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Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*)

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Contraception is becoming a common approach for the management of captive and wild ungulates yet there are few data for contraceptive effects on entire populations. Management-level treatment of mares with porcine zona pellucida (PZP) vaccine resulted in zero population growth of the Assateague Island wild horse population within 1 year of initiation of treatment. Contraceptive efficacy was 90% for mares treated twice in the first year and annually thereafter. For mares given a single initial inoculation, contraceptive efficacy was 78%. The effort required to achieve zero population growth decreased, as 95, 83 and 84% of all adult mares were treated in each of the first 3 years, compared with 59 and 52% during the last 2 years. Mortality rates for mares and foals after the initiation of management-level treatments decreased below historic and pretreatment mortality rates of approximately 5%. Two new age classes have appeared among treated animals (21–25 years and > 25 years), indicating an increase in longevity among treated animals. Body condition scores for all horses, all adult mares and non-lactating mares increased significantly between summer 1989 and autumn 1999 but did not change significantly in lactating mares. These results provide reliable data for the construction of realistic models for contraceptive management of free-roaming or captive ungulate populations.

Introduction

The management of free-roaming and captive ungulate populations often relies on some form of contraception. The use of porcine zona pellucida (PZP) vaccine to inhibit fertility among various free-roaming wildlife (Kirkpatrick *et al.*, 1990a, 1991a, 1992, 1995a; Kirkpatrick, 1995) and captive exotic (Kirkpatrick *et al.*, 1995b, 1996) species has been well documented. Until 1994, studies had focused on contraceptive efficacy and short-term (< 3 years of treatment) safety of the vaccine, and they did not address the larger issue of changes among entire populations and long-term safety (> 3 years of treatment). One of the ultimate goals of any wildlife contraceptive effort, whether captive or otherwise, is to alter entire populations in some manner, which will positively affect habitat, other species or the target population itself.

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In the case of Assateague Island National Seashore, MD (ASIS), the National Park Service (NPS) sought to protect fragile barrier island habitat from a rapidly growing wild horse population (Rodgers, 1985; Stribling, 1989, 1990; Furbish, 1990, 1994) and to protect the health of the wild horses themselves, by limiting population growth, while at the same time protecting the genetic integrity of the population.

From 1988 to 1993, between 10 and 34 mares in a total population that ranged from 129 to 156 animals were treated annually to test the efficacy and safety of the PZP vaccine. In 1994, ASIS began management-level application of PZP immunocontraception with the short-term goal of stabilizing the population at or near 166 animals. This paper reports the efficacy of PZP contraception over an 11 year period, population changes over 5 management years, and associated changes in mortality, the percentage of mares treated annually, and the occurrence of increased longevity and body condition among treated animals. The working hypotheses were that: (i) the population could be stabilized in < 5 years; (ii) the body condition scores of treated mares would increase; and (iii) that annual effort to achieve these goals would decrease over the 5 years.

Materials and Methods

Management plan

In 1994, a management plan was developed by the ASIS Resource Management staff to maximize the potential population-level effects of immunocontraception while protecting the genetic integrity of the wild horse herd. A treatment priority was established: (i) to inhibit older mares (those with three living offspring or two or more surviving generations) from breeding for the remainder of their lives; (ii) to limit treatment of mares with fewer than two living offspring to no more than 3 consecutive years of treatment, after which they would be permitted to have a foal; and (iii) to prohibit any treatment of older mares with no living offspring. The 3 year limit was imposed because it was clear in 1994 that PZP contraception was reversible after a few consecutive annual treatments (Kirkpatrick *et al.*, 1995a), but fewer data were available for reversibility after treatment for longer periods. The long-term treatment of mares was liberalized in 1998 to two surviving offspring or two generations, where offspring was defined as a foal that survived through its first winter. At the onset of management-level contraceptive treatment in March 1994, the population was 166.

