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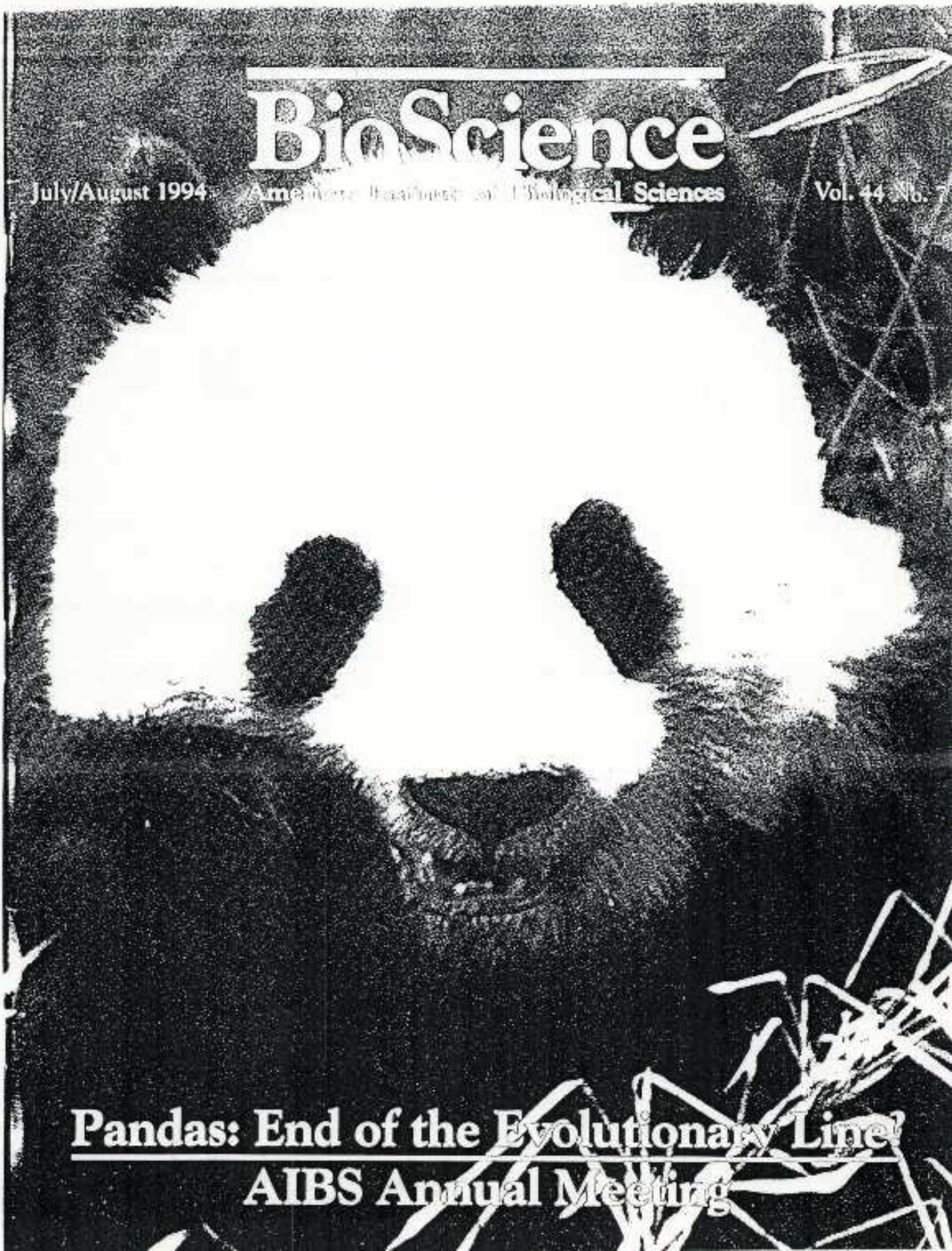

Pandas: End of the Evolutionary Line?

AIBS Annual Meeting

Fiddling with foaling

One team's quest for a contraceptive to manage wild horse populations humanely seems near realization

In 1972, John W. Turner and Jay F. Kirkpatrick, friends since their student days at Cornell, decided while on a backpacking trip in Montana that they wanted to develop contraception for wild horses. They agreed that controlling fertility might be the most humane way to handle a rapidly growing population that had been much abused, says Turner, now a reproductive physiologist at the Medical College of Ohio in Toledo.

