CONTRACEPTION OF BISON BY GnRH VACCINE: A POSSIBLE MEANS OF DECREASING TRANSMISSION OF BRUCELLOSIS IN BISON

Authors: Miller, Lowell A., Rhyan, Jack C., and Drew, Mark

Source: Journal of Wildlife Diseases, 40(4) : 725-730

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-40.4.725

Journal of Wildlife Diseases, 40(4), 2004, pp. 725–730

CONTRACEPTION OF BISON BY GnRH VACCINE: A POSSIBLE MEANS OF DECREASING TRANSMISSION OF BRUCELLOSIS IN BISON

Lowell A. Miller,[1,4] Jack C. Rhyan,[2] and Mark Drew[3]

[1] US Department of Agriculture-Animal Plant Health Inspection Service, National Wildlife Research Center, 4101 LaPorte Ave., Fort Collins, Colorado 80521, USA
[2] US Department of Agriculture-Animal Plant Health Inspection Service, Veterinary Services, National Wildlife Research Center, 4101 LaPorle Ave., Fort Collins, Colorado 80521, USA
[3] Idaho Department of Fish and Game, 16569 S. 10th Ave., Caldwell, Idaho 83607, USA
[4] Corresponding author (email: lowell.a.miller@usda.gov)

ABSTRACT: Preventing pregnancy in brucellosis-infected bison (*Bison bison*) provides a potential means of preventing transmission of disease. To determine whether a gonadotropin-releasing hormone (GnRH) vaccine was effective in reducing pregnancy in bison and to study the safety of injecting GnRH in pregnant bison, a study was conducted at the Idaho Fish and Game Wildlife Health Laboratory in Caldwell, Idaho (USA). Four pregnant and two nonpregnant female bison were given a single injection of GnRH vaccine, and five pregnant adult females were given a sham injection that contained only adjuvant. Three of the GnRH-vaccinated bison that were pregnant at the time of vaccination delivered healthy calves. One treated bison had dystocia that resulted in a dead calf. All control bison delivered healthy calves. After calving, females of both groups were exposed to two bulls. Treated bison were palpated 6 wk after exposure to the bulls, and blood was drawn for pregnancy-specific protein B analysis. The six treated bison were not pregnant. The sham-treated bison became pregnant and delivered viable calves. This study demonstrates that a single dose of GnRH vaccine is effective in preventing pregnancy in female bison for at least 1 yr.

Key words: Gonadotropin-releasing hormone, immunocontraception, GnRH vaccine, bison.

INTRODUCTION

Bovine brucellosis, a bacterial disease caused by *Brucella abortus,* is transmitted among animals, including cattle, bison (*Bison bison*), and elk (*Cervus elaphus*), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or postparturient uterine discharge. Additionally, *Brucella* is shed in milk from infected dams and can be transmitted to calves through suckling. After initial infection, a dam often experiences abortion. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may also result in the shedding of *B. abortus.* The occurrence of venereal transmission of brucellosis in bison is unknown; however, on the basis of a single study in bison (Robison et al., 1998) and studies in cattle (Manthei et al., 1950; Rankin, 1965), it is considered unlikely to be a significant route of transmission. Transmission of the disease in cattle, bison, and elk, therefore, is primarily dependent on the occurrence of pregnancy and exposure to abortion or calving in infected animals.

Rhyan et al. (2002) suggested that permanent sterilization, surgical or chemical, is a disease-management strategy that could be effectively used in *Brucella*-infected bison to greatly reduce the possibility of transmission to other animals. Bison cows could remain persistently infected with *B. abortus,* and, as long as the infected animals were not allowed to become pregnant, they would not be likely to transmit the infection. Therefore, disease prevalence might decrease dramatically as that generation of infected bison disappears. Objections have been raised to permanent sterilization in relation to wild horse immunocontraception, because it might result in the permanent removal of those animals from the gene pool and the creation of a new "unnatural" class of animals (Kirkpatrick and Turner, 1991).

