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Case studies in wildlife immunocontraception: wild and feral equids and white-tailed deer

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Abstract. Non-lethal management methods are required for wild equids that are protected by law and for deer inhabiting areas where lethal controls are not legal or safe. Single or multiple inoculations of porcine zona pellucida (PZP) vaccine have been delivered to wild horses and deer by means of darts. Contraceptive efficacy in horses after two inoculations ranged from 90% to 100%, and after a single inoculation ranged from 19% to 28%. Mares given a controlled-release form of the vaccine had foaling rates ranging from 7% to 20%. No detectable changes in social organization or behaviours among treated horses occurred. Contraceptive effects were reversible after 4 consecutive years of treatment but 5–7 years of treatment resulted in ovulation failure and decreased urinary oestrogen concentrations. Among deer, two inoculations were 70–100% effective in preventing fawns, but one inoculation yielded a contraceptive efficacy of ≤20%, with pregnancies occurring late in the breeding season; a single annual booster inoculation reduced fertility to 20% in the second year. Energy costs of extended breeding seasons were less than those resulting from pregnancy. After two years of treatment, ovaries appeared normal. These studies suggest that PZP immunocontraception can be successfully applied to certain free-roaming populations of wild horses and deer.

Introduction

A unique application of porcine zona pellucida (PZP) vaccines is contraception of certain wildlife species, where legal restrictions or social objections to lethal control have eliminated other options (Kirkpatrick and Turner 1985, 1991). Three species for which there is a need for non-lethal, humane and publicly acceptable population control are the North American wild horse (*Equus caballus*), the feral burro (*Equus asinus*) and the white-tailed deer (*Odocoileus virginianus*). The first two species have almost complete legal protection on public lands in the USA, under the Free-roaming Wild Horse and Burro Act. The white-tailed deer is extremely adaptable and has successfully inhabited suburbs, urban parks, government campuses, and military reservations, where lethal controls are no longer legal, wise or safe (Grandy 1993). This report examines progress to date with PZP immunocontracepton in free-roaming wild and feral equids and white-tailed deer and discusses implications of this research for the directions of future research.

Wild and feral equids

The first application of immunocontraception to free-roaming wildlife occurred in wild horses. A native PZP vaccine was successfully administered to, and proved highly effective in, wild horses on Assateague Island National Seashore (ASIS) and in several areas of Nevada. The vaccine was prepared from pig ovaries according to the procedure described by Liu *et al.* (1989) and stored frozen until use. The most effective vaccine protocol consisted of an initial inoculation of 65 μg of PZP (in 0·5 mL of phosphate buffer solution) emulsified with 0·5 mL of Freund's complete adjuvant (FCA) followed by a second inoculation with a mixture of 65 μg PZP and Freund's incomplete adjuvant (FIA) several weeks later. This protocol yields a contraceptive

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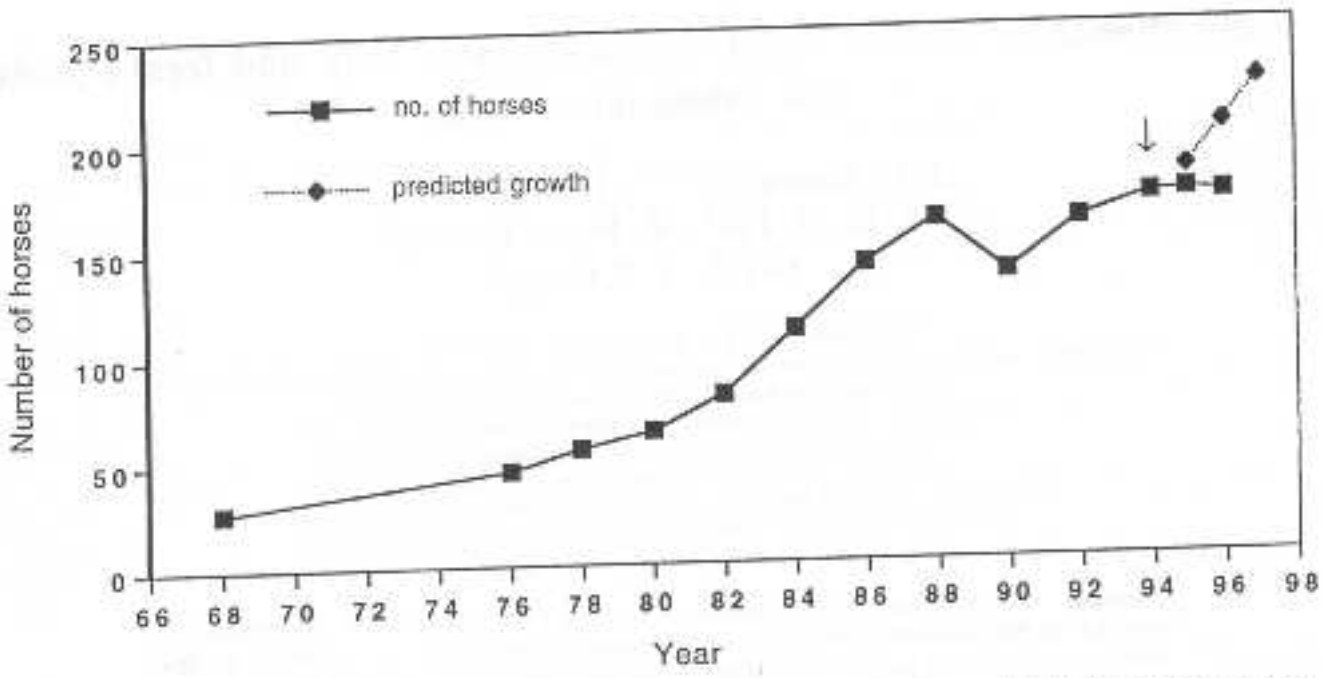

