of 455 total captures).

Pregnancy rates among recaptured control deer (68.2%) did not differ from pregnancy rates among deer at the initial capture (78.4%; two-tailed Fishers exact test, $P = 0.29$; Table 1). These were therefore pooled for comparison with pregnancy rates among recaptured treated females. The total pregnancy rate for treated females (31.4%, including all females that had received any PZP treatments) was significantly lower than that of the pooled untreated deer (77.5%; $\chi^2 = 93.07$, d.f. = 1, $P < 0.0001$), and were significantly ($P < 0.01$) lower within each year, except for 2009 (two-tailed Fishers exact test, $P = 0.13$).

Table 1. Pregnancy rates (number pregnant/total; % pregant given in parentheses) of captured female white-tailed deer, Fripp Island, South Carolina, USA, 2005–2010

'PZP-treated' includes all females that had received any porcine zona pellucida (PZP) treatment

Parameter	2005	2006	2007	2008	2009	2010	Total
First capture	78/92 (84.8)	61/76 (80.3)	18/28 (64.3)	17/24 (72)	6/11 (54.5)	12/14 (85.7)	192/245 (78.4)
Recaptures (controls)	–	4/6 (66.7)	3/6 (50.0)	5/5 (100)	0	3/5 (60.0)	15/22 (68.2)
Subtotal: pooled controls	78/92 (84.8)	65/82 (79.3)	21/34 (61.8)	22/29 (75.9)	6/11 (54.5)	15/19 (78.9)	207/267 (77.5)
Recaptures (PZP-treated)	–	18/33 (54.5)	12/46 (26.1)	12/48 (25.0)	8/31 (25.8)	5/17 (29.4)	55/175 (31.4)

Table 2. Number of initial porcine zona pellucida (PZP) treatments by vaccine formulation and year, Fripp Island, South Carolina, USA, 2005–2009

See text for definition and manufacturer details of vaccine preparations

PZP preparation	2005	2006	2007	2008	2009	Total
PZP–AdjuVac + H/X pellets	6	20	0	0	0	26
PZP–MFCA + H/X pellets	6	0	28	25	8	67
PZP–AdjuVac + C/V pellets	8	0	0	0	0	8
PZP–MFCA + C/V pellets	4	0	0	0	0	4
SpayVac/AdjuVac (aqueous)	22	31	0	0	0	53
SpayVac/AdjuVac (non-aqueous)	26	28	0	0	0	54
Untreated or sham controls	21	4	1	0	6	33
Total	93	83	29	25	14	244

Table 3. Pregnancy rates (number pregnant/total; % pregnant given in parentheses) among female deer treated with SpayVac, Fripp Island, South Carolina, USA, 2005–2008

All preparations adjuvanted with AdjuVac. Note that the 2005 SpayVac preparations were heat-treated to 85°C, whereas the 2006 SpayVac preparations were heat-treated to 75°C. See text for further details on vaccine preparations

SpayVac preparation	2006	2007	2008	Total
2005 aqueous	7/11 (64)	–	–	7/11 (64)
2005 non-aqueous	9/10 (90)	–	–	9/10 (90)
2006 aqueous	–	0/9 (0)	2/8 (25)	2/17 (11.8)
2006 non-aqueous	–	4/11 (36)	2/8 (25)	6/19 (31.6)
Pooled controls	65/82 (79.3)	21/34 (61.8)	22/29 (75.9)	

The aqueous and non-aqueous SpayVac preparations administered in 2006 were effective, whereas those administered in 2005 were not (associated with a modification of the preparation protocol – see Discussion) (Table 3). Pregnancy rates of deer treated with aqueous SpayVac in 2006 were significantly lower than those of controls in both 2007 (0 of 9) and 2008 (2 of 8) (two-tailed Fisher's exact test, $P = 0.0014$ (2007), $P = 0.035$ (2008)). Pregnancy rates among females treated with non-aqueous SpayVac in 2006 did not differ from those of the controls in 2007 (4 of 11) but were significantly lower than controls in 2008 (2 of 8) (two-tailed Fisher's exact test, $P = 0.18$ (2007), $P = 0.035$ (2008)). Pregnancy rates for 2006-treated deer did not differ between aqueous and non-aqueous treatments in either 2007 or 2008. Neither aqueous nor non-aqueous preparations of SpayVac delivered in 2005 differed significantly from the controls in 2006 (two-tailed Fisher's exact test, $P = 0.25$ (aqueous SpayVac), $P = 0.68$ (non-aqueous SpayVac)).

