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The practical side of immunocontraception: zona proteins and wildlife

J.F. Kirkpatrick[a,*], A. Rowan[b], N. Lamberski[c], R. Wallace[d], K. Frank[a], R. Lyda[a]

[a] The Science and Conservation Center, ZooMontana, Billings, MT 59106, USA
[b] The Humane Society of the United States, Gaithersburg, MD, USA
[c] San Diego Wild Animal Park, Escondido, CA, USA
[d] Milwaukee County Zoo, Milwaukee, WI, USA

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ABSTRACT

With shrinking habitat, the humane control of certain wildlife populations is relevant. The contraceptive vaccine based on native porcine zona pellucida (PZP) has been applied to various wildlife populations for 20 years. Prominent efforts include wild horses, urban deer, zoo animals and African elephants, among others. This approach has been successful in managing entire populations and to date, no significant debilitating short- or long-term health effects have been documented.

1. Introduction

About 37 years back, it was demonstrated for the first time that antibodies against zona pellucida (ZP) may be possible for inhibition of fertility (Ownby and Shivers, 1972). Since then, several groups have investigated the potential of ZP-based immunocontraceptive vaccines in a variety of animal models (Kirkpatrick et al., 1996; Gupta et al., 2004). Porcine zona pellucida (PZP) became the antigen of choice due to easy accessibility of porcine ovaries from abattoir and observations that antibodies against PZP react with zona from various species and inhibit *in vitro* human fertilization (Sacco, 1977). Indeed, active immunization studies with purified PZP, employing various animal models have established the efficacy of such an approach to control fertility (Skinner et al., 1984; Mahi-Brown et al., 1985; Sacco et al., 1986). However, interest and investment in zona-related research for human fertility control declined due to the observation in some animal models that infertility is invariably associated with ovarian dysfunction (Skinner et al., 1984; Mahi-Brown et al., 1985; Dunbar et al., 1989; Jones et al., 1992). These studies suggest that PZP-based immunocontraceptive vaccines, at least in some species, may curtail fertility either by interfering in the normal physiology of ovaries or by blocking fertility. This coupled with the variability in contraceptive efficacy and time taken for the recovery of fertility after immunization led to abandonment of PZP-induced human contraception.

Significant population problems, however, exist in species other than humans. Certain wildlife populations may not for societal and other reasons, lend themselves to lethal management approaches, and companion animals pose a serious dilemma in many countries. Captive animal populations in zoological gardens must be regulated because of limited space. Thus in 1989 PZP was tested in a new role—namely, control of wildlife fertility. Happily this use has proven effective, safe and practical. The first species to be targeted was the horse, where PZP was shown to be effective in inhibiting fertility (Liu et al., 1989). PZP was then shown to be effective in free-ranging wild horses and burros (Kirkpatrick et al., 1990; Turner et al., 1996), captive and free-ranging white-tailed deer (Turner et al., 1992; McShea et al., 1997), captive exotic species in zoos

* Corresponding author. Tel.: +1 406 652 9718; fax: +1 406 652 9733.
E-mail address: jkirkpatrick@montana.net (J.F. Kirkpatrick).

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Table 1
Species in which PZP contraception has been successful.

