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Contraceptive efficacy of priming and boosting doses of controlled-release PZP in wild horses

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Abstract

Context. At some sites, wild horse populations have been effectively and non-invasively regulated using remote darting with porcine zona pellucida (PZP) immunocontraceptive vaccines. However, this model has not been widely applied to wild horse herds in western USA, many of which are difficult to access because they roam large areas and are wary of people. Single-treatment, multi-year contraceptive vaccines would significantly broaden the scope for successful contraceptive management.

Aims. The aims of the present field studies were to (1) test the contraceptive effectiveness and longevity of primers incorporating PZP–adjuvant emulsions plus PZP and adjuvant in controlled-release pellets (‘PZP-22’); and (2) compare the contraceptive effectiveness and duration of PZP–adjuvant emulsion-only boosters with those of PZP-22 boosters when administered by hand or remote darting to PZP-22-primed mares.

Methods. Wild horses in herd management areas in Colorado and Utah, USA, were rounded up in 2008 and in Utah again in 2012. Previously untreated females received a single hand-injection of PZP–emulsion plus controlled-release PZP pellets (‘PZP-22’ vaccine) and were then released. In Autumn 2010, 50 Colorado females treated in 2008 were booster-darted with either the PZP-22 vaccine or the PZP emulsion alone. In Utah, 57 previously treated females rounded up in 2012 received hand-injections of either the PZP emulsion–pellet vaccine or the PZP emulsion alone. Maternity was established through field observation of nursing and other close associations.

Key results. Effectiveness of initial controlled-release PZP treatments over 2 years was low relative to previous trials. However, boosters delivered by dart or by hand to PZP-22-primed mares yielded high levels of contraceptive effectiveness for 3 years, with no consistent difference between simple PZP-emulsion boosters and boosters incorporating controlled-release pellets.

Conclusions. Priming mares with PZP-22 extends the efficacy of a subsequent PZP booster to at least 3 consecutive years.

Implications. Regulation of wild horse populations may be achieved with existing contraceptive agents by developing models and management plans that account for the increased longevity of repeated contraceptive treatments.

Additional keywords: feral horse, immunocontraception, porcine zona pellucida, remote delivery.

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Introduction

The 1971 *Wild Free Roaming Horse and Burro Act* (P.L. 92-195) assigned primary responsibility for managing wild horses and burros occupying public lands to the US Bureau of Land Management (BLM), with secondary responsibilities to the US Forest Service (USFS) for managing the species on USFS lands. In 1978, prompted by growth of the herds subsequent to passage of the *Act*, Congress authorised the BLM and USFS to gather, remove and make available for adoption by the public wild horses and burros deemed excess relative to appropriate management levels set by public land use planning processes (*Public Lands Improvement Act*, P.L. 95-514).

Through the fiscal year (FY) 2015, the BLM had adopted out more than 239 000 wild horses and burros to the public (BLM 2016*a*). However, the rate at which horses have been adopted has failed to keep up with the number of horses removed from the range, especially since FY2001, resulting in increases in wild horse numbers in captive-holding facilities and on the range. As of March 2015, more than 45 000 formerly free-roaming wild horses were being maintained in short- and long-term holding facilities at a budgeted cost of US$62 million for FY 2016 (BLM 2016*b*). In addition, the 67 027 wild horses and burros estimated to be living on the range as of 1 March 2016 significantly exceeds BLM estimates of a system-wide appropriate management level (AML, the number of horses and burros defined by the BLM as the number that can thrive in balance with other public-land resources and uses) of 26 715 (BLM 2016*b*). While BLM estimates of AML can be challenged, it is clear that the system is unsustainable as currently being operated.

Both BLM documents and outside reviews have repeatedly identified on-the-range fertility control as a plausible option for reducing the rate of growth of wild-horse herds, thereby reducing pressure on the system of removal and adoption as well as the range itself. In response, BLM has been funding research into practical, safe, and effective wild-horse contraception since the 1970s (Turner and Kirkpatrick 1982; Plotka *et al*. 1988, 1992; Turner *et al*. 1997, 2007; National Research Council 2013). Several potentially suitable immunocontraceptive agents have emerged from BLM-funded research, including porcine zona pellucida (PZP) and GnRH vaccines (Killian *et al*. 2008; National Research Council 2013). Porcine zona pellucida has the deepest research history, having been previously tested and applied for several years at Assateague Island National Seashore, MD, and elsewhere (Kirkpatrick *et al*. 1990; Kirkpatrick and Turner 2002, 2003, 2008; Ransom *et al*. 2011; National Research Council 2013).

