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Factors contributing to the success of a single-shot, multiyear PZP immunocontraceptive vaccine for white-tailed deer

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Abstract: We evaluated 6 different porcine zona pellucida (PZP) preparations used as a single-shot vaccine for multiyear contraception of captive white-tailed deer (*Odocoileus virginianus*). The study compared 2 PZP preparation technologies from ImmunoVaccine Technologies™ (IVT) and National Wildlife Research Center (NWRC) over a 7-year period. The study compared both the use of oil in an emulsion and in suspension delivery, as well as replacement of the oil with an alum adjuvant. The study demonstrated that the oil emulsion adjuvant provided the longest lasting response. PZP isolated by the IVT provides a longer-lasting response than the preparation used by NWRC. The SpayVac™ and IVT-PZP vaccines presented in an emulsion form with AdjuVac™ produce a single-shot immunocontraceptive vaccine lasting up to 7 years.

Key words: human–wildlife conflicts, immunocontraception, *Odocoileus virginianus*, porcine zona pellucida, single-shot PZP vaccine, white-tailed deer

Immunocontraceptive vaccines prevent conception by stimulating production of antibodies that bind with and neutralize proteins or hormones essential for reproduction (Curtis et al. 2008, Miller et al. 2008). Porcine zona pellucida (PZP) vaccines prepared using zonae pellucida isolated from pig ovaries are one of the most studied immunocontraceptive vaccines in wildlife. The zona pellucida is a cellular glycoprotein layer surrounding the mammalian oocyte. During fertilization, sperm bind to receptors on the outer surface of the oocyte and penetrate the zona pellucida. Anti-PZP antibodies block sperm binding and penetration into the ovum and may also interfere with follicle maturation and ovulation (Dunbar 1989, Aitken et al 1996). Females treated with PZP vaccines typically experience multiple infertile estrous cycles throughout the breeding season (Killian and Miller 2000, Miller and Killian 2000, Miller et al. 2000). PZP vaccines have been used to contracept feral horses (*Equus caballus*; Kirpatrick et al. 1990, Kirpatrick et al. 1992), white-tailed deer (*Odocoileus virginianus*; Curtis et al. 2007, Killian et al. 2008, Rutberg and Naugle 2008), coyotes (*Canis latrans*; Miller et al. 2006), domestic sheep (*Ovis aries;* Stoops et al. 2006), and captive exotic species (Frank et al. 2005).

Based on the encouraging results of early investigations, we began studies with the PZP vaccine in white-tailed deer during 1992. We hoped to develop a single-shot contraceptive vaccine that was safe for the animals and had multiyear efficacy. White-tailed deer were then, as they are now, of interest as a target species because of inadequate control methods available to deal with dense urban and suburban deer populations in the northeastern United States (Hussain et al. 2007, Bissonettee et al. 2008, DeNicola and Williams 2008, Mastro and Conover 2008, Ng et al. 2008).

In an early study, we immunized white-tailed deer with a 1-ml injection containing 500-µg PZP in Freund's Complete Adjuvant™ (FCA) followed by multiple booster injections, each with 1-ml injection containing 300-µg PZP in Freund's Incomplete Adjuvant™ (FIA). In the first year, the prime dose was given in August and 2 boosts were given in September and October. In November, the does were exposed to bucks. In the second year, does were given 1 or 2 boosts, depending on their current PZP titer.

We continued the study for 4 more years without additional boosts. The does exhibited 100% contraception the first year, 89% over 3 years, and 76% over 6 years (Miller and Killian 2000). Given the multiple injections and the high dose of PZP required to induce infertility, we recognized that this approach was not practical for field applications. In a later field study, we showed that a prime and boost of 100 ug of PZP emulsified in FCA and FIA could provide multiyear contraception (Curtis et al. 2008).

Few studies have reported that a single-injection vaccine can provide immunization with effectiveness up to 2 years (Fraker et al. 2002, Liu et al. 2005, Locke et al. 2007, Turner et al. 2007, Rutberg and Naugle 2008). In mice, a single injection with a vaccine containing influenza viral peptides and adjuvant (Adjumer®) in mice caused antibody titers to increase gradually up to 6 months, but titers began to decline at 9 months (Payne et al. 1995). Their study suggested that for infertility to be achieved with a single injection, the vaccination would need to be given months before the onset of reproductive activity (i.e., the rut period) so that an adequate titer level could develop for contraception to occur.

Adjuvants are critical in vaccines for the development of high antibody titers in response to vaccination. Our work on a new adjuvant began in 1998 after learning that the U.S. Food and Drug Administration (FDA) would not approve the use of vaccines containing FCA because this adjuvant was associated with significant reactions at the site of injection. Consequently, we developed a new oil-based adjuvant called AdjuVac, containing <200 µg of killed *Mycobacterium avium* per dose. *M. avium* is a common nonpathogenic bacterium to which most wildlife and domestic animals worldwide have been exposed.

The experiments described in the current paper represent the last phase of the study conducted with the PZP vaccine in white-tailed deer. The objectives of the study was to compare the effectiveness of 6 different single shot vaccine formulations, containing different PZP preparations and different adjuvants or delivery systems.