Immunocontraception

Contraception was carried out with PZP vaccine, as previously described (Liu *et al.*, 1989; Kirkpatrick *et al.*, 1990a, 1991a, 1995a; Kirkpatrick, 1995). The vaccine consisted of 0.5 ml PZP, at a concentration of 65 μg protein per 0.5 ml phosphate-buffered saline (PBS), emulsified in 0.5 ml of either Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FIA). FCA was used for the initial inoculation only and FIA was used exclusively thereafter. The total 1.0 ml of PZP–adjuvant was administered via a barbless 1.0 cc dart with a 14 g, 3.8 cm needle, and was delivered by a Model 193 Pneu-Dart® capture gun (Pneu-Dart, Inc., Williamsport, PA). Each mare was located on foot or by vehicle and darted at a range of 20–50 m to inject the antigen i.m. in the hip or gluteal muscles. Darts were recovered and inspected to make sure they had injected all their contents. All mares on ASIS are identified by physical markings and colour patterns, and the locations and band affiliations of each animal are monitored monthly by ASIS personnel. Initial treatments were carried out in March of each year, or in August or September in the case of mares that had already been treated for at least 2 years.

In March 1994, there were 82 mares aged ≥ 2 years. Of these mares, 41 had never been treated during the previous 6 years of contraceptive research; 33 had been treated previously in the course of the research; and eight mature mares had been treated previously but were not to be treated again until after they had foaled. The 41 untreated mares were given a single PZP–FCA inoculation, the aim of which was not to achieve contraception, but rather to establish antigen recognition and thereby make them 'single-inoculation' animals in preparation for subsequent management-level application of the vaccine. One year later, in March 1995, all mares designated for treatment under the management plan were treated with the PZP vaccine as described above. Two-year-old mares received only a single dose treatment in 1995, but in 1996, the 2-year-olds received an initial PZP–FCA inoculation followed by a second PZP–FIA inoculation approximately 2 weeks later. All other mares included in the management plan received a single booster inoculation of PZP–FIA.

Reproductive success was determined as the birth of a foal in the year after treatment, or a positive pregnancy test during the autumn months after treatment, by analysing concentrations of hormone metabolites in urine and faecal samples (Kirkpatrick *et al.*, 1990b, 1991b; Lasley and Kirkpatrick, 1991). Contraceptive effectiveness was measured by the lack of reproductive success during the 12 months after each mare was treated.

Body condition scores

The body condition score of each mare was assessed on a scale of 0 to 5 according to the shape of the rump and, therefore, the amount of fat deposited in the rump area: 0 indicated an animal in very poor condition and 5 represented extreme fat deposits (Rudman and Keiper, 1991). These scores were assessed between July and November in 1989 and again in 1999 by the same method, and the findings were compared.

Results

Overall contraceptive effectiveness during 1988–1999 for the animals given either the standard double-inoculation protocol, then given a single booster inoculation, and the 41 mares given a single-inoculation initial treatment only in 1994, was 88.7% (43 foals produced after 381 treatments). The effectiveness of contraception was 90% (34 foals produced after 340 treatments) when the 41 single-inoculation mares were excluded. The contraceptive effectiveness among the 41 mares treated with single-inoculation treatment in 1994 was 78% (nine foals produced after 41 treatments).

From 1994 to March 1999, the original population of 166 horses remained stable, ranging between 172 and 164 (Fig. 1). On the basis of historic population increases of 5% annually (Keiper and Houpt, 1984), the number of horses in this herd would have increased to 240–260 by March 2000 had immunocontraception not been used.

The percentage of the total number of mares of all ages that required treatment under the ASIS management plan decreased from 95% to 52% in 1999. This decrease was brought about by cessation of treatment of mares after they had finished their third consecutive treatment, as dictated by the management plan, and by not inoculating other animals that had already been treated for several consecutive years.