In a minority of BLM herd management areas (HMAs), wild horses are approachable enough so that contraceptive vaccines can be delivered directly by dart (Ransom *et al*. 2011; Naugle and Grams 2013). However, in others, horses are too wary to approach closely enough for darting, and are thinly dispersed over wide areas (Naugle and Grams 2013). In these areas, either gathers or trapping must be used to make horses accessible for treatment. Where accessing horses for treatment is challenging, the availability of a multi-year, single-treatment contraceptive vaccine would be enormously helpful in effectively and substantially reducing foaling rates and, consequently, rates of population growth.

Evidence for multi-year effectiveness of contraceptive vaccines is mixed. Killian *et al*. (2008) showed that single doses of SpayVac® (Immunovaccine Technologies, Halifax, NS, Canada) and GonaCon™ (USDA/NWRC, Fort Collins, CO, USA) significantly reduced fertility in a small sample of captive horses for up to 4 years. Gray *et al*. (2010) showed that GonaCon-B™ and a formulation of PZP with Adjuvac™ (USDA/NWRC, Fort Collins, CO, USA) reduced fertility in free-roaming Nevada mares for 3 years, although efficacy was modest for both vaccines (reducing fertility from control levels by 25–59%). Turner *et al*. (2001) showed that a single dose of native PZP plus PZP prepared in controlled-release microspheres produced a 1-year reduction in fertility comparable to that of two doses of native PZP administered 3 weeks apart. However, the microspheres tended to clump in the syringe and proved difficult to handle under field conditions (Turner *et al*. 2002). In a subsequent field trial at Clan Alpine HMA, NV, a single injection of 65 µg PZP emulsified in Freund's complete adjuvant (FCA) plus 410 µg PZP and 875 µg QA-21 adjuvant in controlled-release pellets reduced fertility in treated mares by 91%, 73%, and 38% from control levels in Years 1–3 after treatment (Turner *et al*. 2007).

On the basis of the results of the Turner *et al*. (2007) trial and supporting data on captive and non-captive mares (Turner *et al*. 2002; Liu *et al*. 2005), BLM initiated wider use of the PZP emulsion plus PZP/QA-21 pellet vaccine (dubbed 'PZP-22,' based on antibody titer concentrations displayed in Liu *et al*. 2005; National Research Council 2013), treating between 500 and 1000 mares annually between 2007 and 2013. However, the Clan Alpine study was not replicated, and information on the efficacy of BLM administrations at both the individual and population levels was anecdotal. Also lacking are any studies of the effectiveness and longevity of PZP boosters administered to mares primed with PZP-22.

As a part of a larger study to further evaluate the individual- and population-level effectiveness of PZP-22, and to establish the effectiveness and longevity of PZP boosters administered to mares that had previously been primed with PZP-22, we conducted field studies in two BLM herd management areas.

Materials and methods

Study areas

Sand Wash Basin Herd Management Area, Colorado

The Sand Wash Basin (SWB) HMA comprises ~638 km^2 located ~72 km west of Craig, CO, USA. Elevation ranges from 1900 to 2660 m. SWB receives 18–30 cm of annual precipitation, and the climate is typical of the cold deserts of the Rocky Mountain Region, with warm summers and very cold winters. Vegetation types within the HMA include sagebrush (*Artemesia spp*), bunchgrasses, saltbush (*Atriplex* spp.), and pinyon–juniper woodlands. Horses, livestock and wildlife at SWB rely for water on a combination of developed wells, undeveloped springs and seeps and artificial reservoirs.

According to BLM aerial-survey estimates, there were ~463 wild horses on the SWB HMA and 50 wild horses off HMA. Our initial ground inventory after the BLM gather in October 2008 indicated the presence of 194 horses (K. Grams and A. Rutberg, unpubl. data). The appropriate management level at SWB is 163–362 (BLM 2017).

Cedar Mountains Herd Management Area

The Cedar Mountains (CM) HMA comprises 727 km^2 and is located ~80 km west of Salt Lake City, UT, USA. Approximately 425 km^2 within the HMA is part of a congressionally designated candidate wilderness area; vehicles and permanent structures are not permitted within the designated wilderness area. Elevations vary from 1400 to 2460 m. Annual rainfall in the lower elevations averages 20–25 cm, and 25–38 cm of rainfall is received in the upper elevations. Desert shrubs and bunchgrasses are common in the lowlands, with sagebrush and juniper woodlands dominating higher elevations. Water is seasonally scarce, and is concentrated in dry summer months at cattle troughs and a few natural springs.

