

PRESENTATION SUMMARY & POWERPOINT

Proposal to Evaluate the GnRH Vaccine GonaCon for Population Control of Feral Male and Female Dogs

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The Problem

Overpopulation of stray dogs is an unquestioned problem in many parts of the world. Unwanted or unowned dogs often live in deplorable conditions and die prematurely. In the United States, the Humane Society estimates that 6-8 million dogs and cats enter animal shelters each year, and about half of these animals are euthanized.¹ In developing countries, the problem is compounded in disadvantaged or poverty areas where animal shelters and veterinary care are absent or limited, and education on animal welfare issues is lacking.

Stray dogs are also a significant human health concern. A 2005 report by the World Health Organization (WHO) on rabies indicates that overpopulation of stray or uncared-for dogs poses a significant human health concern for rabies in many developing countries in Asia, Africa, South America and the Middle East.¹ WHO estimates that each year as many as 70,000 humans die of rabies and at least 10 million humans receive post-exposure treatments.² In more than 99% of the cases, rabies transmission to humans occurs through bites, scratches or contact with the saliva of infected dogs. Although no data are available, given the number of human cases of potential rabies exposure, one can easily speculate that the number of rabid dogs in developing countries is much higher. To address the problem, WHO recommends that efforts should be made to vaccinate dogs for rabies and significantly reduce dog population density.²

The number of stray dogs in many large cities of developing countries is staggering. In Mexico City, for example, it's estimated that there are more than two million stray dogs. While some efforts are made each year to remove and euthanize dogs or surgically sterilize others, the number of dogs and their rates of reproduction are simply too great to be affected by these removal and sterilization efforts.

The only reliable method currently available to limit dog fertility is surgical sterilization. Spaying and neutering programs typically require a veterinarian and major campaigns may be too costly for impoverished communities or countries to undertake. Additional approaches are needed to effectively control fertility of stray dogs and reduce population densities. A single-shot contraceptive/sterilization vaccine would enable, at minimal cost, treatment of a significantly greater number of dogs than surgical sterilization.

The Opportunity

GonaCon™ is an immunocontraceptive vaccine developed and tested by the National Wildlife Research Center that targets gonadotropin-releasing hormone (GnRH). GonaCon™ contains AdjuVac, an adjuvant made with diluted Johne's vaccine. By blocking GnRH, in either vaccinated males or females, production of sex hormones and gametes ceases and treated animals enter a non-breeding condition. Studies have shown that a single shot of GonaCon™ is safe and highly effective in preventing conception in deer,³ pigs,^{4,5} squirrels⁶ and horses.^{7,8} Recently, this vaccine has also been used successfully to cause testicular regression in male cats.⁹

Gonacon™ has recently undergone some modifications since the first formulation. These modifications have resulted in vaccine that is less costly to produce, and one that has excellent long-term efficacy. In ongoing studies with white-tailed deer, 80% of the females treated with a single vaccination are still infertile after four years. Although tissue reactions to the vaccine were not a significant problem in other species,¹⁰ use of an earlier formulation of GonaCon™ and AdjuVac in three male dogs¹¹ and AdjuVac in captive coyotes resulted in a significant reaction at the injection site. This reaction only occurred in animals given leptospirea vaccine less than one year prior to GonaCon™/AdjuVac. Consequently, further modifications were made and tested in rabbits to develop special formulations of GonaCon™ and AdjuVac for dogs that will provide good contraceptive efficacy without significant reaction at the injection site. Given the success of this vaccine in other species, we believe GonaCon™ will prove to be effective in male and female dogs, and provide a simple alternative to spaying and neutering for addressing the problem of dog population reduction.

The Proposed Research Plan

The proposed study will be a collaborative effort between scientists in the USA and Brazil. The study will be conducted in the College of Agriculture and Veterinary Medicine, Department of Animal Reproduction, São Paulo State University, Jaboticabal, in the State of São Paulo, Brazil. We decided to conduct the study there for several reasons. Dog overpopulation is a very serious problem in Brazil and is associated with serious health threats to humans. In the City of São Paulo, there are estimated to be 1.4 million stray dogs. If the proposed studies are successful, we expect that the Brazilian government will move rapidly to establish national vaccination campaigns to treat stray dogs in several cities and to then provide vaccines to dog owners in poverty areas. It is also less costly to conduct a research trial in Brazil. Study dogs will be acquired free of charge from the Departments of Zoonoses for the cities of São Paulo and Jaboticabal. These former "street dogs" will have been rescued from euthanasia. They will be well cared for and will receive some training. At the end of the study, all study dogs will be spayed or neutered and efforts will be made to find adoptive homes for them.

