University of Nebraska - Lincoln DigitalCommons@University of Nebraska - Lincoln

USDA National Wildlife Research Center - Staff Publications

Wildlife Damage Management, Internet Center for

1-1-2009

No adverse effects of simultaneous vaccination with the immunocontraceptive GonaConTM and a commercial rabies vaccine on rabies virus neutralizing antibody production in dogs

Scott C. Bender

Navajo Nation Veterinary Program, Navajo Nation, Chinle, AZ, USA

David L. Bergman

United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Phoenix, AZ, USA

Krista M. Wenning

United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Phoenix, AZ, USA

Lowell A. Miller

United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, CO, USA

Charles E. Rupprecht

Centers for Disease Control and Prevention, Atlanta, GA, USA

Follow this and additional works at: http://digitalcommons.unl.edu/icwdm_usdanwrc

Part of the Environmental Sciences Commons

Bender, Scott C.; Bergman, David L.; Wenning, Krista M.; Miller, Lowell A.; Rupprecht, Charles E.; Slate, Dennis; and Jackson, Felix R., "No adverse effects of simultaneous vaccination with the immunocontraceptive GonaConTM and a commercial rabies vaccine on rabies virus neutralizing antibody production in dogs" (2009). *USDA National Wildlife Research Center - Staff Publications*. Paper 863. http://digitalcommons.unl.edu/icwdm_usdanwrc/863

This Article is brought to you for free and open access by the Wildlife Damage Management, Internet Center for at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USDA National Wildlife Research Center - Staff Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors Scott C. Bender, David L. Bergman, Krista M. Wenning, Lowell A. Miller, Charles E. Rupprecht, Dennis Slate, and Felix R. Jackson		



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



No adverse effects of simultaneous vaccination with the immunocontraceptive GonaConTM and a commercial rabies vaccine on rabies virus neutralizing antibody production in dogs

Scott C. Bender^a, David L. Bergman^{b,*}, Krista M. Wenning^b, Lowell A. Miller^c, Dennis Slate^d, Felix R. Jackson^e, Charles E. Rupprecht^e

- ^a Navajo Nation Veterinary Program, Navajo Nation, Chinle, AZ, USA
- b United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Phoenix, AZ, USA
- c United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, CO, USA
- d United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Concord, NH, USA
- e Centers for Disease Control and Prevention, Atlanta, GA, USA

ARTICLE INFO

Article history: Received 24 August 2009 Accepted 3 September 2009

Keywords:
Dogs
Rabies
Immunocontraception
GonaConTM

ABSTRACT

Parenteral vaccination campaigns are integral to the elimination of canine rabies. To maximize herd immunity in dogs, immunocontraception provided at the time of rabies vaccination should reduce fecundity and dog abundance. GonaConTM has been used successfully as an immunocontraceptive in a variety of mammals, and by inference, the dog would be an ideal candidate for testing. As an initial step in evaluating a combination-vaccination program, we assessed the effects of GonaConTM on rabies virus neutralizing antibody production in dogs after administration of a veterinary rabies vaccine. Eighteen feral/free ranging dogs were included in this initial study: six were given GonaConTM only, six were given rabies vaccination only, and six received GonaConTM and rabies vaccination. Antibody levels were evaluated over 82 days. The use of the immunocontraceptive GonaConTM did not affect the ability of dogs to seroconvert in response to the rabies vaccine. Thus, GonaConTM provides a potential immunocontraceptive for use in combination with rabies vaccine to increase herd immunity and address dog population over abundance to better manage rabies.

Published by Elsevier Ltd.

1. Introduction

During the last century, extensive parenteral vaccination of host reservoir dog populations accompanied by control of free ranging dogs has led to the elimination of canine rabies in several countries, such as Japan [1] and the United States [2]. Almost a half of a century later, rabies continues to challenge public health systems in developing countries, especially in Africa and Asia, where many of the estimated 55,000 annual human rabies deaths occur. Rabies is a neglected tropical disease [3,4] due in part to a lack of political will brought about by an absence of accurate data on the true public health impact of this zoonosis [5].

The threat of rabies virus transmission to humans from dogs increases when the density of dogs exceeds the threshold density at which canine rabies is maintained. The threshold density for canine rabies has been estimated to be 4.5 dogs/km² [5]. Immunocontraception has been proposed to reduce the density dependence of rabies [6]. Several targets for immunocontraception are avail-

able, such as gonadotropin-releasing hormone (GnRH). Research has suggested that by controlling GnRH, the breeding capability of a dog could be removed [7]. Others have shown that a GnRH vaccine reduced the breeding capability of male dogs for at least 1 year [8]. In other species tested, GonaConTM induced infertility 2–3 times longer in females than males. Thus, we expect the same increased induced infertility in female dogs.