Our ground-based population inventory indicated that there were at least 608 horses on CM when the study began in 2008, with additional horses on adjacent military lands that had not yet been documented. Initial estimates by BLM based on their aerial survey indicated that 191 remained after the December 2008 BLM gather, although subsequent ground-based population inventories indicated that there were at least 265 horses present post-gather. The appropriate management level for CM is set by the BLM at 190–390 (BLM 2017).

Horse identification

Beginning in 2008, information was documented in the field and then entered into a database. This working document included sex, colour, face and leg markings, unique descriptions, mane and tail description, year of birth and the subpopulation in which the horses lived. Each horse was assigned an ID number to establish individual identity. Horses, including bachelors, were grouped by band in the areas in which they were most recently found. The horse list was updated weekly to reflect any changes or additions and was used in the field to help identify horses. Horses were individually recorded in the Wild Horse Identification Management System (WHIMS, US Geological Survey, Fort Collins, CO, USA). Information from the horse list was placed into this database along with individual pictures of each horse. A photo catalogue allowed for viewing each horse, with the pertinent information to that horse.

Horses that were identified during the study but were not observed within the HMA were excluded from the analysis.

PZP treatments

Wild-horse mares were gathered by the BLM and restrained in squeeze chutes for PZP treatments at Sand Wash Basin on 23 October 2008, and at Cedar Mountains on 17 December 2008 and 25–28 February 2012. Porcine zona pellucida treatments were also administered remotely by dart at SWB from 11 September to 17 November 2010. Thus, boosters were administered at SWB 2 years after the initial treatment, and at CM 3.2 years after the initial treatment.

Hand-injections

In October 2008 and December 2008 respectively, 62 mares (2+ years old) at SWB and 70 mares (2+ years old) at CM were gathered and treated with hand-injections of PZP-22, consisting of one priming injection of 100 µg PZP in 0.5-mL phosphate buffer solution (PBS) emulsified in 0.5 mL of modified FCA (mFCA, Calbiochem, LaJolla, CA, USA) (following Kirkpatrick *et al*. 1990), and a second injection of three heat-extruded lactide–glycolide polymer pellets (polymers from Lactel/Durect, Pelham, AL, USA) containing a total of 450 µg of PZP and 900 µg of QA-21 (Antigenics, Lexington, MA, USA), a saponin adjuvant, engineered to release at 1, 3 and 12 months (Turner *et al*. 2008). The 1- and 3-month pellets each contained 100 µg of PZP and 200 µg QA-21, and the 12-month pellet contained 250 µg PZP and 500 µg of QA-21. Both injections were delivered intra-muscularly into the hip, the PZP–mFCA emulsion with a hand-held syringe fitted with an 18-gauge needle, and the pellets with a trocar fitted with a 14-gauge needle. In the squeeze chute, the horses were also freeze-branded on the hip with a marking that identified them as PZP-treated mares. In February 2012, 85 previously untreated mares at CM were similarly treated and released. Of these mares, 23 were 2 years old, and 62 were $\geq$3 years old.

Booster trials

To examine the relative effectiveness of the PZP-22 booster when hand-injected, 58 mares that had been treated at CM in 2008 and recaptured for treatment in February 2012 received a hand-injection of either PZP-22 (with Freund's incomplete adjuvant (FIA, Calbiochem, LaJolla, CA, USA) substituted for mFCA, 'PZP-22–FIA'; $n = 39$) or the PZP–FIA booster alone ($n = 19$).

A parallel test of the PZP-22 booster when delivered remotely, by dart, was conducted at SWB. From 11 September to 17 November 2010, 34 mares received PZP-22–FIA delivered in a Pneu-dart® (Williamsport, PA, USA) dart designed with a rod within the 1.5-inch needle, to simultaneously inject both the emulsion and the dart (see Walter *et al*. 2012 for a similar device). Another 16 mares received only the PZP–FIA emulsion by dart (Kirkpatrick *et al*. 1990). Two dart projectors were utilised for short and longer distances. The Dan-Inject CO2 pistol (Børkop. Denmark) projected darts at a distance of 10–18 m. The Pneu-Dart X-Caliber Gauged CO2 projector rifle projected darts at a distance of 17–45 m. Depending on the tractability of the animal, the Dan-Inject was primarily used for close range.

Observations

Observational data were collected beginning in 2008, focusing on the months from June to October in SWB and April through to December at CM. Observations were conducted from the ground, usually by one but sometimes by two observers. Depending on distance, observations were made through binoculars or 20–60 × 80 spotting scope.

For each band encountered, observers identified all known horses present, described any new horses in the band that had not previously been described, tabulated the age–sex composition of the group, and, using maps and field notes, noted the location of the band. Mares were matched with foals within 2 weeks of birth, as indicated by persistent proximity and nursing. Within limits of visibility and accessibility, the observer also estimated and recorded body condition scores on a 1–5 scale (after Rudman and Keiper 1991) and noted any apparent injuries, lameness, abscesses and other injection-site reactions, or other signs of ill health, for each band member, as well as any reproductive activity or mortalities that occurred.