Before 1971, wild horses on private and public lands were rounded up, shipped to meat-packers, and turned into dog food. "The industry was unregulated and cruel," says Kirkpatrick, a scientist at Deaconess Research Institute in Billings, Montana. Many horses died enroute to the meat-packers.

The Wild Free-Roaming Horse and Burro Act, one of a handful of bills that has passed unanimously in the House and Senate, put an end to such roundups and declared that the animals were to be protected into perpetuity as a symbol of the pioneering spirit of the West. The act, however, did not address ways to control the population—wild herds out west generally experience annual growth rates of 10% to 20%, and they cost millions to manage.

In 1972, Bureau of Land Management (BLM) officials asked Kirkpatrick whether he and Turner could find a way to stop the free-ranging, western horses from reproducing. "I said 'yes,'" says Kirkpatrick. The scientists' aim was an inexpensive, effective contraceptive that could be delivered in one shot with a minimal amount of animal contact. Ideally, it would give at least a breeding season's worth of protection. Two decades later—after a major switch in research focus from hormones to antibodies and a long side trip to Assateague Island, Maryland—the scientists now have a workable strategy.

The wildlife contraceptive research community carefully tracks developments by Kirkpatrick, Turner, and colleague Irwin K. M. Liu, a researcher in the School of Veterinary Medicine at the University of California-Davis, who joined them in 1988. The trio's work on the Assateague horses is the longest running contraceptive study on a free-roaming species. In Assateague, contraception is likely to become a regular management tool in 1995. The trio recently has begun giving their contraceptive to horses in Nevada, and they have gathered data relevant to other species, including deer and burros (see box page 446). Their work has the imprimatur of the National Park Service (NPS), the Bureau of Land Management, and the Humane Society of the United States (HSUS). "They are about five years ahead of everyone else in terms of field and safety data," says Allen T. Rutberg, an HSUS senior scientist for wildlife and habitat protection.

The path to a technique and an island

National Park Service wildlife managers started seriously evaluating ways to control the horse population in the Assateague Island National Seashore in the mid-1980s, says Carl Zimmerman, chief of resource management at the park. NPS owns and manages the horses on 8000 acres of the upper island, which is in Maryland. A separate population of horses living in the Chincoteague National Wildlife Refuge in the southern portion belongs to the Chincoteague (Virginia) Volunteer Fire Company. It is these that are rounded up each July and made to swim across the channel between Assateague and Chincoteague for Pony Penning Day, the famous annual foal auction.

Despite the general NPS policy that defines horses as exotics or alien species in parks, the Assateague horses are considered exceptions. Because of congressional interest in the animals when the park was established, they are managed as "a desirable feral species," says Zimmerman. The public values them for cultural and historical reasons: their ancestors most likely belonged to tax-evading settlers who pastured their animals on the island to avoid the English king's tax on fences.

But there is a limit to how many horses the island can support. "We don't want the animals starving, we don't want to feed them, and we don't want them damaging other natural resources in the park," says Zimmerman. The Park Service is particularly concerned about horses grazing in sensitive marsh habitats and how it might affect other animal communities, particularly small mammals and birds.

The Park Service in 1985 set the population ceiling at 150 horses. That decision was based on the results of a carrying capacity study conducted in the late 1970s by Ron Keiper and Stan Zervanos of Pennsylvania State University, whose results were modified to take into ac-

by Anna Maria Gillis

Assateague horses have adapted to the marshes and dunes. Photos: Anna Maria Gillis.

count herbivory by other large mammals, uneven distribution of the horses over the island, fluctuating vegetative production, and potential problems between horses and park visitors.

When Keiper did his study, there were 60 horses in the park, but by the time the 1985 horse-management plan was completed, the herd was fast approaching the Park Service's population ceiling. Clearly, some human intervention was necessary to check Mother Nature. Once they survive the first two years, when horse mortality is highest, Assateague horses generally live 15 to 20 years. Healthy mares can produce five or more offspring over a lifetime.

In 1986 when Turner and Kirkpatrick began their Assateague work, they focused on the use of steroids to control reproduction. "We spent 15 years working with steroids and met with some pharmacological success, but not logistical success," says Kirkpatrick.