The hypothesis to be tested during this three-year study is that GonaCon™ is safe and effective when used as a contraceptive/sterilizing agent for male and female dogs.

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Specifically, we will determine the duration of sterilization effect when the vaccine is given as a single-shot dose or as a single shot boosted one year later in both male and female dogs. We will also determine if contraindications are associated with use of the vaccine.

A total of 30 male and 30 female mixed-breed dogs, ranging in age from approximately one to eight years will be acquired by the veterinary school from the Zoonosis Control Centers. Dogs will be weighed and evaluated for overall health and reproductive soundness, given health vaccinations, dewormed and ear tattooed for identification. After a period of quarantine and evaluation, suitable dogs will be assigned to the study.

Study dogs will be separated by gender, housed in a pen singly or with a compatible pen mate, and provided food and water *ad libitum*. Each month, dogs will be weighed. Dogs will be randomly assigned to treatments as follows:

	Controls-AdjuVac	500ug GonaCon-Blue	500/500ug GonaCon-Blue
Males	10	10	10
Females	10	10	10

The estrous cycles of females will be monitored and females in estrus will be bred to fertile males. To test the fertility of treated bitches during the three-year study, the males determined fertile in the control group will be maintained as studs. For females receiving the GnRH vaccine, we will take initial data readings for all parameters prior to immunization. Serum samples will be obtained biweekly and assayed for reproductive hormones and antibody titers. Four months post-vaccination, females showing estrus will be bred in paired matings with fertile control males. Pregnant bitches will be spayed.

We expect that from four months post-vaccination until the end of the first year, at least 90% of the females will be contracepted and fail to express estrus in both treatment groups, based on our experience with other species. We expect this rate of contraception to be sustained in the females receiving the boost immunization into the second and third years. We also expect for the group not boosted that contraception will be sustained at the initial level or perhaps drop to 70-80% for the second and third years.

For males, scrotal measurements of testis length and width will be taken at biweekly intervals. At monthly intervals, males will be evaluated for libido, and semen will be collected by masturbation. Semen volume, sperm concentration, percent motile sperm and percent abnormal sperm will be determined. We expect serum testosterone and semen parameters to decline during the first 6-7 months of the study and remain in a regressed state for at least one year. We expect the long-term infertility effects on males to be greatest in males receiving the two-shot regimen. Based on testis histology of treated deer and pigs, we expect that the majority of the two-shot males will be sterilized, perhaps permanently. At the end of the study, dogs will be spayed or neutered, organs weighed and tissues prepared for blinded histological evaluation.

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Although our studies in other species suggest that GonaCon™ will work well in dogs, data are necessary to demonstrate for this species the long-term efficacy of the vaccine and whether or not contraindications occur with its use. These data will make it possible for veterinarians, pet owners and public-policy makers to make an informed decision about the vaccine's use in dogs. We believe the potential benefits of a dog contraceptive vaccine to address the overpopulation problem justify studies to evaluate GonaCon™ in dogs.

References

1. Humane Society of the United States (HSUS). 1999. *Pet Overpopulation Facts*. <http://www.hsus.org/ace/11830>.
2. *World Expert Consultation on Rabies: First Report*. 2005. WHO Technical Report Series 931. World Health Organization Press, Geneva, Switzerland.
3. Miller, L.A., B.E. Johns and G.J. Killian. 2000. "Immunocontraception of White-Tailed Deer with GnRH Vaccine." *Am. J. Reprod. Immun.* 44:266-274.
4. Miller, L.A., J. Rhyan, and G. Killian. 2003. "Evaluation of GnRH Contraceptive Vaccine Using Domestic Swine as a Model for Feral Hogs." In: *Proc. 10th Wildl. Dam. Manage. Conf.* 120-127.
5. Killian, G., L. Miller, J. Rhyan, and H. Doten. 2006. "Immunocontraception of Florida Feral Swine with a Single-Dose GnRH Vaccine." *Amer. J. Reprod. Immunol.* 55: 378-384.
6. Nash, P.B., D.K. James, L.T. Hui, and L.A. Miller. 2004. "Fertility Control of California Ground Squirrels Using GnRH Immunocontraception." *Proc. Vertebr. Pest Conf.* 21: 274-278.
7. Killian, G., L.A. Miller, N.K. Diehl, J. Rhyan, and D. Thain. 2004. "Evaluation of Three Contraceptive Approaches for Population Control of Wild Horses." *Proc. Vertebr. Pest Conf.* 21: 263-268.
8. Killian, G., L. Miller, N.K. Diehl, J. Rhyan, and D. Thain. 2006. "Long-Term Efficacy of Three Contraceptive Approaches for Population Control of Wild Horses." *Proc. Vertebr. Pest Conf.* 22: in press.
9. Levy, J.K., L.A. Miller, C. Crawford, M.K. Ross, K.A. Fagerstone, and H.L. Jordan. 2004. "GnRH Immunocontraception of Male Cats." *Theriogenology.* 62: 1116-1130.