The mortality rate in the total population of mares and foals decreased between 1990 and 1999 despite an overall increase in population during that period (Fig. 2). During the 4 years preceding the introduction of management-level contraception, the mortality rate for all adult horses was > 10% and was approximately 3% for foals. During the 4 years after the start of the management-level contraception, overall adult mortality decreased to < 4%; mare mortality dropped to < 4% and foal deaths were only about 1%. The unusually high mortality rates in

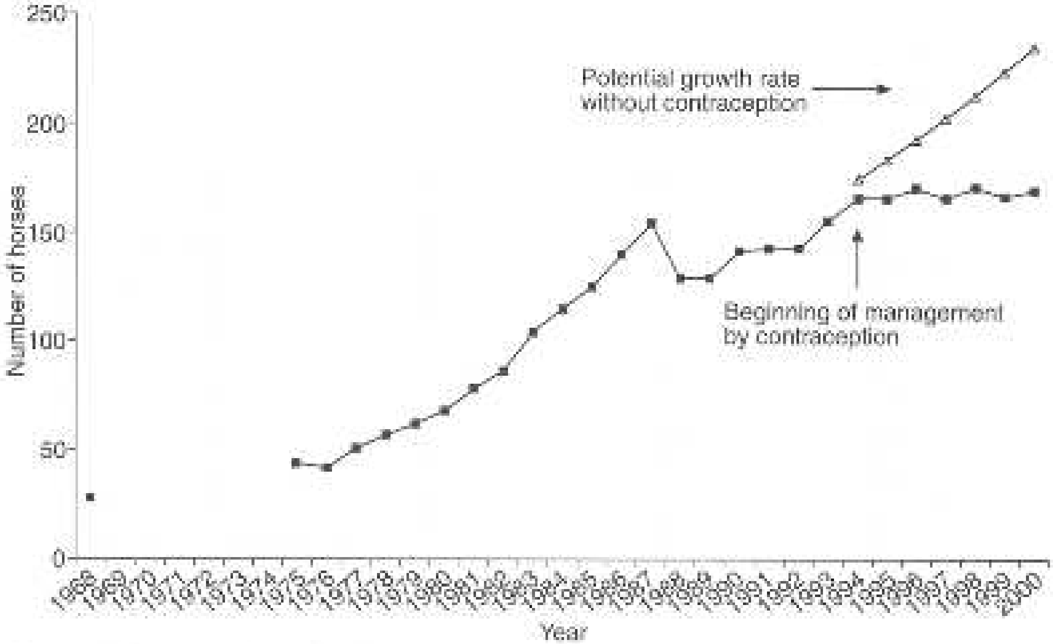

Fig. 1. Population growth curve for the Assatreague wild horses. The sharp decrease between 1988 and 1990 was caused by an outbreak of Eastern equine encephalitis. The absence of growth between 1990 and 1993 was the result of additional mares being treated during the research phase of the study and storm-related deaths. The projected increase in population without contraception, beginning in 1994, was based on historic foaling and mortality data.

