ACC&D
Alliance for Contraception in CATS & DOGS

GONACON™ (GNRH-HEMOCYANIN CONJUGATE) FORMULATIONS

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Product Profile and Position Paper

GnRH-hemocyanin conjugate immunocontraceptive vaccine formulations have been shown to prevent reproduction in multiple mammalian species for extended periods. GonaCon™ and GonaCon-Equine™ are registered with the U.S. EPA for female white-tailed deer and wild/feral horses and burros, respectively. Studies indicate that a GnRH-hemocyanin conjugate immunocontraceptive vaccine has potential for multi-year contraception among free-roaming/feral cats. Work to develop an effective, safe formulation for free-roaming dogs is ongoing.

GonaCon™ (GnRH-hemocyanin conjugate) formulations

CONTRACEPTIVE FOR MAMMALIAN SPECIES

INTRODUCTION

GonaCon™ Immunocontraceptive Vaccine and GonaCon-Equine™ are gonadotropin-releasing hormone (GnRH) immunocontraceptive vaccines approved by the U.S. Environmental Protection Agency (EPA) for use in female white-tailed deer and wild and feral horses/burros, respectively. They were developed by the National Wildlife Research Center (NWRC), the research arm of the USDA-APHIS Wildlife Services program.

Although EPA regulatory approval is limited to these aforementioned products and species, GnRH-hemocyanin conjugate vaccine formulations developed by the NWRC have been evaluated in males and females of many mammalian species, including cats and dogs. Study findings with one vaccine formulation indicate potential for multi-year contraception in cats. This result prompts ACC&D to believe there is great value in further exploring a GnRH-hemocyanin conjugate vaccine as a fertility control tool for cats, particularly community and feral populations. The NWRC is currently working to improve vaccine safety and efficacy for dogs; it is particularly interested in product use to augment rabies prevention efforts among community (free-roaming) canine populations.

This paper was created from a review of published literature, conference proceedings, and representative opinions of relevant organizations. ACC&D did not conduct any clinical or field research; data were obtained from external sources. Cited articles provide more extensive information than contained in this document, and we recommend referencing these materials for further details.

MECHANISM OF ACTION

GnRH is a decapeptide hormone produced by the hypothalamus in the brain of all mammals. It is sometimes called the “master sex hormone” because it controls reproductive processes in both males and females. Without GnRH, the release of gonadal hormones is suppressed, and gametes do not mature.

The GnRH-hemocyanin conjugate formulas developed by NWRC are designed to elicit the creation of antibodies against the GnRH produced by the body, thus suppressing production of gonadal hormones and maturation of gametes. Due to the mechanism of action, in addition to preventing reproduction, the immunocontraceptive vaccine has been found to suppress behaviors driven by sex hormones in deer and other species (Levy 2011, Levy et al. 2011, Miller et al. 2004, Miller et al. 2008b).

In order to be antigenic (i.e., to produce an immune response), a GnRH peptide must be coupled with a carrier protein. The final GnRH-hemocyanin conjugate vaccine contains the GnRH peptide, a carrier protein, and an adjuvant to boost immune response against GnRH. The adjuvant presently used in NWRC formulations is the mineral-oil based AdjuVac™, which contains a small amount (<400 µg/ml) of killed *Mycobacterium avium* (Miller et al. 2004, 2008a; Munks 2012). Intramuscular injections have been the route used in both dog and cat studies to date, although research in other species has explored subcutaneous vaccination. The NWRC aims to develop a product that prompt long-term fertility suppression with a single vaccine injection, a feature that is particularly important for use in wild and feral populations.

STUDIES IN CATS

ACC&D is familiar with three feline studies of a GnRH-hemocyanin conjugate formula developed by NWRC. These studies indicate that while both male and female cats respond to the vaccine, females overall have stronger and more predictable antibody development following vaccination than males (Levy 2011).[1]

A long-term (5-year) study was undertaken with female cats aged 8 to 14 months. Of 15 vaccinated cats, 93 percent (n = 14) were infertile for at least one year, 73 percent (n = 11) for two years, 53 percent (n = 8) for three years, and 40 percent (n = 6) for four years; 27 percent (n = 4) were still infertile at the end of the study (five years). Response to vaccination was accompanied by cessation in estrous cyclicity and weight gain, similar to cats undergoing spay (ovariohysterectomy) surgery (Levy 2011, Levy et al. 2011).

In a long-term study with males, nine of 12 male cats (75 percent) developed high GnRH antibody titers. Among those cats who responded, testosterone production remained undetectable for a median of 14 months, with a range of five to 33 months. Azoospermia (no motile sperm in the ejaculate) was generally observed one to two months after treatment, and normal sperm counts returned two months after GnRH antibody titers fell and testosterone increased (Levy 2006, 2011). In short, while the vaccine caused temporary infertility in a majority of male cats, the proportion of responders was not as high, and the duration of efficacy not as long, as with females.