The potential for interference between simultaneously administered immunocontraceptive and rabies vaccines must be determined before field trials using this tandem vaccination strategy merit consideration. In this study, we evaluated the effects of the immunocontraceptive vaccine GonaConTM on rabies virus neutralizing antibody (VNA) induction in dogs from a commercial rabies vaccine.

2. Materials and methods

2.1. GonaConTM and rabies vaccine

The $GonaCon^{TM}$ immunocontraceptive vaccine (United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort

^{*} Corresponding author. Tel.: +1 602 870 2081; fax: +1 602 870 2951. E-mail address: david.l.bergman@aphis.usda.gov (D.L. Bergman).

Collins, CO, USA) formulation consisted of mammalian GnRH conjugated to a large mollusk hemocyanin protein (keyhole limpet hemocyanin [KLH]) and emulsified with the adjuvant in Dog-AdjuVacTM. Each 0.5-ml dose contained 500 µg of the GnRH conjugate and 85 µg (one-half the standard dose) of inactivated *Mycobacterium avium*. GonaConTM was stored and shipped under refrigerated conditions in pre-loaded, 3 ml Air-Tite luer-hub syringes (Air-Tite Products, Virginia Beach, VA, USA).

The rabies virus vaccine was a commercially available inactivated rabies virus (DEFENSOR 3, Pfizer, Inc., New York, NY, USA). Each 1 ml container was stored under refrigerated conditions at $4\,^{\circ}\text{C}$ until ready for use.

2.2. Experimental animals

Eighteen female dogs of mixed breeds were acquired from dog round-ups conducted by the Navajo Nation Animal Control Program during May 2008. Unclaimed dogs gathered by Animal Control are euthanized 3 days post round-up pursuant to the Navajo Nation Animal Control Laws (Navajo Tribal Code; Title 13, Section 1711, Impounded Animals). Female dogs over 5 months of age were offered for this study instead of euthanasia. Breeding age female dogs were selected based on availability.

Dogs were separated into kennels at the Navajo Nation Animal Control Program's Many Farms Facility, Many Farms, Arizona, USA. Each dog was individually marked with a collar and an attached identification tag. Animal care personnel maintained the kennels twice per day. Dogs were fed on commercial dog food (Ol' Roy: Complete Nutrition Dog Food, Wal-Mart Stores, Inc., Bentonville, AR, USA) and offered water *ad libitum*. All dogs were under direct oversight by an onsite veterinarian. Dogs were randomly assigned to each of three treatment groups.

Group 1: Six animals. All were vaccinated with 0.5 ml of GonaConTM immunocontraceptive. The injection was administered intramuscularly in the upper left hind leg.

Group 2: Six animals. All were vaccinated with a single 1 ml injection of DEFENSOR-3 rabies vaccine. The vaccine was administered intramuscularly to the upper right hind leg.

Group 3: Six animals. Each animal received a single injection of 0.5 ml GonaConTM immunocontraceptive plus a 1 ml injection of DEFENSOR-3 rabies vaccine. The immunocontraceptive injection was administered intramuscularly to the upper left hind leg and the rabies vaccine was administered intramuscularly to the upper right hind leg.

2.3. Determination of anti-GnRH antibody titers

Blood was drawn from the jugular vein of each dog on a weekly basis using a 12 ml syringe equipped with a 20-gauge needle, or with a 6 ml CorVac (Kendall, Mansfield, MA, USA) serum separator tube and a 20-gauge needle. Blood samples were stored on blue ice in an ice chest and then centrifuged. Serum was separated and stored in CryoTubes (Nalge Nunc International, Rochester, NY, USA) at $-70\,^{\circ}\text{C}$ until analyzed.

To evaluate the immune responses of dogs to vaccination with GonaConTM, we used an enzyme-linked immunosorbent assay (ELISA) to measure anti-GnRH antibody titers [8]. In brief, a 96-well plate was prepared by adding 100 ng of BSA-GnRH antigen to each well and then blocking with SeaBlockTM. Fifty microlitres of serially diluted serum was used for each assay. Anti-GnRH antibody was determined by adding rabbit anti-dog IgG to each well, washing, then adding goat anti-rabbit IgG labeled with horse-radish peroxidase (HRP), washing, then developing a color by adding a HRP substrate. The color was proportional to the anti-GnRH anti-

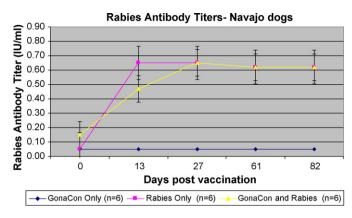


Fig. 1. Mean rabies virus neutralizing antibody titers in dogs vaccinated with DEFENSOR-3 rabies vaccine and GonaConTM.

body titer. BSA-GnRH was added to the ELISA plate, causing only antibodies to GnRH to be detected.