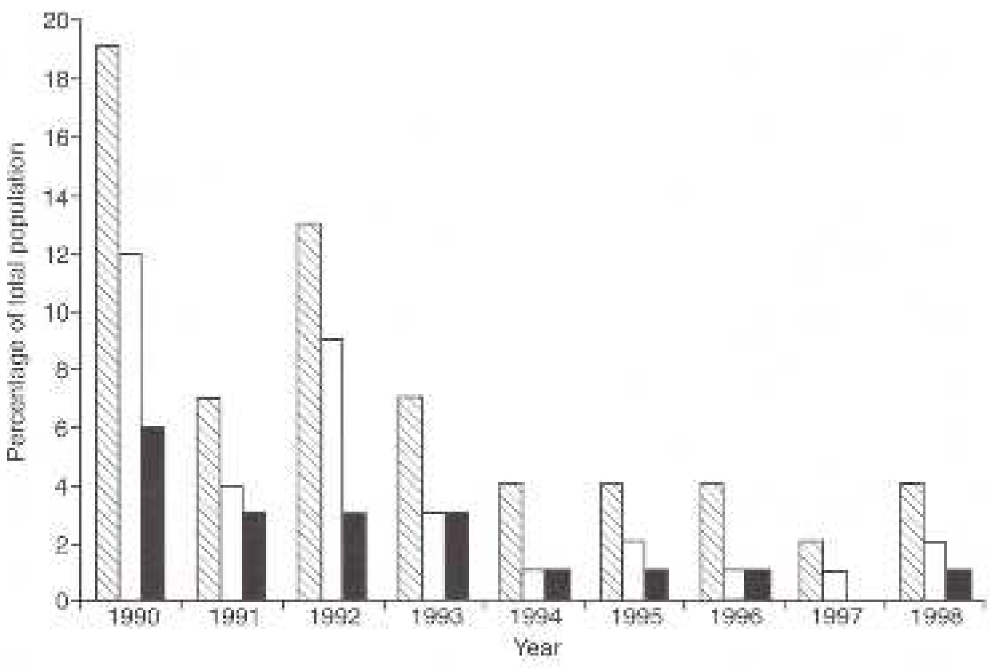

Fig. 2. Mortality rates among Assateague wild horses. The unusually high rates in 1990 and 1992 were the result of an Eastern equine encephalitis outbreak and a large number of storm-related deaths, respectively. ▨: Total population; □: mares only; ■: foals only.

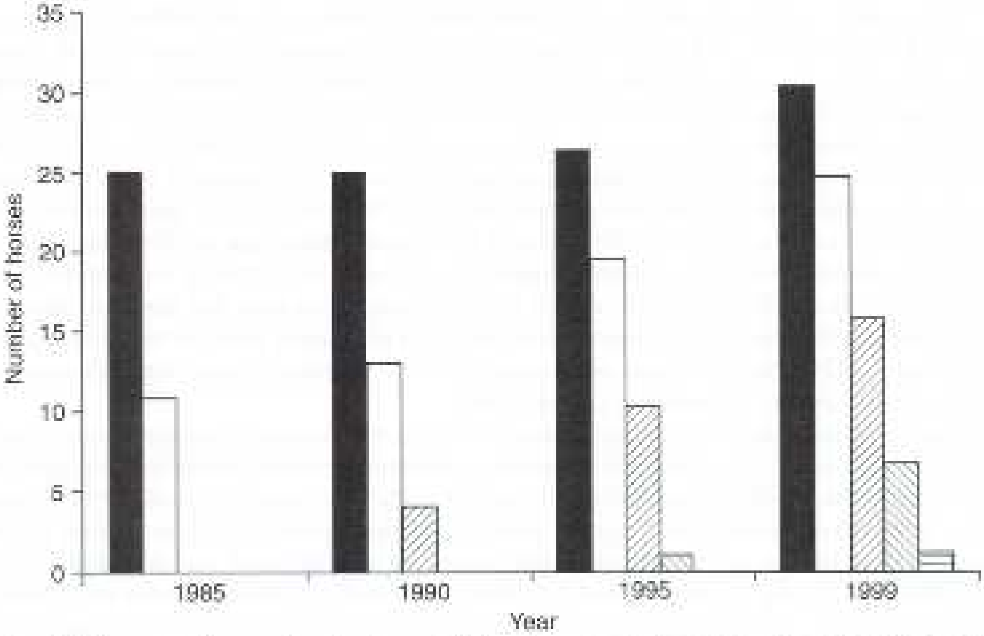

Fig. 3. Changes in the age class structure of the Assateague wild horse herd. The marked change in population trends between 1986 and 1990 was the collective result of horse removals, an outbreak of equine encephalitis, contraception of 26–33 mares for research purposes and storm-related deaths. ■: Age 6–10 years; □: age 11–15 years; ▨: age 16–20 years; ▧: age 21–25 years; ▤: age > 25 years.

1990 and 1992 were the result of an outbreak of Eastern equine encephalitis on the island in 1990 and severe storms in 1992. The 1991 and 1993 mortality rates were normal and representative of the 8-year period before the start of immunocontraception (Keiper and Houpt, 1984).

Changes in the age classes of the entire herd shifted upwards, indicating increased survival (Fig. 3). These changes were not just an expected increase in the age profiles of the treated herd, but rather animals were reaching new and older age classes, indicating increased longevity. The change was greater among mares than stallions, with the former accounting for 83% of the animals reaching the 16–25 year group in 1995, and 67.7% of the animals reaching the same age group in March 1999.