The gonadotropin-releasing hormone

(GnRH) vaccine is generally considered to provide temporary sterilization, because the reproductive activity of the target animal returns as the GnRH antibody titer drops below a protective level. This temporary period of infertility may allow time for *B. abortus* infection to clear.

The use of nonlethal methods to control populations of pest animals is an area of research that is receiving more interest (Fagerstone et al., 2002). Kirkpatrick et al. (1996) pioneered the use of porcine zona pellucida (PZP) for use as a nonlethal, contraceptive approach to pest animal control. The difficulty with the use of PZP in ungulates is that the animals that receive it, although they remain infertile, continue to have estrous cycles. Female white-tailed deer (*Odocoileus virginianus*) vaccinated with PZP have continued to exhibit sexual activity into February, 4 mo beyond the normal breeding season (Miller et al., 2000b). This continuous estrous cycling results in increased activity during early winter, at a time when the conservation of calories is important, although this increased cycling has not resulted in any apparent health problems (Miller et al., 2001). Additionally, it could increase the spread of venereally transmitted diseases, if present and, at least in the case of deer in populated areas, may contribute to increased collisions with automobiles. Prolonging the breeding season of bison in the greater Yellowstone area may be deleterious to the winter survival of dominant bulls and vaccinated cows because of increased activity during fall and early winter.

Immunocontraception using the GnRH vaccine is an alternative to PZP that would not extend the breeding season. The keyhole limpet hemocyanin–GnRH immunocontraceptive vaccine interferes with the release of follicle-stimulating hormone (FSH) and leutinizing hormone (LH), thereby preventing normal function of the ovaries and testes and their production of progesterone and testosterone. Thus, GnRH vaccine can effectively prevent conception in either females or males (Talwar, 1985).

The GnRH vaccine has successfully produced sterility in Norway rats (*Rattus norvegicus*; Miller et al., 1997) and white-tailed deer (Miller et al., 2000a). The immunoneutralization of GnRH produces temporary nonsurgical castration in animals (Meloen et al., 1994; Oonk et al., 1998). In an ongoing study in female white-tailed deer conducted by the National Wildlife Research Center (Fort Collins, Colorado, USA) and Pennsylvania State University (University Park, Pennsylvania, USA), a single injection of GnRH vaccine resulted in infertility lasting up to 3 yr.

The development of immunocontraceptives that are practical to use for wildlife population control must include vaccine delivery systems. Although the administration of an oral form of the vaccine may be necessary in some situations, a long acting single-shot injectable form of the vaccine would have practical advantages over formulations that require two injections. Immunocontraception has typically required at least two doses, given as a prime and a boost. The prime dose prepares the immune system for a repeat antigen exposure and provides only a short-term immune response. The boost immunization can result in an immune response that may last for months to years. To have success with a single injection, the dose and timing of the injection is more critical than when using two injections. This article reports on the immunocontraception of penned bison using the newly developed single-shot GnRH vaccine.

MATERIALS AND METHODS

On 6 June 2002, six 6-yr-old female bison were injected with 1,800 μg of a single-shot GnRH vaccine (GonaCon/AdjuVac™, developed by the National Wildlife Research Center, United States Department of Agriculture, Animal Plant Health Inspection Service, Fort Collins, Colorado—patent pending) in a 1-ml injection given intramuscularly in the hip. Five control bison were injected with the adjuvant,

TABLE 1. Results of contraception in female bison using a GnRH vaccine.

Treatment	Year 1		Year 2		
	Pregnancy status when injected (June 2002)	Calving dates, 2002	Pregnancy rate	PSPB[a] results	Calving dates, 2003
Sham injection	5/5	20 June–26 July	5/5	5/5 positive for pregnancy	4 June–29 July
1,800 μg GnRH/AdjuVac[b]	4/6	28 June–1 July	0/6	0/6 positive for pregnancy	No calves born

[a] PSPB = pregnancy-specific protein B.
[b] GonaCon/AdjuVac, US Department of Agriculture, Animal Plant Health Inspection Service, patent pending.