Methods

Deer Research Center at Pennsylvania State University

White-tailed deer used in this study were born, raised, and maintained at the Pennsylvania State University (PSU) Deer Research Center. The original source of animals for the PSU deer herd was the free-roaming white-tailed deer population from central Pennsylvania. During our study, the PSU deer facility encompassed 54 ha of natural forest habitat and was divided into 9 outdoor paddocks ranging in size from 0.1 to 1.5 ha. Vegetation on the open areas consisted of a mixture of clover and orchard grasses, but most of the land was covered with dense eastern deciduous forest that had little understory vegetation. Deer were kept at a density of 25 to 37 animals per ha. During the nonbreeding season, treated female deer were isolated from males. To test vaccine contraceptive efficacy, 4 bucks of proven sire ability were confined with the does each year from the first week of November through the end of the following February.

PZP vaccine preparations

The heat-soluble PZP preparation used for the PZP was prepared by Dr. Irwin Liu (University of California at Davis) following the method described by Dunbar and Raynor (1980). PZP was provided to NWRC in phosphate buffered saline (PBS) buffer at a protein concentration of 1 mg/ml. It was used in either a 200-µg or 500-µg protein dose prepared as an emulsion with AdjuVac for administration in a 1-ml volume. This preparation will henceforth be referred to as NWRC-PZP.

ImmunoVaccine Technologies™ (IVT) PZP vaccine preparations

ImmunoVaccine Technologies™ (IVT) of Halifax, Nova Scotia, Canada, developed the IVT-PZP preparation using the Yurewicz et al. (1983) procedure to isolate zona. This method is similar to that described by Dunbar and Raynor (1980). The IVT procedure consisted of homogenizing the ovaries in Tris buffer by passage through a commercial meat grinder. The resulting oocytes were cleared from cellular debris by successive passages of the ground tissue through a series of nylon screens of decreasing pore size. The oocytes were then passed

through a homogenizer to release the zona pellucida. We emulsified this PZP with AdjuVac to create the IVT-PZP vaccine. We used SpayVac (an IVT-PZP preparation encapsulated in liposomes; Brown et al. 1997) in 3 other vaccine preparations. These were SpayVac emulsified with AdjuVac, lypohilized SpayVac suspended in AdjuVac, and SpayVac mixed with alum as the adjuvant.

Experimental design

We divided 30 deer into 6 treatment groups of 5 does each (Table 1). The first 4 treatment groups were vaccinated with variations of the IVT-PZP preparation. Group 1 was vaccinated with SpayVac (IVT-PZP encapsulated in liposomes) suspended in AdjuVac. Group 2 was vaccinated with IVT-PZP (without the liposomes) suspended in AdjuVac. Group 3 was vaccinated with lyophilized SpayVac suspended in AdjuVac. Group 4 received SpayVac combined with alum. Lyophilized (freeze-dried) vaccine used in Group 3 evaluated whether a suspension would produce a long-lasting contraceptive effect like that seen with the emulsion in the standard vaccine preparations. The SpayVac in alum used in Group 4 was intended to evaluate the utility of alum suspension as an adjuvant for producing long-term contraception.

All IVT-PZP-treated deer were vaccinated with 200 μg of PZP that contained approximately 35% protein. The IVT-PZP preparation contained some precipitate that became part of the vaccine preparation. Groups 5 and 6 were vaccinated with 200-μg and 500-μg doses, respectively, of NWRC-PZP as measured by Lowry assay. Vaccines were prepared by producing a 1:1 emulsion volume:volume with AdjuVac.

We administered all PZP vaccine formulations in mid-July 2000. We took 10 ml of blood from does in all groups except for Group 2, which was vaccinated during July 2001. Fawning data were available for untreated does exposed to bucks in the Penn State herd during each year of study. While these does were not sham-treated, they were handled in the summer and fall, as were the treated does, when they received health vaccinations. We considered the fawning rates of the untreated does to represent normal fertility rates for does in the Penn State herd.

Laboratory methods

We collected 10 ml of blood by jugular venipuncture from the study deer in July, September, November, and February. We used the sample to determine antibody titers, progesterone levels, and pregnancy-specific protein B in samples collected in February. Anti-PZP titers for groups using IVT-PZP and the NWRC-PZP 200 μg/dose as the antigen were determined by ELISA at the IVT laboratories. This method reports the titer as a percentage of a standard rabbit anti-PZP titer, which is considered 100% (Brown et al. 1997).

Anti-PZP titers for the NWRC-PZP 500 μg/dose vaccine were measured, using ELISA at the NWRC laboratories. Titers were calculated as the last serial dilution in which the treated sample was at least twice the absorbance of the pretreated sample of the same animal. In the NWRC laboratory method to determine anti-

Table 1. Experimental design defining the nature of the vaccine treatments and adjuvants. Groups 1–4 represent PZP produced by IVT mixed with different adjuvant preparations. Groups 4 and 5 represent 200 and 500 μg of PZP from NWRC, both made into an emulsion.