Data analysis

Foaling data inferred from observations of foal–mare associations and behaviour were compiled by mare age (when age data were available) and PZP treatment status. The relationship between age and foaling rates was examined using the linear regression function of IBM SPSS®-22, using study site, age and age squared as independent variables (modified from Ransom *et al*. 2011). Foaling rates were compared among treatment groups and between treatment groups and untreated mares by year after treatment, using Pearson's chi-square tests or, if sample sizes were too small, by two-tailed Fisher's exact tests. Sham controls were not used, because Turner *et al.* (1997) had previously demonstrated that fertility rates among sham controls and untreated controls were virtually identical.

The proportions of untreated and pretreatment mares of known age that foaled in a given year were calculated for each site. Foaling data comprised 170 foals born to mares of known age (2–17 years old) in 311 mare-foaling opportunities at SWB between 2008 and 2014, and 193 foals born to mares of known age (2–24 years old) in 376 mare-foaling opportunities at CM between 2008 and 2015. Because fewer records existed for older mares, ages were consolidated into groups of at least 10 mares in each age category, assigning the median age to each group. Sample size per age group ranged from 12 to 154.

Because of uniformly low foaling rates among untreated 2-year-old females (see below), all comparisons between treatment groups and untreated controls were performed using mares 3 years old and older at the time of foaling. The criterion for rejecting null hypotheses was set at $\alpha = 0.05$.

Results

Effects of age on likelihood of foaling

In both study sites, 2-year-old females were less likely to foal, then older females (Fig. 1). Site, as an independent variable, was not a significant predictor of foaling rate ($P = 0.824$), and was eliminated from the analysis. Because the form of the relationship between age and fertility appeared to differ at both sites, each site was analysed separately. At CM, the probability of foaling followed a fairly strong parabolic relationship with age ($R^2 = 0.817$, $F_{2,5} = 11.14$, $P = 0.014$; Fig. 1*a*). However, at SWB, the age–foaling relationship was flat for mares older than 2 years old relative to CM, and the parabolic relationship was not significant ($R^2 = 0.580$, $F_{2,5} = 3.46$, $P = 0.114$; Fig. 1*b*).

Initial treatments with PZP emulsion and controlled-release pellets

Sample sizes for observation of foaling in treatment groups showed only slight attrition during the study, whereas samples of untreated controls observed for foaling generally grew from year to year as new individuals in the population were identified or were recruited as foals born into the herd during the study aged into the 3+ years age group (Table 1).

In all three priming treatments (CM 2008 and 2012 and SWB in 2008), foaling rates were statistically indistinguishable between treated and untreated mares during the foaling period immediately after treatment, defined as Year 0 (reflecting pretreatment pregnancy rates; Fig. 2). Significantly lower foaling rates were found among mares ≥3 years old in treated mares in Year 1 at CM in the 2008 ($\chi_1^2 = 29.04$, $P < 0.0001$; Fig. 2*a*) and 2012 ($\chi_1^2 = 24.99$, $P < 0.0001$; Fig. 2*b*) treatments, but not in the 2008 SWB treatments ($\chi_1^2 = 3.4$, $P = 0.065$; Fig. 2*c*), relative to untreated controls. In Year 2, foaling rates in treated mares were significantly lower than those in untreated mares for both the 2008 and 2012 CM treatments ($\chi_1^2 = 5.94$, $P = 0.015$; $\chi_1^2 = 5.57$, $P = 0.018$) and the SWB treatments ($\chi_1^2 = 6.2$, $P = 0.013$). Summed over the two post-treatment years, foaling