2.4. Determination of rabies VNA titers

The coded sera were thawed rapidly and heat-inactivated in a 56 °C water bath for 1 h. Rabies VNA titers were determined using the Rapid Fluorescent Focus Inhibition Test (RFFIT), as previously described [9]. In brief, the RFFIT was performed by mixing dilutions of test serum with a constant amount of challenge virus standard (CVS) rabies virus, 50 FFD₅₀/0.1 ml, in multi-channeled slides (LabTek, Thermo Fisher Scientific, Rochester, NY). After allowing the mixture to incubate in a CO2 incubator at 37 °C for 90 min, mouse neuroblastoma (MNA, C1300) cells in Eagle's Minimum Essential Medium with 10% (heat-inactivated) fetal bovine serum (MEM-10) were added to each serum-virus mixture. The serumvirus-cell cultures were incubated for 20 h in a CO2 incubator at 37 °C. Thereafter, the cultures were taken from the incubator, the media was removed, and the slides were washed with PBS, fixed in acetone, and stained with an anti-rabies virus conjugate. The fixed cell cultures were observed under a fluorescence microscope for the presence of fluorescing cells; 20 microscopic fields $(160 \times -200 \times)$ were read for each dilution and compared against a reference slide (virus control; 50 FFD₅₀/0.1 ml), which contained 18-20 fields of undiluted fluorescing cells. The 50% neutralization endpoint was defined as the dilution at which 9 or less of the 20 observed fields contained one or more infected cells. Observed titers were converted into international units by comparison with the standard positive control rabies virus immunoglobulin (IgG) at a concentration of 2 IU/ml.

2.5. Statistical methods

SAS software was used for statistical analysis [10]. The GLM procedure for repeated measures of analysis of variance and Tukey's studentized range were used for comparison. Statistical differences with P < 0.05 were considered significant.

3. Results

The anti-GnRH antibody and rabies VNA titers for each group were determined over an 82-day period (Fig. 1). Titers were analyzed at days 0, 13, 27, 61, and 82 as shown in Table 1. Group 1 was an internal control for the rabies vaccinations administered to Groups 2 and 3. In Group 3, for those individuals that received both vaccines, antibody responses to GnRH and rabies virus were measured (Fig. 2).

Table 1Tukey's studentized range (HSD) test.

Treatment comparison	Difference between means	Simultaneous 95%
Day 0		
G3-G1	0.12000	-0.10731, 0.34731
G3-G2	0.12000	-0.10731, 0.34731
G1-G3	-0.12000	-0.34731, 0.10731
G1-G2	0.00000	-0.21673, 0.21673
G2-G3	-0.12000	-0.34731, 0.10731
G2-G1	0.00000	-0.21673, 0.21673
Day 13		
G2-G3	0.10800	-0.09658, 0.31258
G2-G1	0.60000	$-0.40494, 0.79506^{***}$
G3-G2	-0.10800	-0.31258, 0.09658
G3-G1	0.49200	0.28742, 0.69658***
G1-G2	-0.60000	$-0.79506, 0.40494^{***}$
G1-G3	-0.49200	-0.69658, -0.28742
Day 27		
G2-G3	0.0000	0.0000, 0.0000
G2-G1	0.6000	0.6000, 0.6000***
G3-G2	0.0000	0.0000, 0.0000
G3-G1	0.6000	0.6000, 0.6000***
G1-G2	-0.6000	$-0.6000, -0.6000^{***}$
G1-G3	-0.6000	-0.6000, -0.6000 ***
Day 61		
G2-G3	0.0000	0.0000, 0.0000
G2-G1	0.5700	0.5700, 0.5700***
G3-G2	0.0000	0.0000, 0.0000
G3-G1	0.5700	0.5700, 0.5700***
G1-G2	-0.5700	$-0.5700, -0.5700^{***}$
G1-G3	-0.5700	$-0.5700, -0.5700^{***}$
Day 82		
G2-G3	0.0000	0.0000, 0.0000
G2-G1	0.5700	0.5700, 0.5700***
G3-G2	0.0000	0.0000, 0.0000
G3-G1	0.5700	0.5700, 0.5700***
G1-G2	-0.5700	$-0.5700, -0.5700^{***}$
G1-G3	-0.5700	$-0.5700, -0.5700^{***}$
*** -		

^{***} Comparisons significant at the 0.05 level.

There was a significant interaction between time step (F= 56.59, P<0.0001) and group (F= 16.28, P<0.0001). This interaction was a function of the negative rabies VNA titers for all individuals at time-step-day 0 and the subsequent rise in titers for Groups 2 and 3 post time-step-day 0.