Their first stallion study at Assateague, which stemmed from a study done on wild stallions in Idaho, posed delivery problems. In Idaho bands where the stallions had been injected with microencapsulated testosterone propionate, there had been an 83% reduction in foal production. On Assateague, the delivery protocol was changed to remote delivery of four shots via dartgun, which the stallions began avoiding. Disappointed, Turner and Kirkpatrick switched to the mares. They dosed mares with a progestin known to be a highly effective contraceptive in women. All six of the treated mares foaled the next year.

At the same time that Kirkpatrick and Turner were doing their studies, other researchers were meeting with varying success implanting progesterone and estradiol or the synthetic estrogen ethinylestradiol into wild mares. But to be successful, the natural steroids had to be given at levels too large to be handled easily; the synthetic hormone had efficacies ranging from 75–100%, but the scientists worried that the synthetics might cause cancer in horses as they did in cats.

Obstacles to easy delivery, including the need for field surgery for implants, and environmental concerns made Turner and Kirkpatrick reconsider the practicality of steroids. They thought that public and regulatory agencies were unlikely to consider a method acceptable if there was any chance that the substances might enter the food chain. Today the sentiment against steroids, at least for horses, continues growing in the broader community, says Liu. He notes that the potential environmental effects of steroids were a concern of many scientists attending the contraception in wildlife management meeting last October in Denver.

In a sharp change in research tack, Kirkpatrick and Turner decided in 1987 that the future of wildlife fertility control lay with immunocontraceptives. Having no experience in the area, they began their collaboration with Liu, who was interested in developing a horse contraceptive based on zona pellucida, the protein membrane that coats mammalian eggs.

Liu, who was studying horse reproduction, first considered the approach in 1979 when he heard a radio broadcast announcing that Alex Shivers of the University of Tennessee was working with rabbit zona pellucida to develop a human contraceptive. The premise of the vaccine is that the subject species, when injected with the zona pellucida of another species, mounts an immune response. The antibodies produced then coat the subjects' eggs, blocking fertilization by denying the sperm access to their receptor on the zona. The vaccine theoretically would be reversible because, as antibody levels drop over time, newly developing oocytes would escape antibody coating and could be fertilized. "I wondered if the same approach could be used on horses," says Liu.

In collaboration, Liu and Shivers found that a few infertile mares produced antibodies to their own zona pellucidae. Further studies by Liu and Marietta Bernoco showed that domestic and captive wild mares at the Wildhorse Sanctuary in Shingletown, California, would generate antibodies in response to pig zona pellucida (PZP). With four shots over six weeks, normal mares maintained pregnancy-preventing antibody titers for approximately nine months. Only 2 of 15 mares got pregnant in the first year. (Immunocontraception is still used on some mares in that sanctuary.)

Four domestic mares, which were scrutinized more thoroughly than the wild ones, continued to ovulate normally every three weeks during breeding season despite the PZP treatment. These mares then conceived in the second year after treatment. Ovaries removed from three of these animals at the end of the study were normal. In contrast, in earlier studies by other researchers, dogs and rabbits treated with PZP stopped ovulating and their ova disappeared. The damage is generally attributed to an autoimmune response, says Liu.

With Liu's findings fresh in their

minds and vaccine from his lab in hand, Turner and Kirkpatrick in February 1988 returned to Assateague. By April, the start of the breeding season, they had darted 26 horses with a vaccine prepared from an adjuvant and raw PZP. The protocol required three shots; however, eight of the mares received only two shots.

Kirkpatrick and Turner went back in the fall of 1988 to track treated mares by collecting urine and fecal samples from the sand and marsh and evaluating their chemical profiles. Half a gram of feces or 100 microliters of urine can reveal a pregnancy.

The researchers were the first to use in the field the technique that Bill Lasley of the University of California at Davis had developed to determine the reproductive status of zoo animals without drawing blood. Lasley found that the presence of certain estrogen metabolites in excretions indicated ovulation or pregnancy. Development of a monitoring method that does not require handling the animals has in some ways been more exciting than the actual contraception work, says Kirkpatrick. "We [and other field biologists] now have a way to evaluate reproductive status in free-roaming animals. We can see what the ovary is doing based on the urine."

Although initially it took two tries at running the assay to gather reliable data, the results indicated that the treated horses had been protected from pregnancy. Recalling that day, Kirkpatrick says: "The data tape came rolling off the scintillation counter around 4 p.m. on a Friday afternoon in November 1988, the building was empty, there was no one to tell that the experiment had worked. Besides, we couldn't say anything. We had to wait until August 1989 to count foals to prove conclusively we were right. Sixteen years of my life rolled by on that tape."