Fig. 1. Population growth of the Assateague Island wild horse herd from 1968 to 1996. The decrease observed between 1988 and 1990 was caused by a combination of immunocontraception of 26 experimental mares, two years of losses due to eastern equine encephalitis, and the loss of 12 horses by natural catastrophe. In 1992, 26 additional mares were immunized and in 1994 (↓), all mares ≥2 years old were immunized.

efficacy >90% (Kirkpatrick *et al.* 1990, 1995*a*). Annual booster inoculations of 65 μg PZP+FIA will maintain contraceptive effects without altering social organization and behaviours (Kirkpatrick *et al.* 1991). Treatment for up to three consecutive years is associated with a return to fertility. On ASIS, during a nine-year period, a total of 30 mares received their initial PZP inoculations during the third trimester of pregnancy and all 30 delivered foals. Three mares exposed to anti-PZP antibodies *in utero*, in 1988, have reached maturity and have produced foals of their own (Kirkpatrick *et al.* 1995*b*). In 1994, PZP contraception was incorporated into the ASIS management plan for wild horses (Kirkpatrick 1995). After only two years using PZP contraception as the sole management tool on ASIS a significant population effect has been demonstrated (Fig. 1).

The administration of PZP vaccine to 76 mares, by means of darts, was possible in the dense understories and the marshes of ASIS. In contrast, the vast spaces and large numbers of horses of the American west require a different strategy. In Nevada, in December 1992, 570 horses were gathered by helicopter roundup and 130 adult mares were hand-injected with PZP as they passed through chutes. During the summer of 1994, they were relocated and observed for the presence of foals. Untreated mares ($n = 63$) produced 34 foals (53·9%), sham-injected mares ($n = 20$) produced 11 foals (55%), and mares receiving two inoculations about one month apart ($n = 44$) produced two foals (4·5%) (Turner *et al.* 1996*b*). In a second field trial in Nevada, approximately 900 wild horses were captured in January 1996 and treated as described above. Adult mares ($n = 198$) were given the standard two-inoculation PZP (65 μg) treatment and results will be available in September 1997.

Because contraceptive efficacy may differ between seasonal and year-round breeders, a study of free-roaming feral burros, which breed throughout the year, was undertaken. Feral donkeys (*Equus asinus*) ($n = 16$) were immunized by dart with PZP, in Virgin Islands National Park. Each adult female received 65 μg of PZP followed by a second inoculation three weeks later and a booster inoculation 10–12 months later. Data were not collected until 12 months after the initial inoculation to allow for completion of any pregnancies in progress at the time of inoculations. Only a single treated female (6·2%) produced a foal or was pregnant, while 6 of 11 (54·5%) control donkeys produced foals or were pregnant (Turner *et al.* 1996*c*).

To ensure the safety of the PZP vaccine, immunocytochemistry of equine somatic tissues exposed to anti-PZP antibodies was carried out. Western blot analysis of glycoprotein hormones was performed and no immunocytochemical cross-reactivity using horse anti-PZP or rabbit anti-PZP was detected with heart, kidney, lung, liver, the somatic portion of ovary and testes. Furthermore, rabbit anti-PZP did not cross-react with equine luteinizing hormone, follicle-stimulating hormone, or chorionic gonadotropin (Fayrer-Hosken *et al.* 1996).