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10. Killian, G., J. Eisemann, D. Wagner, J. Werner, D. Shaw, and L. Miller. 2006. "Safety and Toxicity Evaluation of GonaCon Immunocontraceptive Contraceptive Vaccine in White-Tailed Deer (*Odocoileus virginianus*)." *Proc. Vertebr. Pest Conf.* 22: in press.
11. Griffin, B., H. Baker, E. Welles, L.A. Miller, and K. Fagerstone. 2004. "Response of Dogs to a GnRH-KLH Conjugate Contraceptive Vaccine Adjuvanted with Adjuvac." *Proc. 2nd International Symposium on Nonsurgical Methods for Pet Population Control.*

Session III: What's New in Contraceptive Vaccines?

Evaluating the GnRH Vaccine GonaCon for Population Control of Male and Female Dogs

By Dr. Gary Killian

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USDA-APHIS-WS

The Problem

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Overpopulation of Stray Dogs Is a Worldwide Problem

- HSUS estimates 6-8 million stray dogs and cats in USA per year
- Mexico City: 2 million stray dogs
- São Paulo: 1.5 million stray dogs
- World Health Organization indicates stray dogs pose significant human health risk in developing countries, particularly Africa

Feral dogs pose a significant health risk to humans

- WHO estimates that as many as 70,000 humans die of rabies and at least 10 million humans receive post-exposure treatments each year
- In more than 99% of the cases, rabies transmission to humans occurs through bites, scratches or contact with the saliva of infected dogs.

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How is the problem currently being addressed?

- In the USA, half of the dogs brought to shelters are euthanized
- Efforts in developing countries are inadequate because magnitude of problem is too great for resources available
- Efforts in developing countries may be inhumane

What happens to feral dogs in Brazil?



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Some are gathered for adoption or euthanasia



Most gathered dogs are euthanized



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China Massacres 50,000 Dogs in Anti-Rabies Campaign Dogs Being Walked Seized From Their Owners and Beaten to Death on the Spot

By CHRISTOPHER BODEEN, AP



To address the human health concerns.....

The World Health Organization recommends that efforts should be made to vaccinate dogs for rabies *and significantly reduce dog population density*

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How can research address the problem?

Challenges for fertility control of feral dogs

- Unknown history
- Limited opportunity to treat
- Large numbers of animals need to be treated
- Simple approach, administered by trained non-veterinarians
- No post-treatment monitoring
- Treatment needs to be proven safe and effective

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Research Goals for Nonsurgical Sterilization of Feral Dogs

- Proven safe and effective before field use
- Simple to use
- Effective following a single application
- Effective for multiple years
- Inexpensive

Immunocontraceptive vaccines evaluated in other species with good results

- PZP (porcine zona pellucida) vaccines
 - Effective only in females
 - Does not inhibit breeding behavior
- GnRH vaccine (GonaCon)
 - Effective in both females and males
 - Inhibits breeding behavior

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Why evaluate GonaCon™ in dogs?

- Proven safe and effective in pigs, deer, horses, cats, etc.
- Single shot effective for multiple years
- Government approval pending
- Effective in both males and females
- Could be administered on a large scale by technicians
- Relatively inexpensive

Data from domestic pigs

Gilts Infertile After Vaccination August 2001					
Date of Breeding Trial					
GonaCon Treatment GnRH- KLH	October 2001	January 2002	November 2002	August 2003	November 2004
Control	0	0/10	-	-	-
800ug	10	8/10	5/8	5/5	5/5
1600ug	10	9/10	5/9	5/5	4/4*

*One gilt died during the last year of causes unrelated to the study.