The researchers learned in the 1988 study that PZP did not harm pregnant mares and only a two-dart protocol was necessary. Fourteen of the mares had already been pregnant when they were treated, yet gave birth to healthy foals. Three of those foals have since produced young of their own. The remaining mares did not get pregnant, even those that had evaded the third dart.

In subsequent years, some of the previously vaccinated animals were darted with a one-shot booster in March. This treatment was more than 90% effective and had no affect on the herd's social behavior, a major concern to the researchers. Most females continued to go through estrus from April through July or August; some displayed classic mating behavior—tail lifting—and stallions responded. Pecking orders within harems remained the same, with lead mares still determining when the bands moved and grazed. Harem stallions, which normally breed with the mares and protect them from interloping stallions, continued to do so. The stallions still took a proprietary interest in their mares, rounding them up when they strayed.

Little is known about the effects of continuous PZP treatment on the mammalian ovary over several years, says Liu, who notes the longest an individual horse has been studied in the lab is two years. Although the Assateague horses received small amounts of vaccine relative to body size, they may experience some cumulative effect, he says.

In 1990, four females began displaying an estrogen metabolite profile that indicated PZP might be affecting their ovaries. Urine collected at the height of breeding season from the four mares, which had been treated for three years, revealed no evidence of ovulation, and urinary estrogen concentrations were significantly depressed.

The vaccine, however, does appear to be reversible. "We still see cycling estrogen patterns in mares treated for six years, which means the follicles are still functioning," says Kirkpatrick. One mare that was treated for five years and that had stopped ovulating ended treatment in 1992. She began ovulating again in 1993. Another mare, treated for four consecutive years until 1991, got pregnant in 1993 and was expected to foal this year. (Gestation is 340 days.) "It appears that following long-term treatment, it took two years for the antibody titer to drop enough for her to conceive," says Kirkpatrick.

To determine the long-term effects, five animals that were given the last of seven treatments this March will be monitored for at least five years to determine whether they foal again. The National Institutes of Health, which funds Kirkpatrick, is particularly interested in the ovarian functions of these mares. "The horse ovary is a good model for the human ovary. There are similarities between the follicular and luteal phases of the two," says Kirkpatrick. Such information will be important to companies interested in developing a zona pellucida vaccine for women.

Wrapping up in the field

By 21 March, Kirkpatrick and two Park Service employees, Allison Turner and Grace Bottitta, had wrapped up the 1994 darting season. "We darted 76 mares in 17 working days," a point Kirkpatrick made with no small amount of professional pride. "It takes skill, horse sense, persistence, and luck."

In nine years of tracking the Assateague horses, Kirkpatrick has developed an unabashed admiration for the hardiness of the stumpy animals that are erroneously called ponies by the public. Their stunted growth—they stand less than 56 inches at the withers—is a result of living in the harsh environment of a barrier island where fierce storms and hurricanes are common. The horses' diet consists largely of salt marsh cordgrass and American beachgrass, plants that are protein-

Population control for some other animal islanders

Hungry herbivores in national parks on Fire Island, New York, and on St. John, the Virgin Islands, may someday have their population growth curtailed thanks to contraception. The vaccine that prevents pregnancy in horses is also being tested on deer and burros in the field.

Fire Island, which is part of the National Park Service, contains several villages that have problems with a burgeoning deer population munching through the well-groomed landscape. "It's a perfect model for the suburban deer problem," says Allen Rutberg, a scientist with the Humane Society of the United States, which supports the project.

Despite requests from the Park Service not to feed the animals, the deer on Fire Island have become remarkably tame because of handouts. "One is so adapted to humans, it just about goes up to people and puts its nose in their pockets looking for food," says Jay F. Kirkpatrick of Deaconess Research Institute in Billings, Montana, who is working with the deer.

Community interest in finding a humane way to control the deer population made Fire Island an ideal place to try a contraception project, says Kirkpatrick, who works with John W. Turner of the Medical College of Ohio, and Irwin Liu of the University of California at Davis. The locals established a committee of deer monitors, who learned the markings, behaviors, and habits of the animals, making it possible for the researchers to dart particular individuals with the contraceptive and then find them again for follow-up shots and monitoring.