Ungulata	
Perissodactyla	
Horse (*Equus caballus*)	Plains Zebra (*E. burchelli*)
Donkey (*E. asinus*)	Mountain Zebra (*E. zebra*)
Przewalski's Horse (*E. przewalskii*)	Black Rhinoceros (*Diceros bicornus*)
Grevy's Zebra (*E. grevyi*)	Tapir (*Tapirus indicus*)
Artiodactyla	
Addax (*Addax nasomaculatus*)	Gerenuk (*Litocranius walleri*)
Antelope, Roan (*Hippotragus equinus*)	Giraffe (*Giraffa camelopardalis*)
Antelope, Sable (*Hippotragus niger*)	Goat, Mountain (*Oreamnos americanus*)
Banteng, Javan (*Bos javanicus*)	Goat, Domestic (*Capra capra*)
Bison (Bison bison)	Hippopotamus (*Hippopotamus amphibious*)
Blackbuck (*Antelope cervicapra*)	Ibex (*Capra ibex*)
Bongo (*Tauurotragus euryceros*)	Impala (*Aepyceros melampus*)
Camel, Dromedary (*Camelus dromedarius*)	Kudu (*Tauurotragus strepiceros*)
Camel, Bactrian (*Camelus bactrianus*)	Llama (*Lama glama*)
Caribou (*Rangifer tarandus*)	Markor (*Capra falconeri*)
Chamois (*Rupicaapra rupicapra*)	Moose (*Alces alces*)
Cow, Domestic (*Bos primigenius* [Taurus])	Musk Ox (*Ovibus moschatus*)
Deer, White-tailed (*Odocoileus virginianus*)	Nyala (*Tauurotragus angasi*)
Deer, Mule (*Odocoileus hemionus*)	Oryx, Sciimitar (*Oryx dammah*)
Deer, Axis (*Cervus axis*)	Pronghorn, N. A. (*Antilocapra americana*)
Deer, Sika (*Cervus nippon manchuriensis*)	Pudu, Southern (*Pudu pudu*)
Deer, Sika (*Cervis nippon taioanus*)	Serow, Mainland (*Capricornus sumatrensis*)
Deer, Fallow (*Cervus dama*)	Sheep, Big Horn (*Ovis canadensis*)
Deer, Eld's (*Cervus eldi*)	Sheep, Dall (*Ovis dalli*)
Deer, Pere David's (*Elaphurus davidiensis*)	Sheep, Mouflon (*Ovis musimon*)
Deer, Muntjac (*Muntiacus reevesi*)	Tahr, Himalayan (*Hemitragus jemlahicus*)
Deer, Sambar (*Cervus unicolor*)	Wapiti, North American (*Cervus elaphus*)
Dik Dik, Kirk's (*Madoqua kirkii*)	Wapiti, Roosevelt (*Cervus e. roosevelti*)
Duiker, Red Flanked (*Cephalophus rufilatus*)	Waterbuck, Defassa (*Kobus ellipsiprymnus*)
Duiker, Yellow-backed (*C. sylvicultor*)	Water Buffalo, Asian (*Bubalis arnee*)
Edmi (*Gazella cuvieri*)	Wisent, Lowland (*Bison bonasus*)
Eland (*Taurotragus oryx*)	Yak (*Bos mutus*)
Gazelle, Thomson's (*Gazella thomsoni*)	
Elephantidae and Carnivora	
Elephantidae	
African elephant (*Loxodonta africana*)	
Carnivora	
Bear, American Black (*Ursus americanus*)	Bear, Asian Black (*Selenarctos thibetanus*)
Bear, Sun (*Helarctos malayanus*)	Sea Lion, California (*Zalophus Californian*)
Bear, Brown (*Ursus arctos*)	

(Kirkpatrick et al., 1995), free-ranging wapiti (Garrott et al., 1998), and even free-ranging African elephants (Fayrer-Hosken et al., 2000). PZP has successfully inhibited fertility in 80 species of mammalian wildlife, either free-ranging or captive (Table 1). The wide range of species suggests a strong evolutionary conservation of the sperm receptor, at least within family Ungulata. However, there is considerable difference in each species' ability to generate antibody titers and the duration of the antibody response (Frank et al., 2005).

Demonstrating that a contraceptive is effective on a physiological level does not however, ensure that it has practical value in the realm of population control. The remainder of this paper will examine actual progress in population control, safety issues and differences in response by various species. Unless otherwise noted, the PZP vaccine referenced is native PZP as prepared by the general methodology described previously (Dunbar et al., 1980) at doses of 65–100 μg, and administered exclusively in the hip or gluteal muscles.

2. Population management

At least four examples of effective population management with PZP immunocontraception are available. A population of wild horses (*Equus caballus*) on Assateague Island National Seashore, MD, has been managed since 1994 with PZP. Initially all mares were given a 100 μg primer dose of PZP followed-up by a booster dose of 100 μg a year later. All vaccine treatments were delivered remotely, via small 1.0 cc darts. After 3 consecutive years of treatment, animals were withdrawn from contraception until they produced a single healthy foal and then placed back on treatment until extinction. There were 175 horses in the population. After 14 years of immunocontraception, the population has been reduced to 125, with a goal of 120 animals (Fig. 1; Kirkpatrick and Turner, 2008).