Group	Vaccine Treatment	Liposomes	Adjuvant	Adjuvant-Vaccine Mixture
1	IVT-PZP(200 μg)—SpayVac	Yes	AdjuVac	Emulsion
2	IVT-PZP(200 μg)	No	AdjuVac	Emulsion
3	IVT-PZP(200 μg)—SpayVac-lyophilized	Yes	AdjuVac	Suspension
4	IVT-PZP(200 μg)—SpayVac	Yes	Alum	Suspension
5	NWRC-PZP(200 μg)	No	AdjuVac	Emulsion
6	NWRC-PZP(500 μg)	No	AdjuVac	Emulsion

body titers, 100 ng of PZP antigen was placed in each well of a micro-titer plate. Sea Block™ (Pierce Chemical, Rockford, Ill.) was used to prevent binding of antibodies to the plastic in the ELISA plate. Deer serum was serially diluted from 1:1,000 to 1:128,000 in PBS containing Sea Block. Antibodies in the deer serum to the native PZP antigen on the plate were directed with the following linkages: deer anti-PZP binds to PZP on the plate, rabbit anti-deer IgG binds to the deer IgG, goat anti-rabbit-peroxidase binds to the rabbit IgG. Tetramethylbenzidine was used to develop the color, and 2M H_2SO_4 was used to stop the reaction. The color intensity of the sample was read at 450 nm with a Dynatech MR 5000 ELISA plate reader.

Plasma progesterone levels were assayed by the coat-a-tube RIA method (Diagnostic Products, Los Angeles, Calif.), following the manufacturer's recommended procedure. Assays for Pregnancy-Specific Protein B (PSPB) were performed by ELISA on samples submitted to BioTracking LLC (Moscow, Ida.).

Behavioral observations

We monitored estrous cycles in deer to determine the frequency of estrus and to learn about the mechanism of multiyear contraception. To determine when the does were in estrus, trained observers monitored the behavior of bucks toward the treated does. Three observation periods of >30 minutes each were scheduled daily from November 7 through February 12, followed by 2 periods daily until February 28. Behavioral activity by the buck was recorded on an observation sheet for a range of activities, including sniffing genitalia, pursuit of the female, aggressive guarding, and copulation (Killian and Miller 2000).

Ultrasonography and fawning data

We performed trans-rectal ultrasonography in late January or early February of each year to determine which does were in the first trimester of gestation. Blood drawn on the same day was tested for progesterone concentration and PSPB. From May through September, does were observed daily for evidence of fawning. We recorded fawning dates and the number of fawns born and compared them to observations on behavioral estrus to estimate the date of conception.

Definitions of contraception and fertility

Does that were exposed to bucks and showed signs of estrus, but did not fawn were considered contracepted or infertile. Does in each group were monitored for contraceptive longevity and remained on the study until they had twins. Does >2 years old and having 1 fawn were assumed to be sub-fertile because this is less than the average fawning rate of 2 fawns in untreated does in the Penn State herd.

Results

Contraception

The average fawning rate of the Penn State herd for 84 untreated does from 2001 through 2007 was 1.8 fawns per doe, with an average doe age of 4 years (Table 2). Number of fawns born varied with does age. For 2-year-olds, the

Table 2. Fawning data for untreated does in the Penn State University deer herd during the same years that the contraceptive trial was conducted.

Year	% infertile	*n*	Average doe age	% does with 1 fawn	% does with 2 or more fawns	Average fawns/doe
1	0	9	4.0	67	33	1.4
2	0	26	3.1	23	77	1.8
3	0	8	4.7	13	87	2.0
4	0	10	4.2	20	80	2.0
5	0	13	4.2	0	100	2.0
6	0	8	4.6	13	87	1.9
7	0	10	4.5	20	80	1.8

average number of fawns born was 1.7. For does >2 years old, the average number of fawns born was 2.0.

Groups 1 and 2, which combined SpayVac or IVT-PZP into an emulsion with AdjuVac, had the longest contraceptive effect, with 80% of the deer contracepted for 5 to 7 years (Table 3). Unfortunately, Group 1 was terminated after 5 years because of lack of funding, with 80% of the treated deer still contracepted. One doe in Group 2 had twins in year three and was dropped from the study. In year five, 1 of the 4 remaining does in this group had a single fawn, reducing the rate of contraception to 60%. However, because the birth of a single fawn did not represent a complete breakthrough from immunological contraception, all 4 does were kept for another year. In years six and seven, the doe that had a fawn in year five did not fawn, restoring the contraception rate to 80% (Table 2).

Does in Group 3 were all contracepted in the first year, one of the 4 does conceived the second year, two of the 4 does had fawns during the third year, and all fawned during the fourth year (Table 3). Consequently, this group was dropped from the study at the end of year four.