In September 1993, Turner and Kirkpatrick gave 68 deer two shots of the vaccine in the first field study of a remotely delivered immunocontraceptive vaccine for deer. This summer the researchers plan to count fawns to determine whether the vaccine was effective. Studies of fecal samples collected in March from approximately 30 of the treated deer detected only one pregnancy. Based on Turner's studies of captive deer in Ohio—the vaccine was 100% effective in blocking pregnancy for one year—the researchers expect the contraception to work on the Fire Island deer.

But contraception is, as always, a controversial issue. Hunting interests have argued that deer that are not healthy will mount a poor immune response to the vaccine and would, therefore, be more likely than healthy animals to get pregnant and produce poor quality young, says David Jessup, a researcher at the California Department of Fish and Game. He says, "It's a leap in logic that a deer in poor nutritional status might not respond to a vaccine. But it is a theoretical concern."

Hunters also fear that because treated does continue ovulating past the end of the normal breeding season, males might be affected by expending energy mating for longer periods, and treated females might not be as healthy. Hunting concerns that deer quality could be affected are unreasonable, says Kirkpatrick.

Findings of Bill McShea, a researcher at the National Zoo's Conservation and Research Center in Front Royal, Virginia, and a collaborator of Turner and Kirkpatrick, indicate that the well-being of the prime bucks is not adversely affected. The prime bucks gradually lose interest in the females as the winter progresses, allowing the young bucks a chance to mate. McShea has also found that does that received contraception appear healthier and weigh approximately 20 pounds more than control deer.

Jessup, who has had 100% success rates using steroidal implants to control deer fertility, says, until a one-shot vaccine is developed, immunocontraception remains impractical for deer. Once developed, Jessup says, the one-shot vaccine has potential for islands, enclosed parks, and suburban deer populations.

Herbivore contraception is also being examined in the Virgin Islands. On St. John, feral burros—descendants of work animals released in 1953 when they were displaced by cars—wreak havoc on the plants. The burros consume the native plants, which the exotics outcompete, and they spread the seed of the exotic plants they consume.

"Besides helping the Park Service find a solution to its problem, we're here to find out whether the vaccine will be effective in animals that breed year round," says Turner. He and Kirkpatrick began studying burros in February 1991. The study animals received two darts of vaccine initially and some received one-shot followups in 1992 and 1993. Only one of 16 became pregnant after receiving the booster, says Turner.

Turner is now tracking the reproductive pattern of the animals as they come off the treatment. Burros normally produce foals year round. Would contraceptive treatment at a particular time in some way induce seasonal breeding? As the effects of the vaccine wear off, will the burros enter estrus at the same time and get pregnant in clusters? If the answer is "yes" to these questions, treatment will have to be scattered over time to keep birth patterns normal, says Turner.

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poor, abrasive, and salty. The animals also eat poison ivy and greenbriar. Their rounded bellies are the result of water retention, bloating being common when diets are high in salt.

Kirkpatrick, who also has studied wild horses in US deserts, the Australian outback, and New Zealand, says he has never seen horses thrive in a harsher environment. He points out that several years ago the Chincoteague fire department brought in 40 western mustangs to infuse some diversity

into the herd in the southern part of the island. Only five survived the first year.

Kirkpatrick contends that the Park Service ceiling for the horse population as it presently stands would be adequate if humans could be better managed. Hundreds of thousands of adults and children make a pilgrimage to Assateague solely for the purpose of seeing pintos that look just like Misty of Chincoteague, an equid character in a children's classic. The visitors do not do their part to keep the horses wild. Despite enormous warning signs, fingers filled with enticing goodies wave under feral muzzles. On any given day in the park, a ranger could easily write dozens of citations to visitors, says Kirkpatrick.

Food handouts make the animals dependent on humans, the very thing the Park Service is trying to avoid. "Feeding the horses is part of the larger problem of people trying to treat wild animals as domestic ones," says Zimmerman.

But the tamer horses' habits can make life easier for the scientists. Some of the horses that live around the campground are so habituated to humans, they are relatively easy to dart. The darts containing the vaccines are delivered at close range—10 to 15 yards—with a blow gun.