The levels of circulating antibodies in PZP-treated infertile mares vary significantly ($P < 0{\cdot}05$) (Fayrer-Hosken, unpublished observation; Kirkpatrick, unpublished observation). An important question is whether there are substantial differences in the immune systems of the vaccinated horses that relate to these differing antibody titres. Preliminary data in mares, comparing complete and differential blood counts, and levels of circulating antibodies show no relationship to concentrations of anti-PZP antibodies. Thus, the response of an individual animal to a PZP vaccine is probably related to the dose, adjuvant, and route of administration rather than the individual competence of its immune system.

During the course of our studies we have found that the seasonal timing of a single PZP inoculation with wild horses is important. In the northern hemisphere, wild mares breed between April and July. A single inoculation of PZP+FCA was given to 20 mares in Nevada, in December 1992, approximately four months before the onset of the breeding season. In 1994, 14 of those 20 mares were located and 4 mares (28·6%) had foals at their sides compared with 55% of control mares (Turner *et al.* 1996*b*). In a second test of a one-inoculation PZP trial, a single 65 μg PZP inoculation was given to 42 wild ASIS mares in March 1994, immediately before the onset of the breeding season. This treatment resulted in 8 foals (19%), a fertility rate which was significantly lower ($P < 0{\cdot}05$) than the normal foaling rate (45–55%; Kirkpatrick, unpublished observation).

Cervidae

Populations of white-tailed deer (*Odocoileus virginianus*) have increased dramatically in the USA, because of the adaptability of this species to human activity. The idea of trying PZP immunocontraception on this species was based on the success of PZP contraception of captive cervids in zoo collections. The vaccine has already been administered to >70 species of zoo animals and has been shown to inhibit reproduction in sika deer (*Cervus nippon taiouanus*), sambar deer (*Cervus unicolor*), muntjac deer (*Muntiacus reevesi*), axis deer (*Cervus axis*), and Roosevelt's elk (*Cervus elaphus roosevelti*) (Kirkpatrick *et al.* 1992*a*, 1993, 1996). Successful PZP contraception of white-tailed deer, using two inoculations of 65-μg doses, was first demonstrated with captive animals (Turner *et al.* 1992, 1996*a*, 1996*b*) and portions of these studies, utilizing 67 does, specifically examined the effects of: *(1)* one- and two-inoculation protocols; *(2)* antibody titre responses to PZP treatment; and *(3)* reversibility of contraceptive effects. Results indicated that two inoculations of PZP vaccine produced highly elevated and sustained anti-PZP antibody titres and were >90% effective in inhibiting fertility. Also, one or two consecutive years of contraception were associated with a return to normal fertility the next year.

The first field test with free-ranging white-tailed deer was carried out at the Smithsonian Institute's Conservation and Research Center in Virginia. Does ($n = 30$) were live-trapped, weighed, ear-tagged, separated into three treatment groups and then released. Does in Group A ($n = 10$) received a single injection of 65 μg PZP at the time of capture and a month later, in October 1992, the animals received a second inoculation with 65 μg PZP, remotely, by means of darts. Does in Group B ($n = 10$) received a single inoculation of PZP, and does in Group C ($n = 10$) received a single inoculation of saline plus adjuvant. A year later, 0%, 78%, and 82% of does in Group A, Group B, and Group C, respectively, produced fawns. All breeding activity ceased in control animals (Group C) by December, but breeding activity continued into February for animals in Group A and Group B, indicating that their breeding season was extended by one or two months when pregnancy did not occur earlier. This phenomenon had also been observed in studies with captive deer. Parturition dates for fawns born to animals in Group B were focussed in August, indicating that conception occurred during the extended portion of the breeding season, presumably when antibody concentrations decreased below contraceptive levels. Nine of the does in Group B were given a single booster inoculation in 1993 and, in 1994, only two does (22·2%) produced fawns. This latter experiment was significant because it suggested that a single PZP inoculation can provide antigen recognition which, despite limited contraceptive efficacy in Year 1, yields full contraceptive response to a single booster inoculation in Year 2 and thereafter. Ovaries from deer in all three groups showed no evidence of autoimmune disease and there were no differences between groups (McShea *et al.* 1996).