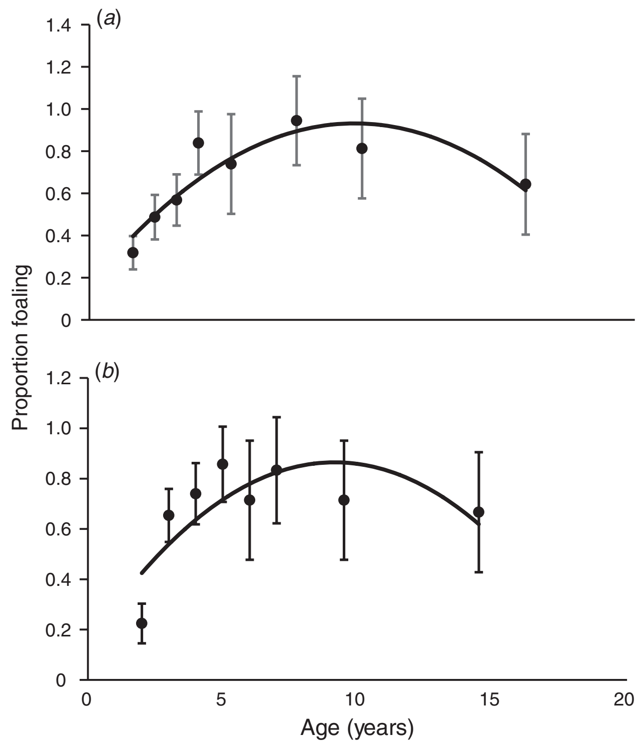

Fig. 1. Proportion of untreated and pretreated mares foaling by age. $N > 10$ for each age category. Error bars represent 95% CI. (*a*) Cedar Mountains, UT, USA, 2008–15 ($Y = -0.005X^2 + 0.126X + 0.165$, $R^2 = 0.817$, $F = 11.14$, d.f. = 2,5, $P = 0.014$). (*b*) Sand Wash Basin, CO, USA, 2008–14 ($Y = -0.009X^2 + 0.157X + 0.144$, $R^2 = 0.580$, $F = 3.46$, d.f. = 2,5, $P = 0.114$).

Table 1. Number of mares 3+ years old for which foaling data were obtained in each treatment group at Cedar Mountains (CM) Herd Management Area, Utah, USA, and Sand Wash Basin (SWB) Herd Management Area, Colorado, USA

At CM, 39 mares received PZP-22–FIA boosters and 19 received PZP–FIA boosters. At SWB, 34 mares received PZP-22–FIA boosters, and 16 received PZP–FIA boosters

Year	Cedar Mountain HMA PZP-22 primer	Cedar Mountain HMA PZP boosters (total)	Cedar Mountain HMA Untreated control	Sand Wash Basin HMA PZP-22 primer	Sand Wash Basin HMA PZP booster (total)	Sand Wash Basin HMA Untreated control
2008	70 hand-treated	–	–	62 hand-treated	–	–
2009	69	–	61	62	–	15
2010	69	–	63	61	50 dart-treated	18
2011	68	–	73	61	50	22
2012	85[A] hand-treated	58 hand-treated	38	–	50	52
2013	83	57	44	–	49	68
2014	80	56	56	–	28[B]	107
2015	–	55	94	–	–	–

[A]Twenty-three (23) mares that were 2 years old at the time of treatment were not included in Year 0 (2012) foaling-rate calculations.
[B]Twenty-one (21) mares were lost to the study because they received additional PZP treatments in 2013. Of the 28 remaining in the study, 17 had received PZP-22 boosters and 11 had received PZP emulsion only.