1 ml, in the hip (control). All control bison and four of the treated bison were pregnant at the time of the injection. Because the GnRH vaccine has the potential to cause abortion, the pregnant bison were vaccinated to determine the safety of the GnRH vaccine. Blood samples were drawn monthly for 4 mo and then every other month for a total of 8 mo. Serum was tested for progesterone by radioimmunoassay and for GnRH antibody by enzyme-linked immunoassay (Miller et al., 2000a).

Two months after calving, a bull was introduced to the pen and allowed to breed the cows for 2.5 mo (17 September–1 December 2002). Six weeks after the bull was removed, both control and GnRH-treated bison were palpated for pregnancy diagnosis, and results were confirmed by serum pregnancy-specific protein B assay (PSPB) testing (Biotracking, Moscow, Idaho).

RESULTS

Analysis of pregnancy and calving data in the control and GnRH-treated bison at the time of GnRH injection and the following year indicated that the GnRH vaccine was successful in reducing reproduction, compared with controls (Table 1). At the time of vaccination, five of the sham-treated cows and three of the six GnRH-treated cows were in the last month of pregnancy. Cows in both groups delivered normal calves the first year; therefore, the GnRH vaccine did not interfere with the pregnancy. None of the GnRH-treated cows became pregnant the year after the vaccination. All control bison conceived, and four had normal calves, with calving dates of 4–30 June 2003. One control cow died on 30 March 2003 but was pregnant at the time of death. During this study, two cows, one each in the treated and control groups, had dystocia that resulted in dead calves.

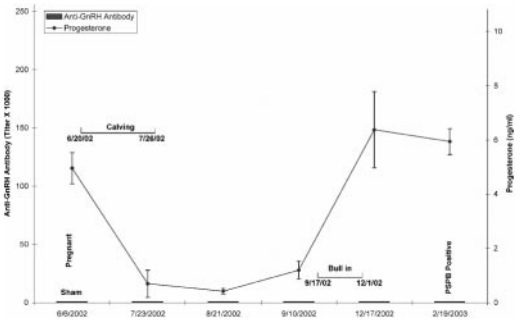

FIGURE 1. Average serum progesterone levels and anti-GnRH antibody titers for control bison cows.

The average progesterone levels for pregnant cows were the same for the treatment and control groups at the start of the study and after calving. After rebreeding, the progesterone level of cows in the control group increased to pretreatment levels, indicating that they became pregnant, and anti-GnRH titers were not detected (Fig. 1). Progesterone levels in the GnRH-treated bison remained at nonpregnant levels (Fig. 2). All control bison delivered normal healthy calves and became pregnant again the second year. One of the five control bison died from accidental causes midgestation. The remaining four controls had normal calves in year 2 of the study.

Three of the six GnRH-treated bison were in late gestation when they were immunized, and all delivered normal calves within 1 mo after treatment. Two of the GnRH-treated cows were not pregnant at the time of GnRH vaccination, as suggest-

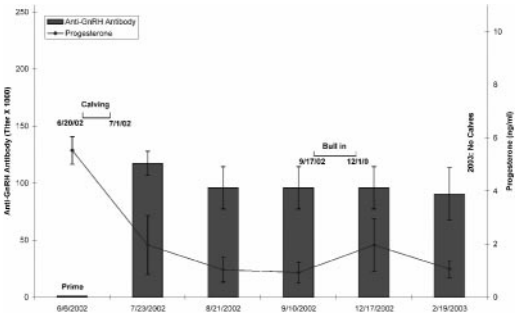

FIGURE 2. Average anti-GnRH antibody titers and serum progesterone levels in bison cows vaccinated late during pregnancy.

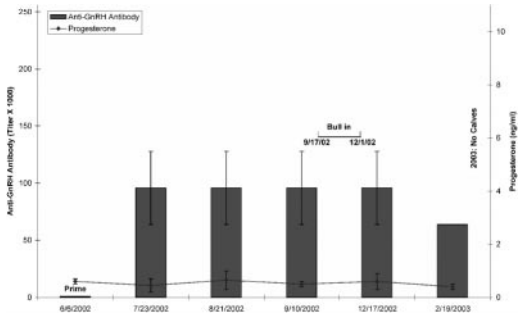

FIGURE 3. Average anti-GnRH antibody titers and serum progesterone levels in bison cows vaccinated when not pregnant.

ed by low progesterone levels at the time of treatment (Fig. 3). The low progesterone values during the postpartum period in the cows that calved were comparable to those of the control cows. However, they did not become pregnant after exposure to the bulls, as indicated by low progesterone levels, the absence of a fetus on palpation, and negative PSPB serum test results.