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Percent Does Contracepted Following GonaCon™ Treatment

Treatment w/AdjuVac	Year Post-Treatment					
	1	2	3	4	5	6
GnRH-KLH 2-Shot*	100	100	80	50	-	-
GnRH-KLH 1-shot	100	60	40	40	20	x
GnRH-Blue 1-shot	100	100	80	80	-	-
GnRH-KLH Lyophilized	100	20	20	20	0	x

*2 of 3 does given GnRH-KLH 2-shot in 1998 contracepted for 6 years

Are there any known side effects in dogs?

- Early formulation of GonaCon™ adjuvant used in coyotes and 3 male dogs resulted in severe tissue reaction
- Both groups had received puppy shots containing Leptospira bacterin
- Coyotes not receiving the Leptospira bacterin 2 years previously did not have an injection site reaction with AdjuVac

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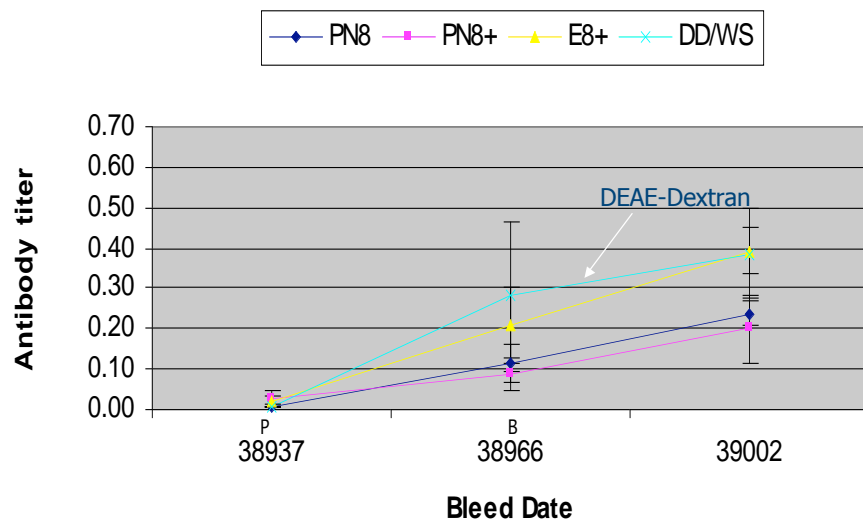
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Remedy: Develop Charged Adjuvants

- After GnRH conjugation to the mollusk carrier protein there are negative charges available
- 4 water soluble positive charged adjuvants were developed at NWRC
- Tested in rabbits for ability to generate antibody titers and tissue reaction