The truly wild horses must be tracked on foot. They spend three quarters of the year distributed on the island's bayside marshes, inhospitable areas rarely seen by visitors. At summer's height, many horses abandon their mosquito-ridden marshes and cross to the oceanside. Often they can be found up to their withers in the surf, away from voracious insects. However, they also tend to join the campground horses in a seasonal migration to areas with less vegetation and to a short strip of state-park land in the middle of the Maryland part of the island. It is the only area where spraying to control mosquitoes is allowed. "For two weeks last summer, we had 100 horses in a four-mile stretch. Stallions were competing for mares on the beach, and it was at times dangerous [to the public]," says Zimmerman.

Kirkpatrick and the Park Service horse managers generally can predict the location of particular bands. Kirkpatrick follows their comings and goings by using their hoof tracks and manure piles. In tracking the horses, Kirkpatrick occasionally dresses differently to deceive the animals that have been darted several times. After spending hours tracking a band on foot through knee-deep marsh muck and across waterways the locals call guts, he and his colleagues sometimes get thwarted when horses walk guts too deep for the humans to comfortably cross in the cold. "The horses will cross back and forth all day long to avoid us," says Kirkpatrick.

When they get close enough to deliver the vaccine, Kirkpatrick and his colleagues use a lightweight rifle capable of shooting the dart with its 1.5-inch needle up to 40 meters, three times the range of the blowgun. They only take aim at the haunch, avoiding spots like the flank, where the dart could enter the peritoneal cavity. "The only indignity these animals deserve is a shot in the rump," says Kirkpatrick.

In the field, Kirkpatrick continuously refers to a cross-reference flip file that includes photos of and details about every horse on the island, a scientist's version of a parental brag book. (Robert Garrott of the University of Wisconsin says Kirkpatrick knows the Assateague horses better than some people know their own children.) The information contained in that book is the result of decades of work by Keiper, Kirkpatrick, Turner, Park Service staff, and others. The book lists the dams, granddams, and greatgrandams of most of the NPS horses and gives a reasonable idea of their sires and grandsires. Their breeding, foaling, and living habits and their identifying marks are recorded. An identifying number assigned to each animal gives its lineage.

Such detailed breeding information, rare for wild animals, would do a racing stable justice. "The island and the horses are an incredible scientific resource," says Kirkpatrick. The availability of so much information, the ability to identify animals, and the horses' inability to leave the study site has helped him and his colleagues to carry out the contraception experiments and to assess their worth. The data has also made it possible to make observations about long-term reproductive success.

This spring marked the end of darting for experimental purposes at Assateague, says Kirkpatrick. Any future darting will be strictly to manage the population.

Management at Assateague

Sixty of the 76 mares darted this spring were treated for management purposes. These 60 mares, which had never been darted before, each received one shot, instead of the usual two-shot protocol, to induce the production of antibodies and to prime them for future booster shots. The shots may also significantly slow the population's growth next year.

"We have some evidence that a one-shot protocol works on Assateague," says Kirkpatrick. In March 1992, 13 mares were given the single standard dose of 65 micrograms of PZP in sustained-release microspheres, and only one became pregnant. He suspects that the success was due to timing of delivery—antibody levels were maintained at sufficient levels to get through the breeding season, which ends in August.

In reviewing its horse plan in fall 1993, the Park Service decided that additional population growth was not in the best interest of the horses and other island resources, says Zimmerman. To limit population growth, the park decided to immu-

J. F. Kirkpatrick retrieving contraceptive darts.

nize all females over the age of two years as a one-time measure, says Zimmerman. "We're locked into 1994 births. But we hope to have near zero population growth in 1995."

In the meantime, park officials are seriously revising the horse management plan and hope to have it in place by March 1995. In keeping with the requirements of the National Environmental Policy Act, the Park Service must prepare an environmental assessment evaluating the implementation of a long-term fertility control program.

The proposed plan has as its first goal reduction of the herd from 165 animals to 150 animals. It also suggests that research be done to determine whether the herd number can be dropped further.

Under any plan, all animals would at some time have the opportunity to reproduce, says Zimmerman. "There are seven lineages on the island. We don't want any of these to die out."

Between mares foaling and some of the older horses dying, the population might settle around 175 individuals by the year's end, says Kirkpatrick. It could take four to five years of contraception to get the herd down to the current population target.

Brian Underwood, a National Biological Survey scientist based in Syracuse, New York, says the Park Service ought not do anything drastic in trying to control the population. He has done the most recent population modeling on Assateague and found that a drastic change, such as moving half the animals off the island, would likely stimulate compensatory reproduction and survival mechanisms. He says, "Compensatory mechanisms could drive management completely haywire."