Four of the 5 does in Group 4 each had 1 fawn in the first year (Table 3). Consequently, this group was removed from the study after the first year. Groups 5 and 6 received 200 μg and 500 μg of NWRC-PZP, respectively, in an emulsion made with AdjuVac. For Group 5, 1 doe had 2 fawns the first year, for an 80% contraception rate (Table 3). However, because all 5 does produced 2 fawns in the second year, Group 5 (200 μg of NWRC-PZP) was subsequently removed from the study. Does receiving 500 μg of PZP were 100% contracepted the first year, but 4 of 5 does produced fawns the second year (Table 3). This 20% contraception rate remained until year five, when the remaining doe produced 2 fawns.

The number of fawns that could be produced by the different groups, had they not be contracepted, was estimated from the average fawning rates of untreated does in the herd during the same years. This estimate of fecundity for the 5 years when Group 1 was on the study was 46 fawns compared to 64 fawns for 7 years of study with Group 2 (Table 3). Considering that actual numbers of fawns produced for these 2 groups during the periods of study were 2 and 4 respectively, fecundity was reduced greater than 90% by the vaccines containing IVT-PZP.

Antibody titers

Comparing the anti-PZP titers of all of the groups, Group 1 (SpayVac; Figure 1a) had the greatest initial immune response of all groups, with a peak antibody titer at 18 months and a titer of 80 to 100% of the rabbit standard during the first 2 years. Although the titer began to drop after 18 months, it was sustained at 40% to the end of the 5-year study. The rise in antibody titer for Group 2 IVT-PZP (Figure 1b) was slower than that observed for Group 1, but by year three, both groups had similar titers. The antibody response patterns for Group 2, and to a lesser extent for Groups 1 and 3, included a self-boosting of titers, which was evident as titers increased without additional antigen injections (Figures 1a and 1b). Notably, for Groups 1 and 2, the high rates of contraception were maintained even with lower titers in years four through seven.

Deer in Group 3 that were given freeze-dried SpayVac suspended in AdjuVac, were 100% contracepted in the first year, but contraception rates dropped off to 75%, 50%, and 0% in years two, three, and four, respectively (Table 3). Titers for Group 3 were high through year two, but dropped in years three and four (Figure 1c), indicating that freeze-dried SpayVac in suspension was less effective for long-term contraception than the non-freeze-dried emulsion form of SpayVac. SpayVac with alum adjuvant produced a large variation in the immune responses, and only 1 doe produced a good immune response (Figure 2a).

Titers for Group 5, (200-μg NWRC-PZP mixed with AdjuVac) were relatively high in the first year (Figure 2a), with an 80% contraception rate. However, average titers dropped dramatically in year two, and all of the does conceived. Antibody titers determined for Group 6, treated with (500-μg NWRC-PZP mixed with AdjuVac™) were very high immediately after injection, but declined over the next 2 years (Figure 2b). In contrast to the IVT-PZP used in Groups 1 and 2 (Figures 1a and 1b), the NWRC-PZP-treated does in Groups 5 and 6 did not appear

Table 3. Number of contracepted female deer that gave birth and number of fawns produced by them. All does were given a single vaccine dose of 200 μg on July 10, 2000, except Group 3 does, which were immunized in July 2001.

Fawning Year	IVT PZP preparations								NWRC preparations			
	Group 1 SpayVac-AdjuVac $n = 5$		Group 2 IVT-PZP-AdjuVac $n = 5$		Group 3 Lyophilized SpayVac-AdjuVac $n = 4$		Group 4 SpayVac-Alum $n = 5$		Group 5 NWRC-PZP (200 μg)-AdjuVac $n = 5$		Group 6 NWRC-PZP (500 μg)-AdjuVac $n = 5$	
	Females	Fawns	Females	Fawns	Females	Fawns	Females	Fawns	Females	Fawns	Females	Fawns
Year 1	0	0	0	0	0	0	4	4	1	2	0	0
Year 2	0	0	1	0	0	0	–	–	5	10	4	6
Year 3	0	0	1	1	2	2	–	–	–	–	4	2
Year 4	1	1	1	2	–	–	–	–	–	–	4	2
Year 5	1	1	1	1	3	6	–	–	–	–	5	2
Year 6	–	–	1	1	–	–	–	–	–	–	–	–
Year 7	–	–	1	1	–	–	–	–	–	–	–	–
Total fawns		2		6		8		4		12		12
% Reduction in fecundity[1]		96		94		75		43		25		74

[1]Fecundity = expected rate of fawn production for the number of does in the treatment group for the duration of the study, using the average fawning rates of untreated does (Table 2) for the same years. Percentage reduction in fecundity was determined by dividing the difference between the expected fawning rate and the actual fawning rate by the expected fawning rate.