An exception to the results of treatment of cows during late pregnancy was bison B-40, which had been given the GnRH vaccine during midpregnancy. This bison cow was injected with the vaccine on 6 June 2002 and delivered full term on 6 November 2002, with dystocia, resulting in a dead calf. The progesterone level progressively dropped from 7.0–3.5 μg/ml of serum during the first 2 mo after vaccination and leveled off at 3.4 μg/ml of serum during the third month, 2 mo before the birth of the dead full-term calf. This cow had a positive PSPB result, low progesterone levels, and was not pregnant on palpation.

The anti-GnRH data in treated bison indicated that a protective antibody titer was reached by the first time blood was collected (47 days after vaccination). The mean titer at this time was 112,000, decreasing to a mean titer of 72,000 by the end of the study. Antibody titers of 64,000 or greater have been shown to be consistent with contraception (Miller et al., 2000a).

In the beginning of the study, similar progesterone and PSPB levels in control and treated groups suggested that cows in both groups were pregnant. Calving dates were comparable in the control and treated cows, indicating synchronous breeding cycles (Table 1). However, in the second year, elevations in progesterone levels in the control group suggested that they became pregnant, and low progesterone levels in the treated group suggested infertility (Figs. 1–3).

DISCUSSION

The GnRH vaccine induces infertility in female mammals by reducing the release of FSH and LH, which, in turn, interferes with either the normal estrous or ovulatory cycle or reduces progesterone concentration during early pregnancy, which may interfere with maintenance of pregnancy. Stevenson (1997) stated that GnRH controls the amount of progesterone produced by the corpus luteum (CL), which maintains pregnancy for 200 days of the mean 280-day gestation in cattle. After 200 days, the ovary containing the CL can be removed without interfering with pregnancy, which indicates that pregnancy is not maintained by pituitary GnRH; the placenta apparently takes over the production and maintenance of progesterone. Bison have a gestation period similar to that of cattle.

Our results indicate that the GnRH vaccine can be administered safely during the

last third of pregnancy. Protective levels of anti-GnRH antibody require 30–45 days to develop, which suggests that the vaccine could be safely administered at ≥170 days of gestation without negative effects on the fetus. This was shown to be the case in the three bison treated late in pregnancy.

One cow was vaccinated during the second trimester of pregnancy and delivered a full-term dead calf on 6 November. It is unknown whether the GnRH vaccine contributed to death the fetus. There was a decrease in progesterone levels in this cow after vaccination that could have contributed to the loss of viability of the calf. Because the bull was with the cows from 17 September to 1 December, it is unlikely that this cow could have rebred. However, anti-GnRH antibody titers were sufficient in this cow to prevent pregnancy. One control bison also had a similar late full-term dead calf; thus, it is uncertain whether the vaccine caused the death of the calf in the vaccinated cow.

All control and treated cows were tested for pregnancy by palpation and serum progesterone and PSBP levels during February 2003. Bison B-40 had a positive serum PSPB test at this time but a low progesterone level and was not pregnant on palpation. Thus, the PSPB test was incorrect. This is consistent with reports that retained placentas following abortions can cause a false-positive PSPB result for several months (Sasser et al., 1986). The bison will be monitored for 2 more years to determine the duration of the contraceptive effect.

This study demonstrates that a single injection of GnRH vaccine is effective in preventing contraception in female bison for at least 1 yr. Booster injections lengthen the contraceptive effect in white-tailed deer (Miller and Killian, 2000), and lengthening the contraceptive effect in bison may be achieved similarly. Use of the GnRH vaccine in *Brucella*-infected bison should effectively reduce transmission of disease by reducing pregnancy rates and subsequent abortion or parturition.