In the case of urban white-tailed deer (*Odocoileus virginianus*) inhabiting Fire Island National Seashore, NY, the fawn production and total population has declined significantly over the period of time that immunocontraception

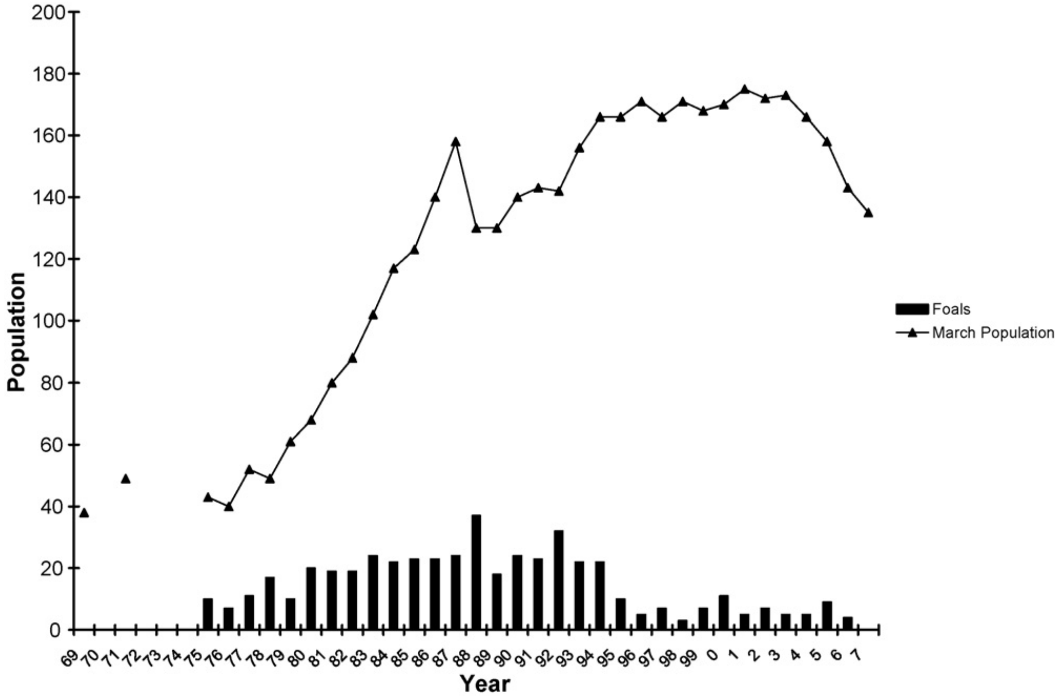

Fig. 1. Population changes of the Assateague Island National Seashore, Maryland, USA wild horse herd over a 38-year period. Contraceptive management began in 1994, as described in the text.
Adapted from Kirkpatrick and Turner (2008).

was applied. The protocol for treatment was the same as with wild horses. The decline was more precipitous in these deer than in horses, primarily because of the shorter life span of deer (Naugle et al., 2002; Rutberg and Naugle, 2008). A second urban deer project, at the National Institute of Standards and Technology, in Gaithersburg, MD, saw similar declines in fawn production and the total population, over a 12-year period. In these studies several different adjuvants were tested, with varying effectiveness (Rutberg and Naugle, 2008). In African elephants (*Loxodonta africana*), the prospect of actually reducing populations is unlikely, but stabilization of populations has been accomplished (Delsink et al., 2007). Similar outcomes have been observed in two captive fallow deer (*Cervus dama*) populations (Deigert et al., 1993). Among a free-ranging population of North American wapiti (*Cervus elaphus nannodes*), herd growth rate was reduced significantly (Shideler et al., 2002).

There has been much speculation about the necessary efficacy of an immunocontraceptive, if it is to be considered useful for population management. In the case of African elephants, an efficacy of 100% was achieved over 7 years (Delsink et al., 2007). Contraceptive efficacy was consistently in the 94–95% range among wild horses (Kirkpatrick and Turner, 2008) over a 15-year period, and somewhere in the 85–90% range in white-tailed deer for approximately the same period of time (Rutberg and Naugle, 2008).