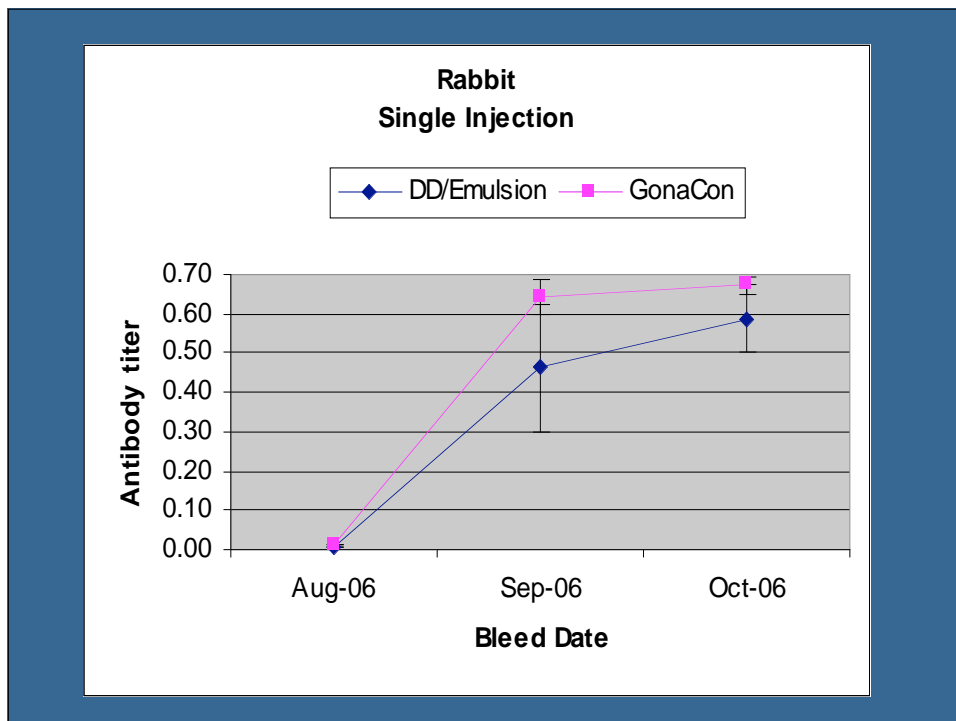
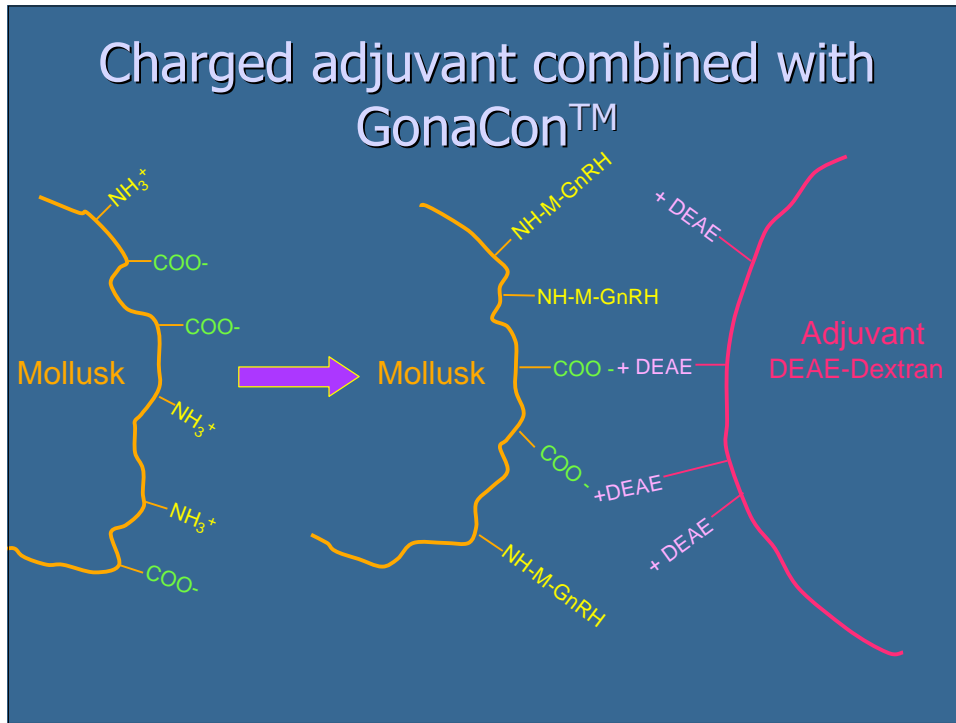
Charged Adjuvant Evaluation in Rabbits



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GonCon/Dual Adjuvant

- Needs to be tested for injection site reaction in dogs
- Needs to be tested for long-term contraceptive effect
- Single shot needs to be compared to a two-shot vaccine

Snapshot Proposed Research

HYPOTHESIS:

GonaCon™ is safe and effective when used as a contraceptive/sterilizing agent for male and female dogs.

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Objectives

- a. Determine the duration of sterilization effect of the vaccine as a single-shot dose or as a single shot boosted one year later.
- b. Determine the vaccine's effectiveness in both males and females.
- c. Determine if contraindications are associated with use of the vaccine.

Experimental Plan

- Healthy mixed-breed dogs acquired by the University of São Paulo veterinary school from the Zoonosis Control Centers
- Assign dogs to treatments
- Observations made for efficacy and contraindications

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Treatments Evaluated

	Controls- AdjuVac	500ug GonaCon- Blue	500/500ug GonaCon- Blue
Males	10	10	10
Females	10	10	10

End points measured

- Collect blood for antibody and hormone titers
- Monitor injection site and dogs for contraindications
- Monitor estrous cycles and semen parameters
- Establish fertility from breeding trials
- Spay and neuter dogs for histological evaluation
- Place experimental dogs up for adoption at conclusion

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Expected results

- Single-shot GonaCon™ Blue
 - > 80+% Contraception 3 years in females, 1-2 years in males
- Two-shot GonaCon™ Blue
 - > 90+% Contraception for 4-8 years in females, 3-5 years in males

Justification for Evaluation of GonaCon™

- Urgent worldwide need to control feral dog populations
- Current methods to control fertility by surgical means inadequate
- GonaCon™ given as a single shot highly effective in other species

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