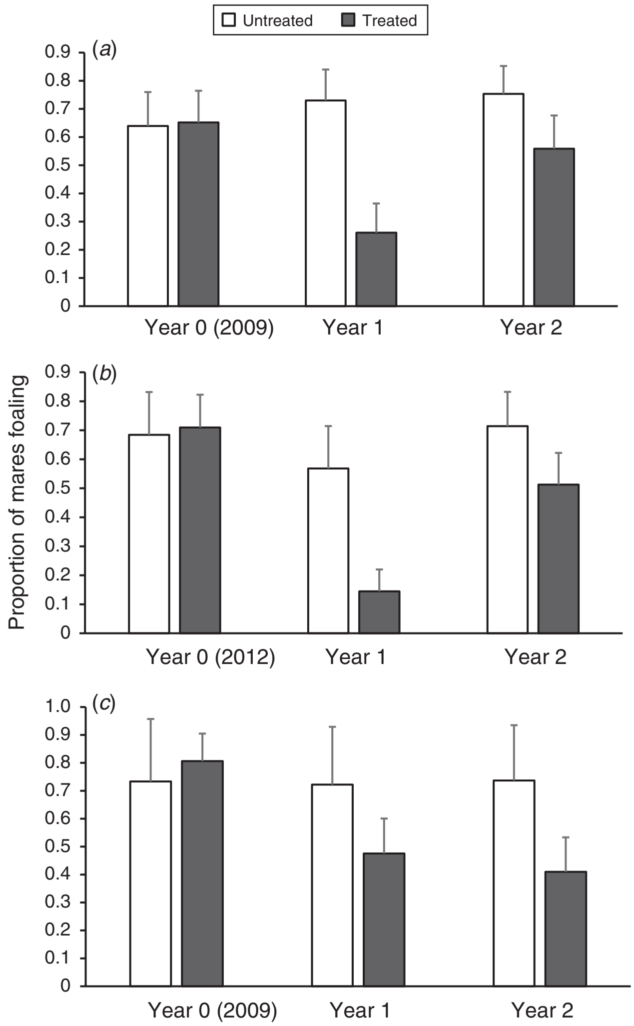

Fig. 2. Foaling rates in mares ≥3 years old. Error bars represent 95% CI. (*a*) Cedar Mountains herd-management area (HMA), UT, USA, following December 2008 administration of porcine zona pellucida (PZP)-22 primers by hand. Year 0 = 2009. (*b*) Cedar Mountains HMA, UT, USA, following February 2012 administration of PZP-22 primers by hand. Year 0 = 2012. Twenty-three PZP-22-treated females that were 2 years old in 2012 were excluded from the Year 0 analysis. (*c*) Sand Wash Basin HMA, CO, USA, following October 2008 administration of PZP-22 primers by hand. Year 0 = 2009.

rates among treated mares were reduced by 39% at SWB in 2008, by 45% at CM in 2008, and by 50% at CM in 2012.

No injection-site reactions were observed in mares after priming via hand-injection.

PZP booster treatments

At both SWB and CM, PZP boosters were associated with significant reductions in foaling rates among mares ≥3 years old every year for 3 years after treatment (Fig. 3).

At SWB, foaling rates in mares boosted with PZP by remote darting averaged 25.2% over 3 years (range 22.0–32.1%) versus 74.9% (65.4–79.4%) in untreated mares (Fig. 3*b*), i.e. foaling rates in booster-treated mares were reduced by 66% relative to those of untreated mares. In each year, foaling rates were significantly lower in treated mares than in untreated mares (Year 1, $\chi_1^2 = 19.46$, $P < 0.0001$; Year 2, $\chi_1^2 = 34.94$, $P < 0.0001$; Year 3, $\chi_1^2 = 20.00$, $P < 0.0001$). Mares darted with PZP-22–FIA showed lower foaling rates (averaging 17.8% across 3 years) than did mares darted with PZP–FIA emulsions alone (averaging 39.5% across 3 years), but there were no significant differences in foaling rates between the two treatment groups in any year.

Injection-site reactions were seen in 32 of 47 darted SWB horses (68.1%) observed in the weeks following darting. Of these reactions, 5–8 were draining abscesses (uncertainty owing to observation quality), of which at least five had resolved by the last time the horse was observed in the 2010 field season (15–52 days after darting). The remainder were swellings and granulomas averaging 2.75 cm (s.d. = 0.87 cm) in diameter. The two treatment types did not differ from one another in either the frequency of swellings and granulomas or in the frequency of draining abscesses.

At CM, hand-injected PZP boosters were also highly effective, with foaling rates of treated mares across 3 years averaging 14.9% (ranging from 7.0% to 20.0%) versus 67.5% (range 56.8–71.4%) for untreated females ≥3 years old, i.e. foaling rates in booster-treated mares were reduced by 78% relative to those of untreated mares (Fig. 3*a*). Treated mares had significantly lower foaling rates than did untreated controls in all years (Year 1, $\chi_1^2 = 30.09$, $P < 0.0001$; Year 2, $\chi_1^2 = 30.38$, $P < 0.0001$; Year 3, $\chi_1^2 = 35.03$, $P < 0.0001$). At CM, no consistent differences in efficacy emerged between the two treatment groups, and there was no significant difference in foaling rates between the PZP-22–FIA and the PZP–FIA-only group in any treatment year. No injection-site reactions were observed in hand-boosted mares.

Discussion

Mares hand-injected with PZP-22 primers generally demonstrated lower foaling rates than did untreated control mares. However, the effectiveness and longevity of PZP-22 were much lower at both CM and SWB than was reported in Turner *et al.* (2007) for wild horses in Clan Alpine. Several differences in manufacturing and protocol may account entirely or in part for the differences.

(1) *Treatment month of administration.* The 1-, 3- and 12-month pellet-release times were engineered to optimise effectiveness of vaccines delivered December to February. The highest 2-year efficacy of primers in the present study occurred when vaccinations were delivered in February (CM 2012), followed by vaccinations delivered in December (CM 2008), with those delivered in October (SWB 2008) being least effective. However, even with optimal administration timing, the efficacy levels observed in these trials following Year 1 did not match those observed at Clan Alpine, suggesting that timing alone is not a sufficient explanation for the differences.
(2) *Differences in timing of antigen release*. Subsequent *in vitro* testing of the heat-extruded controlled-release pellets of batches used in this trial was undertaken to ascertain whether changes in pellet-release characteristics originally described in Turner *et al.* (2007) and Turner *et al.* (2008)