Far more important than efficacy, at least within reasonable limits, is the percent of the adult female population that can be treated. It is risky to generalize about how large a proportion of the target population must be treated to achieve particular population goals, largely because each discrete population has its own age profile, sex ratio, mortality rate, reproductive rate and growth rate characteristics. Thus, determination of what proportion of the population must be treated is site-specific. Nevertheless, there are sufficient data to provide broad guidelines. In the case of wild horses, where contraceptive efficacy was about 95%, it was necessary to treat between 45 and 84% of the adult female population annually in order to achieve a population reduction (Kirkpatrick and Turner, 2008). The salient point is that immunocontraceptives with less than 100% efficacy can in fact be used to achieve significant population effects.

3. Safety

3.1. Treatment during pregnancy

The safety of any contraceptive is an important issue for public acceptance. After 35 years, there is ample safety data for PZP-based immunocontraceptive vaccines. In the initial trials with wild horses, 60% of treated animals were pregnant at the time of PZP treatment, as determined by urinary estrone conjugate concentrations (Kirkpatrick et al., 1988). The treated pregnant mares produced healthy foals whose survival rates were not different from untreated populations (Kirkpatrick et al., 1990). Subsequent studies confirmed the safety of PZP for pregnant mares (Kirkpatrick and Turner, 2002, 2003). PZP treatment did not change seasonal birth patterns, foal survival or subsequent fertility of foals exposed to PZP during gestation. The safety of immunizing against PZP (400–600 μg doses) in pregnant African elephants has also been studied, with no untoward effects noted among calves (Fayrer-Hosken et al., 2000; Delsink et al., 2007). Among white-tailed deer, treatment with PZP did not affect ongoing pregnancies or the health or survival of offspring (Thiele, 1999).

3.2. Reversibility of contraceptive effects

Reversibility of contraceptive action is also an important consideration. Subsequent to immunization with PZP-based vaccine, return to fertility has been documented in wild horses (Kirkpatrick et al., 1991; Kirkpatrick and Turner, 2002). After 3 consecutive years of PZP treatment, mares required a mean of 3.7 years to return to fertility, with a range of 1–8 years. Reversibility has also been documented among horses treated for 4–5 consecutive years, but not after 7 consecutive years (Kirkpatrick and Turner, 2002). However, animals treated with PZP for 7 consecutive years remained in good condition for 14 years following their last inoculations and continue to cycle and ovulate. Reversibility of PZP contraceptive effects has also been documented after 1–3 years of treatment in white-tailed deer (Turner et al., 1996), and in elephant cows (Fayrer-Hosken et al., 2000; A. Delsink, Unpublished data).

It is difficult to generalize regarding reversibility of contraceptive action mediated by PZP immunization as there are clear species differences. Anti-PZP antibody titers are closely correlated with infertility and for each species there is probably a threshold titer level above which animals fail to conceive. This threshold is 60% of serum standard references for horses (the serum standard reference consists of pooled serum from horses with high anti-PZP titers and which never conceived) (Liu et al., 1989), and 50–60% in deer (Walter et al., 2002) but is unknown for other species. A single annual booster suffices for sika deer (*Cervus nippon*), 5 species of bear (*Ursidae*), sea lions (*Zalophus californian*), Mountain goat (*Oreamnos americanus*), fallow deer (*C. dama*), and bighorn sheep (*Ovis canadensis*). However, muntjac deer (*Muntiacus reevesi*) require a booster every 6 months, while zebra and at least 17 other species, representing *Perissodactyla*, *Artiodactyla*, and *Cervidae* require booster inoculations every 7–9 months for effective contraception (Frank et al., 2005).

At the other end of the spectrum, there is mounting evidence that members of *Ovidae* and *Capridae* maintain contraceptive antibodies for longer durations than other taxon groups, and a single year's treatment (primer plus booster) have provided 3–4 years of contraception (Frank et al., 2005). Retrospective studies at the San Diego Wild Animal Park indicate that antibody titers remain high in Himalayan tahr (*Hemitragus jemlahicus*) and Mouflon sheep (*Ovis musimon*). The prospective studies at the Milwaukee County Zoo with Dall sheep (*Ovis dalli*), and domestic goats (*Capra hircus*) indicate the same prolonged antibody titers (R. Wallace, Unpublished) 2 years after a single year's treatment.