Records indicated that the freezer at Fripp Island failed in 2007, resulting in the prolonged thawing of native PZP doses used in primers in 2007. Pregnancy rates of deer treated in 2007 with PZP–MFCA emulsions plus H/X pellets (55%) were significantly higher than those in deer similarly treated in 2006, 2008 and 2009 (17.2%; two-tailed Fisher's exact test, $P = 0.012$) (Table 4). Because we believe that the PZP used in the 2007 priming emulsions was rendered ineffective by prolonged thawing, the 2007 treatment cohort was removed from the analysis.

Excluding the 2007 treatment cohort, a single treatment of PZP–adjuvant emulsion plus H/X pellets was highly effective for 2 years, with diagnosed pregnancy rates of 4.0% in Year 1 and 26.1% in Year 2 (Table 4). Although sample sizes were small, there was some evidence of effectiveness in the third year after treatment as well. The efficacy of H/X pellet vaccines primed with Adjuvac emulsions did not differ from that of H/X pellet vaccines primed with MFCA emulsions (two-tailed Fisher's exact test, $P = 0.69$). Among deer treated with C/V pellets, two of eight deer were pregnant in the first year after treatment (reported in Turner *et al*. 2008).

Across a variety of initial treatments administered in 2005–2007, including non-aqueous and aqueous SpayVac administered in 2005 and 2006, H/X pellets with either AdjuVac or MFCA emulsions, and C/V pellets with MFA, remotely delivered boosters of simple native PZP–FIA or PZP–MFCA emulsions provided an additional year of effectiveness. From 2007 to 2010, only 2 (11.8%) of 17 previously treated deer that received remotely delivered boosters were pregnant after 1 year; one of three remotely boosted deer was pregnant after 2 years. The two deer diagnosed as pregnant after 1 year had received initial doses of non-aqueous SpayVac in 2005; however, sample sizes were too small to establish whether this trend was significant.

Discussion

The present study has demonstrated that both SpayVac and a combination vaccine comprising timed-release polymer PZP pellets primed with a native PZP–adjuvant emulsion provide single-treatment, multi-year contraceptive efficacy in white-tailed deer. These data are the first to show that timed-release PZP polymer pellets can extend vaccine longevity when delivered simultaneously with a priming dose in white-tailed deer. They also provided further field confirmation of earlier data, showing the long-term efficacy of a single treatment of aqueous SpayVac-adjuvanted with AdjuVac, and the moderately reduced efficacy of the non-aqueous Spay-Vac preparation (Locke *et al*. 2007; Miller

Table 4. Pregnancy rates (number pregnant/total; % pregnant given in parentheses) of female deer treated with a porcine zona pellucida (PZP)–adjuvant emulsion plus heat-extruded pellets in 2005–2010
See text for definition and manufacturer details of vaccine preparations

PZP preparation	Initial capture	Year 1	Year 2	Year 3
PZP–MFCA + H/X pellets (2007)	19/27 (70.4)	4/11 (36.4)	7/9 (77.8)	–
PZP–MFCA + H/X pellets (2005, 2008, 2009)	29/37 (78.4)	1/18 (5.6)	4/11 (36.4)	0/2 (0)
PZP–AdjuVac + H/X pellets (2005–2006)	19/25 (76.0)	0/7 (0)	2/12 (16.7)	0/6 (0)
Subtotal, without 2007 cohort	48/62 (77.4)	1/25 (4.0)	6/23 (26.1)	0/8 (0)
Total	67/89 (75.3)	5/36 (13.9)	13/32 (40.6)	0/8 (0)

et al. 2009). They also demonstrated that both AdjuVac and MFCA are highly effective substitutes for FCA.