Body condition scores were significantly higher in 1999 than 1988. For example, the mean ± SEM scores for all horses in 1999 and 1988 were 3.04 ± 0.06 and 2.47 ± 0.43, respectively ($t = 2.85$, 46 df, $P = 0.0064$).

For all adult mares, the mean body condition scores were 2.96 ± 0.08 and 2.33 ± 0.35, respectively ($t = 4.01$, 31 df, $P = 0.0004$) for these 2 years, and for non-lactating mares the scores were 3.07 ± 0.05 and 2.42 ± 0.38, respectively ($t = 5.71$, 18 df, $P = 0.0001$). However, the body condition scores for lactating females did not differ significantly between 1999 and 1988 (2.0 ± 0.55 and 1.96 ± 0.60, respectively; $t = 1.0$, 4 df, $P = 0.373$).

Discussion

Contraceptive efficacy after management-level treatment with PZP was about the same, approximately 90%, as in earlier research trials with the ASIS horses (Kirkpatrick *et al.*, 1990a, 1991a, 1995a) and captive and wild cervids (Turner *et al.*, 1992, 1996; McShea *et al.*, 1997).

The high degree of efficacy of the single-inoculation treatment given to the 41 previously untreated mares in 1994 (78%) was unexpected, and was probably a result of three factors. Firstly, the initial inoculations were given in March, just before the breeding season. Keiper and Houpt (1984) showed that 73% of ASIS foals are born between April and June, so that the single inoculation of the PZP vaccine in March would only need to stimulate contraceptive antibody titres for a period of 3 months to achieve significant inhibition of fertility. A second factor is that wild horses, unlike white-tailed deer, do not appear to have an extended breeding season after contraception. McShea *et al.* (1997) demonstrated that the breeding season of PZP-treated white-tailed deer can be extended by as much as 2 months. In contrast, it was demonstrated that wild horses have a very discrete breeding season that does not appear to lengthen even when nutritional planes are increased or pregnancy does not result (Kirkpatrick and Turner, 1983). Thus, the onset and cessation of the breeding season for ASIS mares are rigidly set and were not extended by contraception.

A third possible reason for the success of the single-inoculation treatment was the use of FCA as the adjuvant. This particular adjuvant is considered widely to be the best available with regard to the ability to stimulate significant antibody titres (Leenaars *et al.*, 1994). The use of FCA is contra-indicated in some other animal species because it may cause false positive tuberculosis tests, but as no definitive test exists for equids, FCA has no health testing implications in horses. Some concern has been expressed over possible abscess formation resulting from the use of FCA. However, after 381 treatments, only two abscesses were noted among the treated ASIS animals, one of which occurred after treatment with FIA rather than FCA. In both cases, the abscesses were < 2.5 cm in diameter and they drained and reduced within a week of formation. We attribute the low rate of abscess formation to the exclusive inoculation into hip and gluteal muscles rather than the neck muscles. In other species, the adjuvant of choice is modified Freund's Adjuvant (Calbiochem, La Jolla, CA), which no longer results in false tuberculin tests and which appears to impart contraceptive effectiveness when given with PZP (J. F. Kirkpatrick, unpublished).

Both the 90% effectiveness of the PZP contraception over 5 years and the unexpected high efficacy of the single-inoculation PZP–FCA treatment regimen in 1994 are important observations when considering the development of population models for captive or wild ungulate fertility control. Estimates of contraceptive efficacy in the past have been theoretical (Garrott *et al.*, 1992; Hone, 1992; Garrott, 1995; McCullough, 1996; Muller *et al.*, 1997), but these data provide a sound factual basis for using a 90% effectiveness figure. In addition, herds of wild horses will exist where the administration of two inoculations in the first year is not easy or even possible, whereas a single inoculation may be feasible. The contraceptive efficacy achieved by the single inoculation, if given in March, provides additional data for population models.