Underwood's preliminary analysis does reveal a decline in the rate of population growth during the period of contraceptive experiments from 1986 to 1991. "What I can't say is to what degree immunocontraception is responsible and to what degree encephalitis is," he says. There were major outbreaks of equine encephalitis, borne by the salt-marsh mosquitos, in 1989 and 1990. The disease may have been responsible for approximately 30 horse deaths; only a small fraction were confirmed as having the disease. Also, during that period, some horses were transferred to the Chincoteague Wildlife Refuge, Underwood adds.

Immunocontraception was chosen for Assateague because it seems to be the least intrusive way to manage the herd, says Zimmerman, adding that the approach may not be appropriate in all settings. However, wildlife managers elsewhere along the east coast also are evaluating their horse plans, says Bruce Rodgers in the NPS regional office in Atlanta, Georgia. Approximately $300,000 has been appropriated for 1994 to 1996 to develop a management plan for feral horses on Cumberland Island National Seashore, Georgia, and on Cape Lookout and Cape Hatteras, North Carolina.

Going west

Nevada is not Assateague. Currently, the Bureau of Land Management handles 26,600 wild horses and burros in 100 herd management areas across 19 million acres in the state. A medium-sized area is 400,000 acres, 50 times bigger than the Assateague Island National Seashore. Few horses are known by name, markings, and quirks.

Like the Assateague mares, the Nevada mares reproduce at high rates. Half the mares get pregnant each year, and population increases of 15% to 20% are common. In one particularly good year for the horses, population increased by 27% in some herd areas, says Thomas Pogacnik in BLM's Nevada office. Losses are generally 5% per year. The animals have few predators—a mountain lion sometimes gets a foal. But once the horses get past that vulnerable stage, they live until their teeth wear out, competing with the deer and antelope.

To keep the horse population down, BLM runs the Adopt-A-Horse program, which places 8000 animals each year. Putting the horse up for adoption costs the agency $600 to $800 per horse. Immunocontraception, if it can be managed for such a large area where the habits of individual herds are not well known, could cost BLM less.

With eventual savings in mind, BLM is spending $225,000 on research. Most goes to PZP vaccine work done by Kirkpatrick, Turner and Liu in Nevada. The rest goes to behavior, mortality, fertility, and parentage studies. Pogacnik says studies conducted by the University of Nevada at Reno show that the herds are genetically diverse.

To set population targets for horses, cattle, and wildlife, BLM is determining the range's carrying capacity. That study should be finished later this year.

Immunocontraceptive studies underway in northeast Nevada involve several different protocols. In December 1992, 51 horses received the standard protocol—two shots of raw PZP with adjuvant delivered 30 days apart—found to be effective on Assateague. Analyses of feces collected in September 1993 from 14 treated mares indicate that none of these mares were pregnant. The researchers also tried two different single-shot protocols—PZP and adjuvant and PZP in sustained-release microspheres. Preliminary studies indicated these protocols were unsuccessful, possibly because the vaccines were delivered five months before breeding season began. Antibody titers could have dropped sufficiently to allow fertilization before the end of the 1993 breeding season. "The ideal time to deliver the shot is when roundups are prohibited [by BLM]," says Kirkpatrick.

The decision to use immunocontraceptives in Nevada came from a task force made up of representatives from the government, scientific community, and animal welfare organizations. BLM went with the PZP approach because it had a track record on Assateague and in domestic horses. "We're not prepared to take a risk with another approach yet," says Pogacnik.

In particular, BLM is avoiding approaches that require surgery. In the late 1980s, a fertility control study on BLM land that involved hormone implants garnered a large amount of negative press attention. In the study by University of Minnesota researchers, horses were herded into unfamiliar territory, surgery was done on mares to implant hormones, and the horses were then released. The disoriented animals were unable to find water and at least 48 died of dehydration. Animal welfare organizations lobbied BLM to end the studies, and BLM field officers recommended to BLM that the research be canceled. Even Nevada's governor got involved. The study was canceled.

When the furor settled, BLM was still faced with a growing horse population. Sen. Harry Reid (D–Nev.) "went out on a limb and pushed for the appropriation of funds so that contraceptive research could continue," says Kirkpatrick.

When the pilot research studies are completed this December, if immunocontraception seems like a management tool that will work on the range, another task group will be put together to produce a management plan. It is unlikely that the technique would be used on all herds. "We'd likely target the 20 or 30 herds that are captured regularly," says Pogacnik.