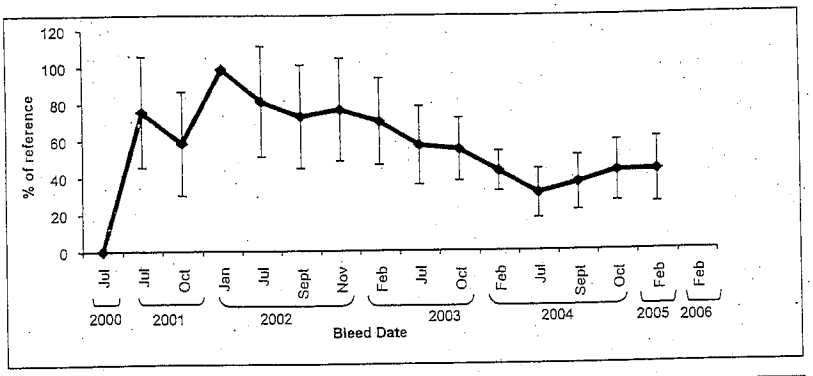

Figure 1a. Group 1: Ab titer (PZP Lipo06sp). SpayVac as an emulsion with AdjuVac.

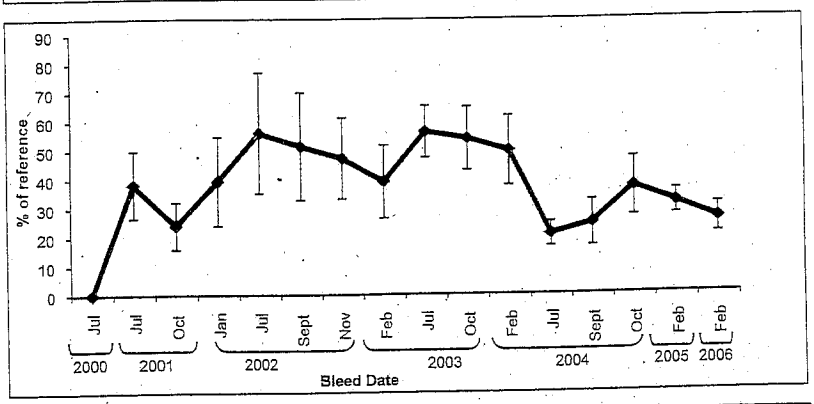

Figure 1b. Group 2: Ab titer (PXPb200spwpd). IVT-PZP as an emulsion with AdjuVac.

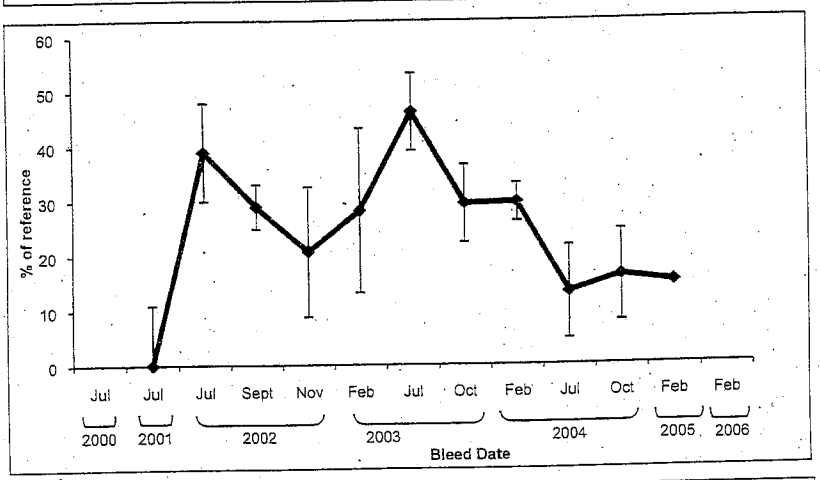

Figure 1c. Group 3: SpayVac, freeze-dried, as a suspension in AdjuVac.

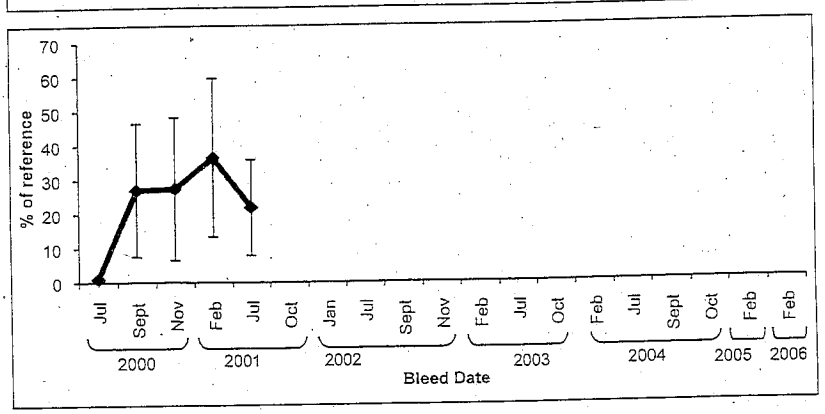

Figure 1d. Group 4: SpayVac absorbed onto alum.

Figure 1, a–d. Each group represents a different PZP with different adjuvant preparations and delivery methods. Antibody titers are presented as a percentage of a 100% anti-PZP rabbit standard. July 2000 to February 2006 represents the month-years post treatment throughout the study. The graph ends at the point that all deer in the group became pregnant and the group was dropped from the study. Each data point represents the mean ± SE.