A second important feature of the present study was the determination that the extension of the breeding season among does that were inoculated with a contraceptive dose of PZP did not impart a harmful energy cost. The deer in this study were captured a year after treatment and the weights of does that received a contraceptive dose of PZP and extended their breeding seasons were significantly higher than those that did not receive PZP and became pregnant. In other words, the energy costs of pregnancy and lactation are far greater than those of an extended breeding season.

A second, larger-scale field trial with free-roaming deer is currently underway on Fire Island National Seashore in New York. These deer are acclimatized to human presence, allowing delivery of the vaccine in Pneu-Darts (Williamsport, PA, USA) fired from a blowgun. In Year 1 (1993), 74 does were given

either 1 inoculation ($n = 6$) or 2 inoculations of 65 μg PZP remotely. Of these 74 does, 62 were adults in 1992 and 90% of these produced fawns in May–June of the year before treatment. A year later, only 39·2% of these deer produced fawns. This was a significant reduction over fawning rates observed the year before treatment, but a surprisingly low contraceptive effectiveness compared with our results with captive deer. We believed that the primary cause for this relatively low contraceptive effectiveness was incomplete injection by some of the darts. In 1994, of these 73 does, 40 were given a single booster inoculation and another 76 previously untreated does were given two inoculations using an improved dart. In 1995, of the total deer treated in both years of the study, only 20% produced fawns. Among the does given booster inoculations, 15% produced fawns and among the 76 does receiving the initial two inoculations in 1994, only 28% produced fawns (Fig. 2). These results are encouraging and have led to the initiation of additional PZP contraceptive trials with free-roaming deer at the National Institute of Standards and Technology, in Gaithersburg, Maryland, and in a city park in Columbus, Ohio.

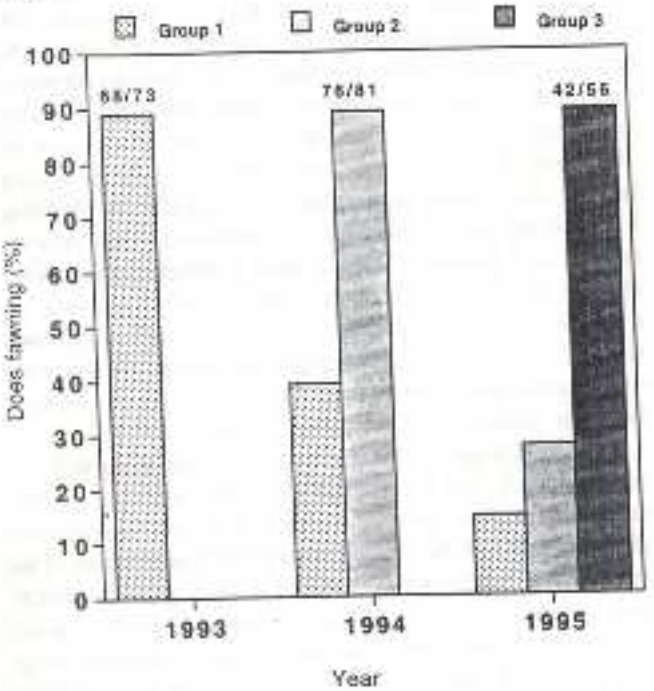

Fig. 2. Contraceptive efficacy of white-tailed deer contraception on Fire Island National Seashore. Group 1 illustrates the fawning rate before treatment for 1993 and the fawning rates for 1994 and 1995 after one year and two years of treatment respectively. Group 2 illustrates the fawning rate before treatment for 1994 and the fawning rate for 1995 after one year of treatment. Group 3 indicates the fawning rate before treatment for deer treated for the first time in 1995. The numbers at the top of each bar indicate the number of animals receiving two inoculations the first year out of the total deer treated in that group.

Current and future research

There are two important dimensions to the immunocontraception of wildlife that supercede other concerns. The first is its practicality, which to a large extent hinges on the availability of a one-inoculation form of the vaccine, particularly one that can act as a contraceptive for >1 year. A possible method for enabling the use of a one-inoculation PZP vaccine in a two-injection protocol is to incorporate the second dose of antigen–adjuvant into non-toxic, biodegradable lactide–glycolide microspheres which, on contact with tissue fluids, erode resulting either in a slow continuous release of antigen or in pulsed releases of antigen (Eldridge *et al.* 1989). The lactide–glycolide is metabolized to lactic acid and carbon dioxide. The pattern of release is important because a slow continuous release of antigen has the potential to cause immunotolerance instead of raising antibody titres to contraceptive levels.