Will the zona-pellucida procedure take off?

Liu, Kirkpatrick, and Turner have now turned their attention to developing a successful one-shot protocol. Once a one-shot horse vaccine is developed that can be used on any horse population at any time of year, "our project will have reached its logical scientific conclusion," says Kirkpatrick.

Instead of working with sustained-release microspheres, they have switched to a pulsed-release microsphere vaccine that they hope will provide two or three years of contraception with a single shot, says Liu. From a single application, doses of PZP would be delivered at the time of darting, one month after the initial injection, and then a year after the initial injection. Liu is currently collecting blood from domestic horses injected with these microspheres to determine whether the horses respond by producing antibodies.

Robert Warren of the University of Georgia is also tweaking the PZP vaccine to make it easier to deliver. He and his colleagues are coupling Liu's PZP with an adjuvant that can be freeze-dried. The combination will be placed in a biobullet, a hollow container of highly compressed cellulose that dissolves when it enters an animal. The biobullets are smaller, more accurate, and safer than darts, says Warren.

The Humane Society believes that the PZP vaccine, remotely delivered by dart, is the safest, most effective, and most practical application currently available, says Rutberg. He adds that the society is unwilling to endorse a method with either environmental or behavioral side effects or one where the delivery cannot be carefully controlled in the field.

Other potential immunocontraceptives involve the use of purified pig zona, recombinant forms of zona pellucida from an array of species, sperm membrane proteins that cause females to produce antibodies to sperm, and gonadotropin-releasing hormone (GnRH) with a carrier. GnRH normally is released by the hypothalamus and goes to the pituitary, where it stimulates the release of luteinizing hormone and follicle-stimulating hormone, both key to egg and steroid production. "Blocking GnRH would shut down entire reproductive systems and in many cases reproductive behavior," says Rutberg.

The Denver Wildlife Research Center, a division of the US Department of Agriculture, is exploring ways to deliver vaccines orally. Others are exploring the use of bacterial and viral agents to deliver contraceptives.

Of the many approaches, Kirkpatrick's is clearly the one that has the upper hand politically, adds Garrott, who has worked with horses and now is evaluating contraception for elk. Like many others in the field, he will use contraceptive material produced in Liu's lab for some studies.

Garrott predicts that wildlife managers, who traditionally deal with manipulating survival, will someday manage by manipulating reproduction. "But it is too soon to say whether PZP will be the standard approach," he says, adding that currently there may be too much

emphasis on remote delivery, reversibility, and prevention of behavioral effects in fertility control for animals.

Garrott considers sterilization to be a reasonable alternative in some situations. Warren, too, says that until an efficient, easy-to-use contraceptive is ready, stallion vasectomies out west or steroids for mares may be reasonable choices.

Before getting too excited about PZP's potential, Garrott adds, more population-level studies will have to be done. Kirkpatrick, Turner, and Liu have enough data to show that the vaccine works at the level of individuals, he says, but they have not shown that it will have a population effect. How many animals in a large population will have to be darted (and how often) to cause significant reductions in numbers? Molecular biologist Kenneth Tung at the University of Virginia is concerned about whether a contraceptive vaccine would be sufficiently effective for a population with great genetic diversity. He points out that immune responses to any antigen are genetically controlled, and in any outbred population, some animals respond well to a vaccine and others do not.

William Lance, president of Wildlife Pharmaceuticals in Colorado, has been following the field of animal contraception since the 1970s. Last year he decided that his company would not pursue the immunocontraceptive market because he expects there to be legal and regulatory roadblocks, especially if the idea of using the vaccine for game species becomes popular. Lance says the vaccine might languish as battles arise among state and federal interests arguing over which body has the right to make management dec sions, and as animal welfare an hunting organizations argue whethe contraceptive vaccines should b used.

Zonagen, a Texas-based compan that is developing recombinant PZ vaccines for the human, pet, an livestock markets, is less pessimi tic. "The technology is adaptable t wildlife," says company presider Joe Podalski.

Kirkpatrick is concerned abou the ethical values of those who w use any vaccine as a manageme tool. "Right now, [our team] large controls what is happening in th field because we're the only on who do it. What if some manage come along who want to elimina animals that they perceive to pests? They could quietly and co tinuously contracept a popul tion until it died [out]."