At time-step-day 0, there was no difference in mean rabies titers by group. For time-steps 1–4 (days 13, 27, 61, and 82) there were statistically significant differences for the same treatment

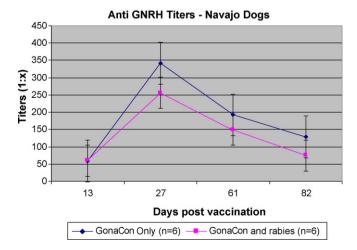


Fig. 2. Post GnRH antibody titers following $\mathsf{GonaCon}^\mathsf{TM}$ and rabies vaccination in dogs.

group comparisons (between Group 3, Group 1 and Group 2). For time-steps 1–4 (days 13, 27, 61, and 82), there was no statistically significant difference (i.e., no evidence of interference) in mean rabies VNA titers for the Group 2 (rabies only) and the Group 3 (rabies + GonaConTM) comparison.

4. Discussion

To our knowledge, this is the first use of the immunocontraceptive GonaConTM in female dogs. We have demonstrated the potential to use this immunocontraceptive in breeding age female dogs without affecting parenteral rabies immunization in 100% of vaccinated animals.

Throughout the world, there is a critical need to control population growth in dogs, especially in areas where canine rabies remains endemic. The current practice of euthanizing large numbers of dogs is a social concern [11], plus it is labor intensive limiting its potential across broad geographic areas. Alternatives to euthanasia have focused on chemical and surgical sterilization [12,13], each possessing positive and negative attributes. Currently applied dog population control methods have been unable to affect a sufficient sized cohort of dogs within a reasonable time frame to have an impact on the local population.

More effective rabies control measures in dogs may be possible through injectable immunocontraceptive products administered in tandem with rabies vaccination programs. Ultimately, the factors that would influence the population-level response to immunocontraception are the proportion of females that can be treated, the incidence of non-responders among treated dogs, the fertility of untreated dogs, compensatory responses such as increased reproductive rates in untreated animals and increased survival of treated individuals, including rates of mortality, emigration and immigration. Given that no interference was identified between GonaConTM and a commercial rabies vaccine in this immunogenicity trial, future research should determine the potential effects of immunocontraception upon duration of immunity and efficacy against relevant challenge viruses.

Acknowledgments

The authors acknowledge Wildlife Services and Navajo Nation personnel especially I. Begay, P. Bender, M. Chavez, R. Files, and S. Johnston for animal care assistance. The authors thank the Navajo Nation Animal Control Program for their assistance with the animals and use of their facilities, and T. Atwood and T. Fry for assistance with data analysis. In addition, the authors thank W. Wunner for his critical review of the paper.

References

- [1] World Health Organization. World survey for rabies VI for 1964. Rabies/Inf./67.1, World Health Organization, Geneva, Switzerland; 1966.
- [2] Held JR, Tierkel ES, Steele JH. Rabies in man and animals in the United States, 1946–1965. Public Health Rep 1967;82:1009–18.
- [3] Meslin F-X, Fishbein DB, Matter HC. Rationale and prospects for rabies elimination in developing countries. In: Rupprecht CE, Dietzschold B, Koprowski H, editors. Lyssaviruses. Berlin: Springer Verlag; 1994. p. 1–26.
- [4] Warrell DA, Warrell MJ. Human rabies: a continuing challenge in the tropical world. Schweizerische Medizinische Wochenschrift 1995;125:879–85.
- [5] Knobel DL, Cleaveland S, Coleman PG, Fevre EM, Meltzer MI, Miranda MEG, et al. Re-evaluating the burden of rabies in Africa and Asia. Bull World Health Org 2005:83:360–8.
- [6] Hanlon CA, Rupprecht CE. Considerations for immunocontraception among free-ranging carnivores: the rabies paradigm. In: Kreeger TJ, Tech Coord. Contraception in wildlife management. USDA APHIS Tech Bull No. 1853; 1993, p. 185–94.
- [7] Ladd A, Tsong YY, Walfield AM, Thau R. Development of an antifertility vaccine for pets based on active immunization against luteinizing hormone releasing hormone. Biol Reprod 1994;51:1076–83.

- [8] Miller LA, Rhyan J, Killian G. GonaConTM, a versatile GnRH contraceptive for a large variety of pest animal problems. Proc Vertebrate Pest Conf 2004;21:269-73.
- [9] Smith JS, Yager PA, Baer GM. A rapid reproducible test for determining rabies
- neutralizing antibodies. Bull World Health Org 1973;48:535–41.

 [10] SAS Institute Inc. SAS 9.1.3 help and documentation. Cary, NC, USA: SAS Institute Inc.; 2000-2004.
- [11] Purswell BJ, Kolster KA. Immunocontraception in companion animals. Theriogenology 2006;66:510-3.
- Howe L. Surgical methods of contraception and sterilization. Theriogenology 2006;66:500-9.
- [13] Kutzler M, Wood A. Non-surgical methods of contraception and sterilization. Theriogenology 2006;66:514–25.