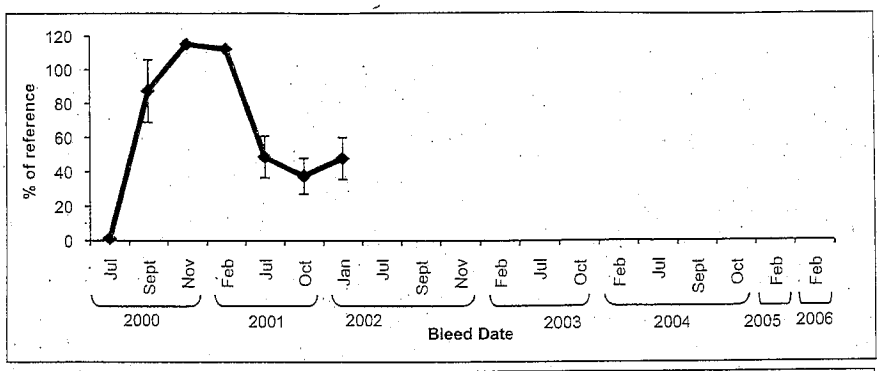

Figure 2a. Group 5: 200 µg of NWRC-PZP presented as an emulsion with AdjuVac, antibody titer presented as a percentage of a rabbit standard as 100%.

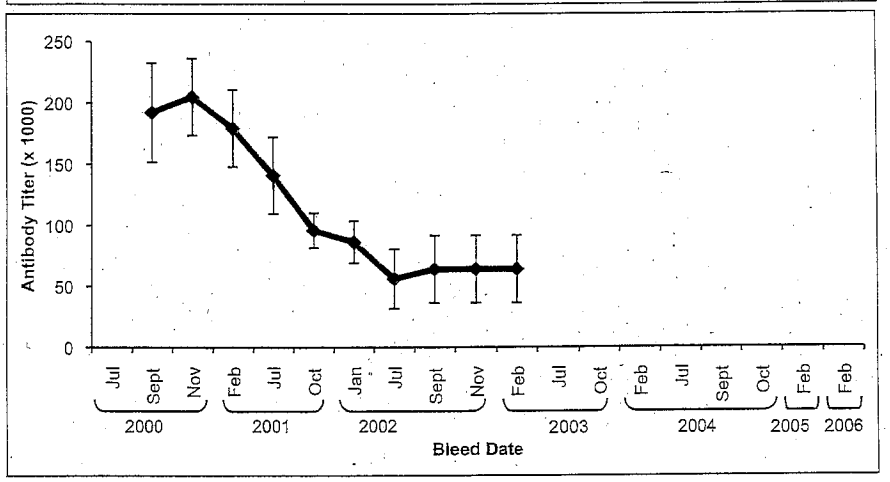

Figure 2b. Group 6: 500 µg of NWRC-PZP presented as an emulsion with AdjuVac antibody titer presented as a 1/1000 –1/256,000 serial dilution end point.

Figure 2 a–b. Groups 5 and 6 represent 2 concentrations of the NWRC-PZP vaccine serial dilution end point. July 2000 is the date of the vaccination, with July 2000 to February 2006 as the months and years of post-treatment throughout the study. The graph ends at the point that all deer in the group became pregnant, and the group was dropped from the study. Each data point represents the mean (±SE).

to stimulate self-boosting, and the decreasing of antibody titers (Figures 2a and 2b) resulted in greater pregnancy rates in subsequent years (Table 3).

Behavioral observations

PZP-treated deer had multiple estrous cycles every year of the 5-year study, with the multicycling ceasing only when they became pregnant. The number of estrus events for all PZP-treated deer ranged from 1.5 to 3.0 per season, with an average breeding season length of 150 days. This compares to 0.2 to 0.5 estrus events for does in the untreated herd and an average breeding period of 42 days per season. The 4 does in IVT-PZP Group 2 averaged 1.75 estrus events in year seven of the trial, demonstrating that cycling was still occurring, although none of the does had fawns.

Progesterone and pregnancy-specific protein B determinations

Blood samples taken from deer anually in July, September, November, and February were assayed for serum progesterone concentrations. Despite the fact that the PZP-treated deer showed signs of estrus and breeding behaviour during November and December, the only consistent elevation in serum progesterone observed was in the February blood samples of SpayVac Group 1, IVT-PZP Group 2, and NWRC-PZP 500-µg Group 6 (Figures 3a, 3b and 3c). This consistent increase in serum progesterone in February in the PZP-treated does was not associated with pregnancy.

Blood serum was taken from 6 adult, proven-breeder does that were not bred during the 2006–2007 breeding season. Because these unbred, untreated does showed a similar elevation of serum progesterone in February (5.6 ± 0.4 SE), we concluded that this elevation of progesterone may be normal for cycling, nonpregnant does as they return to an anestrous state in the nonbreeding season.