3.3. Long-term effects

Debilitating long-term effects have not been noted in any species of wildlife treated with PZP. In the case of wild horses, long-term effects (15–20 years) of treatment include a significant improvement in body condition (Turner and Kirkpatrick, 2002), significantly increased longevity (Turner and Kirkpatrick, 2002; Kirkpatrick and Turner, 2007, 2008), and decreased mortality (Turner and Kirkpatrick, 2002). These positive long-term effects are probably not a direct physiological effect of treatment, but rather are likely to be a secondary effect of the decrease in physiological costs of pregnancy and lactation.

In white-tailed deer there is an extension of the breeding season by 2 or 3 months (McShea et al., 1997; Thiele, 1999), which may cause greater energy expenditure. However by mid-summer, non-pregnant treated females weighed significantly more than untreated pregnant animals, but these weight differences disappeared by the subsequent fall (Walter et al., 2003). In one study a pulse of late births corresponding to the extended breeding season occurred in August, but this pulse of late births disappeared in subsequent years. Other studies in deer under different conditions did not find substantive differences between treated and untreated animals (Miller et al., 2001; Curtis et al., 2007).

3.4. Injection site reactions

Injection site reactions, including abscesses and granulomas, are a concern with any vaccine. Among 381 PZP treatments in wild horses over 19 years, only three abscesses appeared (0.7%). Sterile granulomas of about 10 mm were common at the injection site. In a separate study, 175 remote treatments led to a single abscess among mares (J. Kirkpatrick, Unpublished data). After 1185 treatments in 25 species of captive animals in zoos, 16 abscesses were noted (1.3%). Twelve of these abscesses followed treatment with Freund's Complete adjuvant, three with Freund's incomplete adjuvant, and one with Freund's Modified adjuvant (Lyda et al., 2005). These abscesses appeared in 1–3 days, were about 25 mm in diameter and drained within 2 weeks without complications.

Among free-ranging white-tailed deer, only 2 of 353 deer (0.5%) developed abscesses (Naugle et al., 2002). These abscesses were similar in size to those described in horses and disappeared within 5–14 days, without complications. In another study, 14 treated deer all showed granulomas at the injections site but no data were given for abscesses (Curtis et al., 2007). Delivery of PZP vaccine by remote darting may force hair follicles and surface dirt into the puncture wound, and there is no way to determine if the few abscesses that did occur were the result of vaccine/adjuvant or other causes.

3.5. Ovarian histology

A review of the literature indicates that there are significant differences among species regarding the effect of anti-PZP antibodies upon ovarian histology. The early work of Sacco et al. (1986, 1987, 1991) and others (Bagavant et al., 1994) revealed no persistent ovarian damage from PZP inoculations despite temporal inflammation in non-human primates. However, significant ovarian disruption has been noted in dogs (Mahi-Brown et al., 1985), rabbits (Wood et al., 1981), mice (Lou and Tung, 1993) and domestic sheep (Stoops et al., 2006). Studies with horses (Liu et al., 1989) and white-tailed deer (McShea et al., 1997) revealed no changes in ovarian weights after immunization with PZP. A study with white-tailed deer by Curtis et al. (2007) revealed no changes in ovarian weights, nor changes in Graafian follicles, but eosinophilic oophoritis occurred in 6 of the 8 deer studied, and there was a significant decrease in secondary follicles. It was noted however, that the eosinophilic oophoritis was also associated with normal ovulation in non-treated animals. Stoops et al. (2006) reported significant ovarian disruption in domestic sheep, with atrophic changes including the absence of growing follicles, reduction in primordial follicles, and the presence of abnormal granulosa cells. It was not known whether these changes were transitory or permanent. This phenomenon in sheep may possibly be associated with the high and prolonged antibody titers seen in the *Ovidae* and *Capridae* species.

The difficulty in evaluating such differences is compounded by the fact that there are obvious species-specific responses to PZP, consistency in the purity of PZP vaccine and various doses used in these studies. Additionally, there is evidence that oophoritis, at least in mice, is a transient phenomenon (Lou et al., 1995) associated with immunization. While ovarian damage may be transient or permanent, it is a consideration that must be addressed for each species. In some cases (white-tailed deer for example) permanent infertility is generally viewed as desirable, but this would not be the case for valuable exotic species, or wild horses and elephants, where reversibility is preferred.