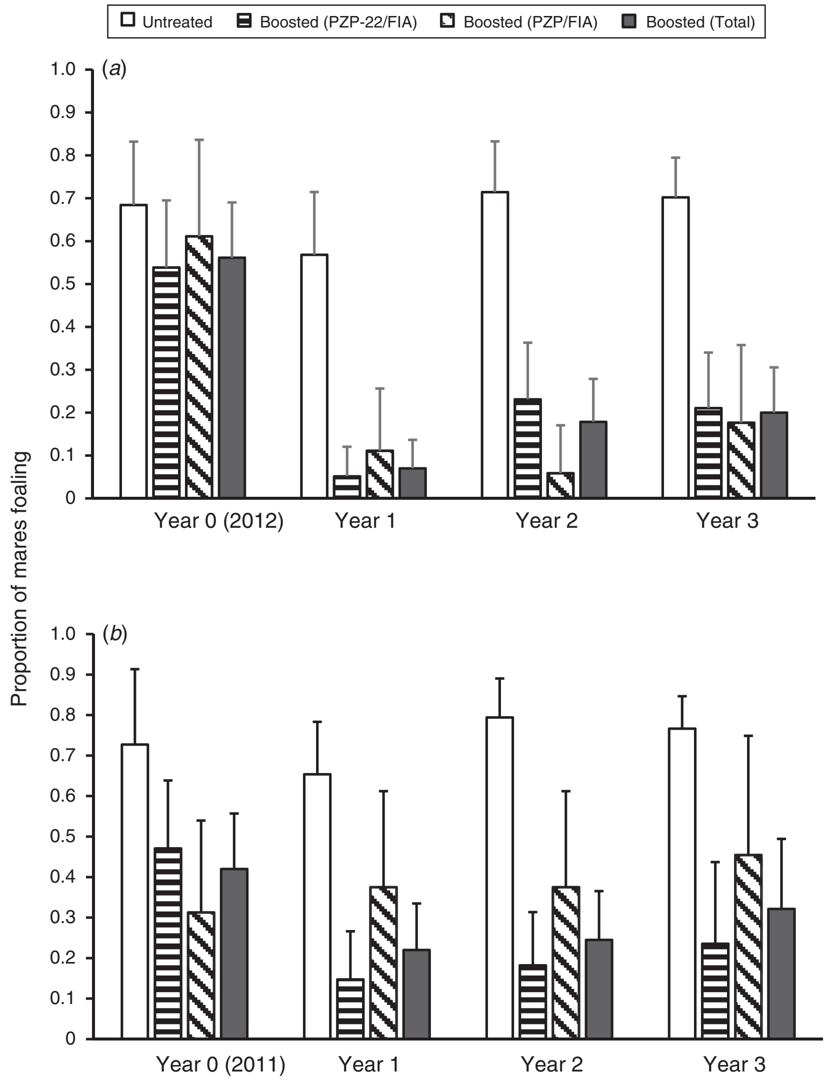

Fig. 3. Foaling rates in mares ≥3 years old. Error bars represent 95% CI. (*a*) Cedar Mountains herd-management area (HMA), UT, USA, following February 2012 administration of PZP-22–FIA or PZP–/FIA boosters by hand. Year 0 = 2012. (*b*) Sand Wash Basin HMA, CO, USA, following Autumn 2010 administration of PZP-22–FIA or PZP–FIA boosters by dart. Year 0 = 2011. Note that the Year 0 (2011) foaling rates for treated mares are the same as those for Year 2 in Fig. 2*c*.

contributed to suboptimal PZP-22 performance. Comparison of release characteristics of pellets produced before and after 2007 revealed that post-2007 pellets of the 12-month delay type released prematurely and more gradually than was expected (J. W. Turner, unpubl. data). Possible contribution of polymer-batch and pellet-manufacture variations to this circumstance are under investigation.

(3) *Method of injection.* PZP-22 at Clan Alpine was delivered by jab stick, and both the emulsion and pellet components were delivered together at the same injection site, whereas emulsion and pellets were delivered by two separate injections in all hand-injection protocols of PZP-22 followed in the present study. Although being an implausible explanation for the differences, this hypothesis also receives weak support from the improved PZP-22 efficacy relative to the PZP–FIA emulsion associated with dart delivery in booster trials (described below).