In an initial trial to determine if continuous release of PZP would yield contraceptive antibody concentrations, as opposed to tolerance, white-tailed does ($n = 3$) were given an initial inoculation of 65 μg of native PZP and, on the same day, an osmotic mini-pump containing 65 μg of PZP was implanted subcutaneously in the neck of each animal. The pump was designed to release PZP continuously for 28 days at a rate of approximately 2·5 μg day^{-1}. Anti-PZP antibody titres were 76·3% of positive reference serum several weeks after placement of the pumps and 7–10 months later were 69·5%, indicating that continuous release of PZP will elevate titres to contraceptive levels (Turner *et al.* 1996*a*).

In a subsequent experiment, domestic mares ($n = 5$) were inoculated intramuscularly with an initial dose of 65 μg PZP together with 65 μg PZP contained in 50-μm microspheres designed to release the antigen over a six-week period. Antibody concentrations were compared in domestic mares ($n = 5$) inoculated with two doses of 65 μg of PZP given three weeks apart. Over 31 weeks, antibody concentrations did not differ significantly between the two treatment groups at Weeks 3, 14 and 31, but were significantly higher in the two-inoculation group at Week 5 and Week 8 than in the one-inoculation group (Kirkpatrick *et al.* 1996).

In December 1992, adult wild mares ($n = 20$) were captured in Nevada and hand-injected with a single inoculation comprising 65 μg of PZP emulsified in FCA together with 65 μg PZP incorporated into microspheres. In 1994, 14 of those 20 mares were relocated and 4 (28·6%) had foals at their sides compared with >50% of control mares with foals. In 1992, a similar preparation was remotely administered to wild ASIS mares ($n = 14$) by means of darts. One dart failed to inject. That mare, plus one other mare produced foals following treatment. The differences in fertility were significant

($P < 0.05$) comparing either 2 foals/14 mares or 1 foal/13 mares with foaling rates for untreated mares (Kirkpatrick *et al.* 1995*a*). In January 1996, adult mares ($n = 68$) were captured and given a second generation of a potential long-acting form of the vaccine. The long-acting form of the vaccine comprised 65 μg of native PZP in phosphate-buffered saline, emulsified with FCA, together with lactide–glycolide microspheres containing 65 μg of PZP and the adjuvant Carbopol. Results from this trial will not be available until 1997.

The second issue is that of long-term effects in general, and reversibility of contraceptive effects in particular. The public's concern over certain free-roaming wildlife species, and the conservation value of rare and endangered exotic species in captivity make the issue of reversibility of PZP immunocontraception very important, at least in some species. For example, the issue of reversibility is paramount in wild horses in the USA, and certainly in rare exotic species in captivity in zoos, but it is less of an issue in white-tailed deer. Thus far, in wildlife, reversibility has been documented in addax, ibex, and muntjac deer, and among white-tailed deer and domestic and wild horses, but most studies have involved only the effects of short-term treatment (1–3 years). Only in wild horse have reversibility studies been longer, up to seven years of treatment. Reversibility has been documented in mares treated for 1, 2, 3, and 4 years (Kirkpatrick *et al.* 1992*b*, 1995*c*). Ovulation rates for mares treated from 1, 3 and 7 consecutive years declined from 73·3% (11/15) to 55·5% (5/9) and 10% (1/10) respectively. One mare treated for 5 consecutive years ovulated during Year 6 and Year 7 but has not yet conceived. During the breeding season, urinary oestrogen concentrations declined during seven years of PZP treatment. The percentage of mares with normal urinary oestrogen concentrations (100–385 ng mg^{-1} creatinine) decreased to 46%, 33%, 40%, and 0% after 2, 3, 6, and 7 years of treatment respectively. Several mares with depressed urinary oestrogen concentrations continued to demonstrate cyclic peaks and nadirs in oestrogen concentration, suggesting continued follicular activity (Kirkpatrick *et al.* 1995*b*). This study indicates that long-term PZP treatment is associated with a decline in ovarian oestrogen production and ovulation rates. However, reversibility of the conditions of low oestrogen production and ovulation failure, and infertility, are unknown and will require 5–7 additional years to assess.

Conclusions

PZP immunocontraception appears to have great potential in certain populations of wildlife, because: *(1)* it has a contraceptive efficacy >90%; *(2)* it can be delivered remotely, via darts; *(3)* its effects are reversible after short-term use; *(4)* it is effective across many species; *(5)* it lacks debilitating side effects on health, even after long-term treatment; *(6)* it has minimal effects on social behaviours; and *(7)* the vaccine and the antibodies induced in response to it cannot be passed through food chains.

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