Although both oil-based adjuvants consistently worked well, other permutations showed mixed results. Previous work on the C/V pellets has shown some contraceptive effectiveness, but vaccine release rates and antibody-titer levels were very inconsistent (Turner *et al*. 2008; J. W. Turner, unpubl. data). Preparing PZP for SpayVac at 85° apparently denatured the protein, rendering it ineffective. The non-aqueous SpayVac preparation, which eliminates the need for freezing during storage and transport, also showed some contraceptive effectiveness, but apparently less than SpayVac prepared with the standard water–oil emulsion, which does require freezing. Because of the small sample sizes, further comparative testing of the emulsion and oil-based formulations is in order.

Evaluating vaccine efficacy in the field has both disadvantages and advantages. It is more difficult (if not impossible) to obtain in free-ranging animals the routine health, physiological, and immune-response data that can be obtained from captive animals (e.g. Miller *et al*. 2001). Also, under natural conditions, subjects die or disappear, and even when they remain within the study area their behaviour may prevent regular, unbiased recapture for sampling. Thus, the samples do not necessarily involve the same individuals each year, leading to fluctuating sample size and inconsistencies and biases in the data. Multiple field objectives also can compromise testing; because a secondary objective of the study was to induce and measure population reduction (A. T. Rutberg, R. E. Naugle and F. Verret, unpubl. data), subjects that became pregnant were selectively boosted after 2 years. Consequently, the high third-year efficacy rates for the H/X-pellet treatments are probably over-estimates; this sample is highly biased, comprising only animals that had not fawned in the previous 2 years.

In contrast, efficacy tests conducted under conditions of use in the field present more realistic challenges, and the data should better reflect the results that would be obtained in the actual management situations. Moreover, because of the costs and constraints of working on captive large animals such as deer, much larger samples are possible in field studies; sample sizes of four to eight animals per group have been typical in contraceptive studies of penned deer, for example, which significantly reduces precision of efficacy estimates and power to distinguish among treatments (e.g. Miller *et al*. 2000*a*, 2000*b*, 2009). Penned and free-ranging deer may also differ with respect to nutrition, antigen exposure, number and biological characteristics of potential mates, and number and seasonal distribution of mating opportunities.

The efficacy of the standard aqueous SpayVac preparation and that of the H/X-pellet preparations that we observed compared favourably to published estimates of efficacy for a one-treatment protocol using GonaCon (Gionfriddo *et al*. 2009, 2011*a*). Pooled results of two recent field trials in which a single hand-injection of GonaCon was administered to female white-tailed deer in July–August, yielded 22% fawning rates in Year 1 after treatment ($n = 50$), and 55% fawning rates in Year 2 after treatment ($n = 42$). These reductions were matched or exceeded in the present study by the standard aqueous SpayVac treatment (0% pregnancy rates in Year 1, and 25% pregnancy rates in Year 2) and by the primer emulsion–HX pellet combination (4% pregnancy rates in Year 1 and 26% pregnancy rates in Year 2). The capture and treatment of deer in the present study also occurred 5 months earlier in the annual breeding cycle than did those in the GonaCon study, suggesting that the PZP vaccines may at this time show greater seasonal flexibility in the timing of administration, and potentially longer duration of action. Note that in most regions the period during which deer can be captured and treated most efficiently is winter.

Management implications

With multiple options for single-treatment, multi-year immunocontraceptive vaccines available for white-tailed deer, and regulatory and safety concerns over adjuvants apparently resolved, future work will focus on developing mechanisms for remote delivery; prototype darts for delivery of the emulsion–controlled-release pellet vaccine are being tested in white-tailed deer at Fripp Island and in wild horses at Sand Wash Basin Herd Management Area in Colorado. Cost and availability of vaccines remain concerns; the range of landscapes and habitats in which contraception might be effective in controlling populations is yet to be determined; and significant regulatory and political barriers remain before the vaccines can be widely applied to manage urban and suburban deer populations. The registration of GonaCon for use on deer by the US EPA represents a major landmark in the management use of deer contraceptives; to be genuinely useful, other approaches will have to follow the trail broken by GonaCon.

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