(4) *Difference in adjuvant.* PZP vaccine prepared before 2005 utilised FCA, and, thereafter, mFCA was used. Similar anti-PZP titer responses to primer-dose PZP treatment with these two adjuvants have been reported (Lyda *et al*. 2005). However, contraceptive efficacy was not determined, and it is possible that the antibody type and quality generated by these two adjuvants differed sufficiently to influence the immune memory generated by pellet-based boosters (M. Wooten, pers. comm.). In light of the limited future for FCA (related to tissue reactions and false-positive TB tests) and the promise of alternative multi-target adjuvants (e.g. Lu *et al*. 2015), comparison of mFCA with such novel adjuvants to address the above issue may be warranted.

Although the initial PZP-22 treatments tested in the present study showed disappointing effectiveness, a single PZP booster (either the PZP–FIA emulsion or PZP-22–FIA) effectively reduced fertility across three consecutive years. Whether delivered by dart, as at SWB in 2010, or by hand, as at CM in 2012, PZP boosters reduced foaling rates in treated mares by roughly 65–72% relative to untreated control mares over the 3 years. We do not know, because the studies had to be ended 3 years after boosting, whether some contraceptive effectiveness might persist into a 4th year.

Most previous research on PZP in western wild horses has presented data only on the effectiveness and longevity of the initial treatment (Turner *et al.* 1997, 2001, 2007). Working on wild horses at Little Book Cliffs Wild Horse Range, CO, USA, and the Pryor Mountain Wild Horse Range, MT, USA, Ransom *et al.* (2011) examined the relationship between the number of consecutive years of treatment (up to 4 years) with PZP emulsion boosters and the interval between the last booster and the first foaling. This interval ranged from 7 to 18 months, with some trend towards longer intervals as the number of consecutive years of treatment increased, although the sample size for 3 and 4 consecutive years of treatment was very small and the trend was not significant. In their study of reversibility of PZP treatments of wild horses at Assateague Island National Seashore, MD, USA, Kirkpatrick and Turner (2002) showed that most mares receiving two initial injections and up to one subsequent annual booster returned to fertility within 1 year, whereas mares receiving three or four consecutive years of treatment experienced delays of 3–4 years in return to foaling. Although our measures of the longevity of vaccine effectiveness differ from those used in other studies, namely annual foaling rates of treated mares relative to those of controls, rather than interval between last booster and first foaling, as used by Ransom *et al.* (2011) and Kirkpatrick and Turner (2002), our results appear to parallel those for Assateague mares receiving three or more consecutive years of treatment. Thus, even if the initial effectiveness of PZP-22 was low relative to previous studies, the combination of a PZP-22 primer and a single PZP booster appears to have yielded effectiveness comparable to three or more years of consecutive treatments (i.e. 4+ injections) of Assateague mares initially treated with the PZP–mFCA emulsion primer.

Our study failed to show that the addition of the controlled-release pellets to the PZP emulsion increased the efficacy and longevity of the booster. At CM, addition of pellets in hand-administration did not increase the efficacy or longevity of the PZP emulsion over the 3 years of the study. Dart delivery of the booster was associated with a doubling of efficacy across all 3 years at SWB, but because the sample is small, this test warrants replication.

Conclusions

Long-term management of wild horse populations with a non-invasive reversible contraceptive will require repeated treatments over the life of the wild mare. Thus, we believe that the research emphasis placed on the effectiveness and longevity of the primer vaccination may be misguided. The data presented here, as well as in Kirkpatrick and Turner (2008), suggest that population models should be constructed that allow for increased longevity of vaccine effectiveness with successive boosters. In practice, the initial application of contraceptives to a wild horse herd may produce only a modest reduction in population foaling rates, but sustained contraceptive applications over time could result in a much greater reduction in population foaling rates, even with current technology.

At Assateague, the initial management step was to treat every mare with a single primer of PZP–FCA emulsion (Kirkpatrick and Turner 2008). Although the primer alone was only somewhat effective as a contraceptive, subsequent boosting yielded increased effectiveness, and, ultimately, a reduction in herd numbers. This lesson needs to be applied in Western herds.

Also encouraging is the demonstration of management flexibility in PZP-22 application. First, the data suggest that the interval between initial and booster treatments, i.e. 2 years at SWB, and 3.2 years at CM, does not obviously influence effectiveness or longevity of the booster over the range of times tested. Second, the SWB data indicate that PZP-22–FIA can be effectively delivered remotely, at least matching the effectiveness of the PZP–FIA emulsion. However, until technical improvements and additional tests show that the controlled-release pellets significantly increase booster efficacy, managers can employ the much less costly PZP–FIA emulsion as a booster following administration of the PZP-22 primer.

Continuing to improve vaccine longevity is essential. The more individual mares are darted, the more difficult they become to re-treat (Kirkpatrick and Turner 2008; Naugle and Grams 2013). As the controlled-released pellets are refined, re-darting can be performed less frequently, making contraceptive control more cost effective.

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