3.6. Blood chemistry

Few studies with wildlife and native PZP have examined blood chemistry. Miller et al. (2001) and Curtis et al. (2007) have examined 22 and 28 blood parameters, respectively, in PZP-treated deer. Both studies revealed a few but significant differences between treated and untreated animals. However, none of these values suggested a physiological abnormality. Thirty-four parameters were examined in PZP-treated Dall sheep and domestic goats prior to inoculation, at the time of peak titers and at 1 year following treatment. There were few significant changes (R. Wallace, personal communication), and all were within the normal range for untreated control animals. Of particular importance in these studies were the unchanged values for liver enzymes (R. Wallace, personal communication).

4. Future perspectives

Although, with the exception of African elephants, contraceptive efficacy has been below that necessary for an acceptable human contraceptive, the degree of efficacy has been sufficient to manage entire populations of free-ranging animals successfully. Thus far, studies with a variety of species suggest that no significant short- or long-term debilitating side effects result from PZP treatment. After 20 years of application to wildlife, PZP appears to come reasonably close to meeting the characteristics of the ideal wildlife contraceptive. These include remote delivery, contraceptive reversibility, safety in pregnant animals, lack of behavioral effects, no passage through the food chain, no debilitating long-term health effects, relatively low cost, and at least 90% efficacy (Kirkpatrick and Turner, 1991).

Although this review attempts to summarize a large body of work with PZP and non-human species, caution should be exercised when examining the historical efficacy and safety of PZP in wildlife, because the purity of the immunogen, the size of the dose and the adjuvant used all have significant implications for efficacy. Similarly, doses used over past years for studies in non-human primates and wildlife have a wide range. Various investigators have used PZP doses as small as 65 μg to as high as 5000 μg per injection.

Perhaps the greatest difficulty in evaluating and comparing historical work with PZP comes with regard to purity of the immunogen. Until recently, there has been no standardized protocol for preparation or quality control testing of native PZP. Early preparations were prepared with commercial meat grinding equipment or laboratory homogenizers. In these cases, several soluble enzymes from ovarian cells are released into the preparation. The early work of Mahi-Brown et al. (1985), which later set the stage for interest in PZP as a dog contraceptive, probably used quite impure PZP and a good deal of the ovarian disruption reported in that study might well have been the result of antibodies against ovarian tissue enzymes. In one case, a preparation sold commercially and used in at least one wildlife study did not present a gel electrophoresis pattern even remotely similar to most other native PZP. The creation of the ganged razor blade device by Bonnie Dunbar (then at the Population Council, NY) led to an increase in purity of native PZP preparations. Rather than grind tissue, this instrument which is known to some as the “zonamatic”, slices the ovarian tissue at the precise spatial distances needed to release oocytes with a minimum of tissue damage to other cells. While no native PZP preparations can be considered 100% pure, two-dimensional chromatography has confirmed the relative purity of preparations made with the “zonamatic”. The same diversity exists with regard to quality control. Only some of the many PZP preparations used in wildlife have been subjected to a standardized qualitative and quantitative analysis and pathogenic bacterial and viral screens.

Different modes of delivery of the PZP vaccine can also result in different efficacies. There is some evidence in white-tailed deer that hand-injection, particularly with the primer doses, can produce greater efficacy than dart-delivered vaccine during the first year of treatment (Rutberg, 2005), but differences disappear after multiple years of treatment. This is probably the result of better efficiency in delivering the entire dose intramuscularly.

5. Conclusion

Despite the difficulties in comparing data from historical studies, it is now clear that native PZP has an important role to play in the management of wildlife populations. The recent standardization for native PZP preparation, quality control and adjuvant use has produced a data-base that adequately addresses species differences, ability to manage whole populations and safety issues with some confidence. Both the high degree of efficacy and the complete absence of long-term debilitating effects suggest that native PZP may come closer to meeting the characteristics of the ideal wildlife contraceptive (Kirkpatrick and Turner, 1991) than any other available contraceptive. The single largest shortcoming is the need to provide annual booster inoculations, but this may soon be overcome by a long-acting form of the PZP vaccine, that has already shown promise (Turner et al., 2008) and may soon be available for remote delivery. The second urgent scientific gap is the absence of an effective recombinant form of the vaccine, which would expand the number of animals that could be treated by virtue of a larger supply of antigen, now made almost impossible by the labor-intensive nature of PZP production.

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