In contrast to serum progesterone concentrations, PSPB levels with a mean of 0.3 (non-

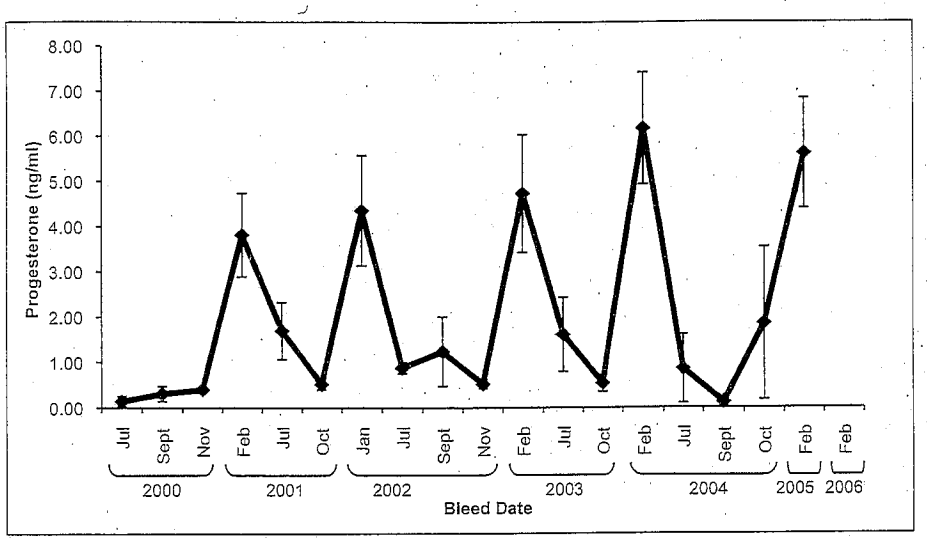

Figure 3a. Group1: Progesterone data of SpayVac presented as µg/ml.

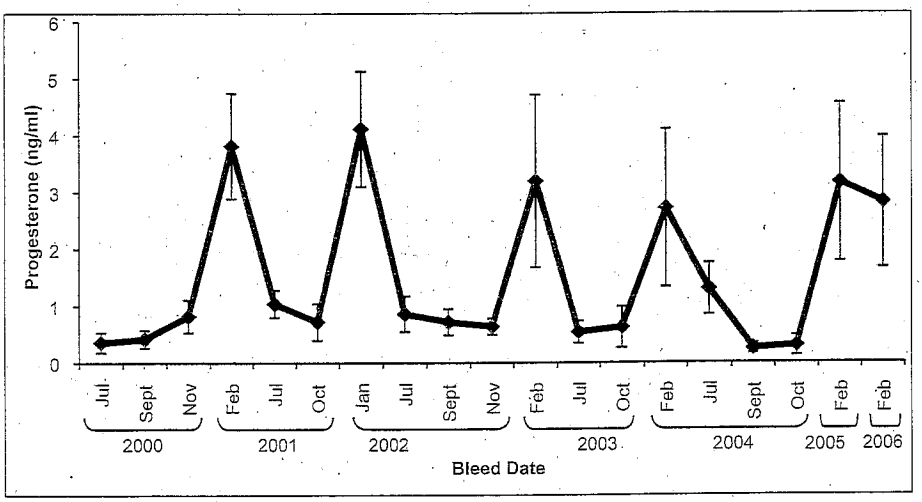

Figure 3b. Group 2: Progesterone data IVT-PZP presented as ng/ml.

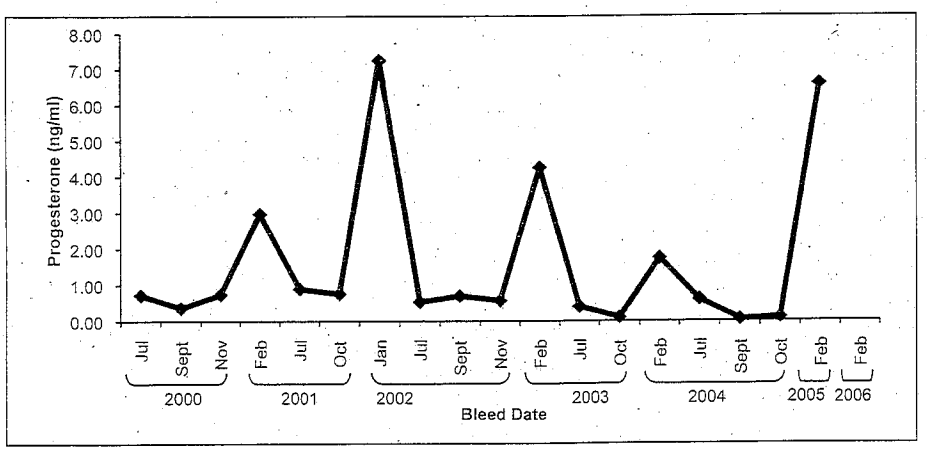

Figure 3c. Group 6: Progesterone data of NWRC 500 µg presented as ng/ml.

Figure 3 a–c. Progesterone peaks present in the January–February bleed in the SpayVac group (3a), the IVT group (3b), and the 500-µg NWRC group (3c). July 2000 to February 2006 represents the month and years of post-treatment throughout the study. Each data point represents the mean (±SE).

pregnant values >1.0) were well-correlated with ultrasound and fawning results. The PSPB levels were correlated with fawning ($r = 0.83$, $P < 0.0001$).