The effect of contraception on the total ASIS population has been marked, with achievement of zero-population growth within the first year of management, and continued over 5 years. Even within an extremely conservative management plan that permits a certain level of successful reproduction for genetic reasons, it was possible to stabilize growth of the herd and thereby meet the first management objective.

The failure to reduce the size of the population after 5 years of management-level treatment has three obvious causes. The first is the fairly conservative treatment schedule, which permits a low level of successful reproduction to ensure genetic integrity of the herd. It ensures that all mares have an equal opportunity to make a genetic contribution to the herd by permitting some reproduction. A second cause is the increased longevity occurring among the treated mares. It is to be expected that the mean age of a population undergoing management by contraception will increase and that the percentage of animals in older age classes will increase. However, the creation of entirely new age classes within the population was unexpected. The cessation of foaling and lactation as a result of contraception, particularly among older mares, which were already losing body mass as a result of the ageing process, was associated with the attainment of new and older age classes, which, in turn, will delay mortality. This increase in longevity has important implications for the use of immunocontraception in wild and captive ungulates. Basically, contraception can still be used to decrease the size of a herd, but the time needed to achieve a significant decrease will be longer than originally thought, particularly when there is concern about the genetic integrity of the herd. In the case of the ASIS herd, significant mortality due to ageing has been delayed and significant decreases in population size will not occur until a new 'age of mortality' is reached. On the basis of available data, this new mortality peak will probably occur between 20 and 25 years of age, rather than at 15–20 years.

The third reason for failing to reduce the size of the population rapidly relates to mortality rates. Normally, as population size increases in most mammalian species, mortality, and particularly mortality of the young, also increases (Christian, 1971; Krebs, 1978). The opposite scenario has occurred on ASIS. During the years of management-level contraception (1994–1999), the population remained static, but between 1988 and 1994 it increased from 126 to 166. Thus, mortality rates should have increased correspondingly or at least remained the same. The tendency for reduced mortality was primarily among mares, indicating that the reduction in physiological stress from not undergoing pregnancy or lactation underlies the higher survival rates in this group. The reduction in foal mortality is harder to explain, but may be related to better body condition of the mothers. Assateague Island is a harsh environment and an earlier study of body condition of all ASIS horses (Rudman and Keiper, 1991) showed that adult females and non-lactating adult females were in fair to good condition, whereas lactating females were classed as thin. In 1999, by comparison, scores had improved to fat for all horses, including adult females and non-lactating females. Clearly, something had improved body condition in the ASIS horses, despite an increase in the number of animals. This finding indicates that continued contraception had a positive effect on body condition scores and, by implication, the health of the horses.

The effort necessary to achieve the desired degree of change in the population within the context of this management plan is clearly intense during the first 3 years, but after this time the effort necessary to maintain the effect decreases as a result of the long interval between the last of the three consecutive treatments in younger mares and the time at which they produce their first post-treatment foal. Apart from a reduction in the number of animals to be treated, the timing of the treatments (March versus August) increases efficiency. In March, there are few humans on ASIS and even fewer on the marshes of the island, where the horses spend most of their time. At this time of year the horses are extremely wary of humans, so successful treatment requires hours of effort for each horse. It appears that after 2 consecutive years of treatment, the third treatment can be carried out in August or September, or probably at any time of the year, and the contraceptive effect will extend throughout the following breeding season. In August and September the horses spend much of their time on the beaches to avoid insects and even in the camping areas in close association with humans. Thus, treatment requires much less effort than in March.

The reduction in effort may be temporary. The management plan was liberalized to permit the long-term treatment of mares with only two surviving offspring to avoid resurgent breeding. However, even with that added burden, the new and older mortality age of an increasing number of mares is approaching and the death of these animals should reduce the annual effort so as to balance the increase from the need to treat a greater number of younger mares.

Conclusion

This study of 5 years of treatment under a management plan provides the first population-level data for contraception in wild horses, and may be useful in developing new models. Previous models have not accounted for changes in age class structure, mortality or the degree of effort required to achieve particular goals. Models can now be refined and improved on the basis of these data.

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