LITERATURE CITED

FAGERSTONE, K. A., M. A. COFFEY, P. B. CURTIS, R. A. DOLBEER, G. J. KILLIAN, L. A. MILLER, AND L. M. WILMONT. 2002. Wildlife contraception. Wildlife Society Technical Review 02–2. 29 pp.

KIRKPATRICK, J. F., AND J. W. TURNER, JR. 1991. Reversible contraception in non-domestic animals. Journal of Zoo and Wildlife Diseases 22: 392–408.

———, ———, I. K. LIU, AND R. FAYRER-HOSKEN. 1996. Applications of pig zona pellucida immunocontraception to wildlife fertility control. Journal of Reproduction and Fertility Supplement 50: 183–189.

MELOEN R. H., J. A. TURKSTRA, H. LANKHOF, W. C. PUIJK, W. M. M. SCHAAPER, G. DIJKSTRA, C. J. G. WENSING, AND R. B. OONK. 1994. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. Vaccine 12: 741–746.

MANTHEI, C. A., D. E. DETRAY, AND E. R. GOODE. 1950. *Brucella* infection in bulls and the spread of brucellosis in cattle by artificial injection. Proceedings of the American Veterinary Medical Association 87: 177–184.

MILLER, L. A., AND G. J. KILLIAN. 2000. Seven years of white-tailed deer immunocontraception research at Penn State University: A comparison of two vaccines. Ninth Eastern Wildlife Damage Management Conference, State College, Pennsylvania, pp. 60–69.

———, B. E. JOHNS, D. J. ELIAS, AND K. A. CRANE. 1997. Comparative efficacy of two immunocontraceptive vaccines. Vaccine 15: 1858–1862.

———, ———, AND G. J. KILLIAN. 2000a. Immunocontraception of white-tailed deer with GnRH vaccine. American Journal of Reproductive Immunology 44: 266–274.

———, ———, AND ———. 2000b. Long-term effects of PZP immunization on reproduction in white-tailed deer. Vaccine 18: 568–574.

———, K. CRANE, S. GADDIS, AND G. J. KILLIAN. 2001. Porcine zona pellucida immunocontraception: Long-term health effects on white-tailed deer. Journal of Wildlife Management 65: 941–945.

OONK, H. B., J. A. TURKSTRA, W. SCHAAPER, M. ERKENS, M. H. SCHUITEMAKER-DEWEERDM, J. H. M. VAN NES VERHEIJDEN, AND R. H. MELOEN. 1998. New GnRH-like peptide construct to optimize efficient immunocastration of male pigs by immunoneutralization of GnRH. Vaccine 16: 1074–82.

RANKIN, J. E. F. 1965. *Brucella abortus* in bulls: A study of twelve naturally-infected cases. Veterinary Record 77:132–135.

RHYAN, J. C., AND M. D. DREW. 2002. Contraception: A possible means of decreasing transmission of brucellosis in bison. *In* Brucellosis in elk and

bison in the Greater Yellowstone Area, T. J. Kreeger (ed.). Greater Yellowstone Interagency Brucellosis Committee, Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 99–108.

ROBISON, C. D., D. S. DAVIS, J. W. TEMPLETON, M. WESTHUSIN, W. B. FOXWORTH, M. J. GILSDORF, AND L. G. ADAMS. 1998 Conservation of germ plasma from bison infected with *Brucella abortus.* Journal of Wildlife Diseases 34: 582-589.

SASSER, R. G., C. A. RUDER, K. A. IVANI, J. E. BUTLER, AND W. C. HAMILTON. 1986. Detection of pregnancy by radio immunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentration during gestation. Biology of Reproduction 35: 936–942.

STEVENSON, J. S. 1997. Clinical practice in large animals. *In* Current therapy in large animal theriogenology, R. S. Youngquist (ed.). W.B. Saunders Co.

TALWAR, G. P. 1985. Immunobiology of gonadotropin-releasing hormone. Journal of Steroid Biochemistry 23: 795–800.

Received for publication 29 January 2004.