Injection-site reactions

There were no injection-site reactions in any of the treatment groups observed throughout the study.

Discussion

This is the first study of a single-injection PZP vaccine for white-tailed deer that results in contraception rates of 80% for 5 to 7 years. Based on the performances of the different vaccine preparations, it is clear that the long-lasting contraceptive effect is related to the vaccine design and the adjuvant used. The longest contraceptive effect was achieved in Groups 1 and 2 that included PZP produced by IVT and was made into an emulsion with the NWRC adjuvant AdjuVac. Both of these groups showed periodic boosting of the antibody titer, which may be central to the long-lasting contraceptive effect.

Because the PZP-contracepted does continued to cycle throughout the 7-year study, we conclude that successful PZP immunocontraception depends on long-lasting, high titers of high-affinity antibodies and is unlikely the result of PZP-immune complexes resulting in ovarian damage.

The immune system is unable to maintain the high antibody responses for long periods in the absence of a stimulating antigen. Therefore, for a successful immunocontraceptive response, the antigen must be retained in the immune system for months or years (Burton et al. 1994). The follicular dendritic cells in the lymph node that drains the site of injection may provide an answer for the long-term immune response found with some antigens. To induce immunocontraception that lasts months to years, there must be a continued stimulation of these B-cells by the injected antigen (Burton et al. 1994).

Antigen preparation

Differences between the methods used to harvest oocytes for the IVT and NWRC antigen preparations may have affected the length of contraceptive response. For the NWRC preparation, the oocytes in the ovaries were cleanly released by 2 beds of 500 razor blades, which enabled the rapid isolation of oocytes with minimal disruption of other ovarian tissue. In contrast, for the IVT preparation, the ovaries were processed in a meat grinder as the initial step for isolating the oocytes. Although most of the remaining steps were similar, the initial material in the IVT preparation probably contained more ovarian tissue. In contrast to the long-term efficacy achieved with the IVT-PZP groups, the NWRC-PZP preparations performed well only for 1 to 2 years.

Adjuvants

The oil-based adjuvant AdjuVac was an important component of the single-injection vaccine, contributing to both the antibody titer produced and the long-term contraceptive response. The benefit of AdjuVac was clearly evident when we compared it to the poor response when alum was used as the adjuvant.

The liposomes in SpayVac increased the long-term contraceptive effect in other studies and species. In this study, the IVT-PZP performed almost as well as the SpayVac, although it is possible that the addition of AdjuVac diminished the need for the liposomes in SpayVac. Presumably, the liposomes facilitated a significant immune response when the vaccine was combined with a less-effective adjuvant.

Multi-year contraceptive effectiveness has occurred in gray seals (*Halichoerus grypus*; Brown et al. 1997), fallow deer (*Dama dama*; Fraker et al. 2002), and white-tailed deer (*Odocoileus virginianus*; Locke et al. 2007) as a result of a single administration of SpayVac using Freund's Complete Adjuvant. For horses, Liu et al. (2005) demonstrated a multiyear contraceptive effect when PZP was injected with Freund's adjuvant, and Turner et al. (2007) also was able to achieve a multiyear contraceptive effect with PZP in a slow-release microbead. However, in the Liu and Turner studies, the contraceptive effect was limited to 2 years, as we observed with the NWRC-PZP prepared by Liu, which is considerably less than the 5–7 years achieved with the IVT-PZP in this study.

Emulsion

This study showed that an adjuvant prepared with the antigen in the form of an emulsion provides longer lasting contraception than that observed with a suspension of freeze-dried PZP. The antigen administered in a single dose must be retained in the body long enough to produce specific antibodies that will bind with the antigen to form immune complexes (Bachmann et al. 1996). It appears that a stiff emulsion (similar to mayonnaise) is important for the retention of the intramuscular dose of vaccine.

The seasonal breeder: endogenous PZP antigen results in self-boosting

It is possible that the success of the PZP-IVT vaccines is affected by the fact that deer are a seasonal multicycling animal. Each season when does enter a breeding condition, it is likely that the zona pellucida antigen is presented to the immune system, resulting in a self-boosting effect. It is possible the particulate nature of the IVT-PZP preparations resulted in a longer-boosting effect and contributed to the longer contraceptive effect. There does not appear to be any long-term negative health effect from the PZP contraception (Miller et al. 2001).

Immunocontraception holds the promise to help resolve deer–human conflicts (Bingham 2007). The possibility of a single-injection PZP contraceptive that would be effective as a contraceptive for multiple years increases the usefulness of an injectable contraceptive for the reproductive control of wildlife.

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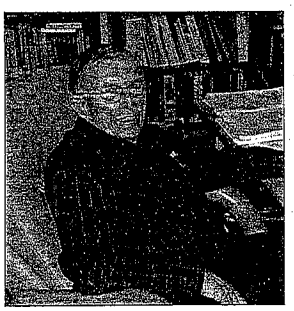

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