No cats displayed any immediate injection site or systemic reactions (e.g., inflammation or tenderness), as has been the case with dogs. Late-onset (two years post-treatment) injection-site reactions developed in five of 15 treated female cats (33 percent). The masses were persistent and waxed and waned for the duration of the study, but they neither drained nor appeared painful (Levy 2011). While the masses appeared to be benign, they nonetheless prompt some concern given indications that cats may be at increased risk of developing granulomatous reactions at the site of injections using oil-mycobacterial vaccines, and that vaccine-induced granulomatous reactions are associated with malignant injection-site sarcoma development in some cats (Munson et al. 2005).

STUDIES IN DOGS

There is great interest in a GnRH-hemocyanin conjugate vaccine for dogs, particularly as part of population and rabies control efforts in free-roaming populations (see Griffin et al. 2004, Bender et al. 2009, Massei et al. 2013, Vargas-Pino et al. 2013, Massei and Miller 2014). In summary, studies to date have shown that dogs are particularly susceptible to developing adverse reactions at the site of the GnRH-hemocyanin conjugate vaccine injection. Reducing the *M. avium* concentration in the AdjuVac™ adjuvant has been found to reduce reaction severity, but other components of the vaccine formulation (e.g., the type of mineral oil used) also need to be considered, while ensuring that the efficacy of the vaccine is not compromised. Consequently, while NWRC's work is ongoing, there is not currently a product considered safe or appropriate for initial or continued field-testing in dogs.

Three male beagles, aged 2-4 years, took part in the first canine study of an NWRC-developed GnRH-hemocyanin conjugate vaccine. Each suffered significant adverse injection site reactions, and only two demonstrated short-term infertility. The authors' conclusion: "a single injection of this vaccine formulation is neither safe (due to the severe local reactions), nor effective at inducing long-term suppression of reproductive function in dogs" (Griffin et al. 2004).

Subsequent studies were conducted with female mixed-breed dogs in Navajo Nation (U.S.), Mexico, and Nepal. Research evaluated the safety of a GnRH-hemocyanin conjugate vaccine and/or efficacy of simultaneous rabies and immunocontraceptive vaccination, and each used a slightly different GnRH-hemocyanin conjugate vaccine formulation. There is no indication that simultaneous vaccination with rabies and GnRH-hemocyanin conjugate vaccines suppresses either rabies virus antibody protection or anti-GnRH immune response (Bender et al. 2009; Vargas-Pino et al. 2013).

[1] Less predictable immunocontraception of males compared with females has been observed in other species as well, possibly related to more difficulty in blocking non-cyclic secreted GnRH in males compared with cyclic secretion in females (Levy 2011). In other species tested, GonaCon induced infertility 2–3 times longer in females than males (Bender et al. 2009).

The most recent field study, which took place in Nepal in 2013, found that 31 of 39 dogs (79%) treated with the GnRH-hemocyanin conjugate vaccine developed sterile abscesses at the injection site and required veterinary care. The remaining eight dogs (21%) demonstrated temporary limping or swelling. This high adverse reaction rate was subsequently attributed to a higher concentration of *M. avium* included in the adjuvant, due to an administrative mistake by the supplier (Massei et al. 2013). The vaccine used in Mexico, in a 2011 study, contained the lowest concentration of *M. avium* to date. Although immediate and long-term injection site reactions were less severe than in other studies, they still occurred (Vargas-Pino et al. 2013).

Work to date highlights the fact that NWRC remains in the research and development phase of producing a GnRH-hemocyanin conjugate vaccine for dogs that is at once safe, effective and long-lasting, and compatible with rabies vaccines. As noted above, there is not currently a product considered appropriate for field-testing in dogs.

DISCUSSION AND RECOMMENDATIONS

ACC&D recognizes that a permanent non-surgical contraceptive or species-specific sterilant that can be administered as a single treatment and without anesthesia would be ideal for community, stray, and feral cats and dogs. As an intermediate step, ACC&D believes that a safe and effective GnRH-hemocyanin conjugate vaccine has great potential to expand population control options for these population cohorts. Such a product may also have the potential to advance rabies control efforts among community dogs by helping to humanely manage dog populations. The fact that GonaCon is administered as a single injection not requiring anesthesia makes it feasible for field use, with no need for surgical facilities or equipment. It is also anticipated to be highly affordable; the NWRC has made cost a priority in formula development.

For many stakeholder groups, suppression of sex hormones is desirable, as behaviors driven by sex hormones are often viewed as a nuisance. Multi-year non-surgical contraception may also be more socially and culturally acceptable in some communities than removing sex organs. A multi-year injectable product can be a great boon if permanent sterilization is not an option at the scale needed to achieve population management goals.[2] Shorter lifespans, on average, among free-roaming animals than their pet counterparts might mean that a multi-year injection would prevent reproduction for a significant portion of the animal's lifespan. For those animals whose lives exceed the duration of the contraceptive vaccine, recapture and revaccination against rabies could potentially coincide with a contraceptive "booster" vaccine. However, there is also concern that a booster vaccine, while inducing a more robust immune reaction, could also prompt more significant adverse injection-site reactions. This concern warrants study for the species in question.

In light of these aforementioned considerations, ACC&D is interested in the possible applications of GonaCon formulations for population management of community/feral cats and dogs, as long as the chosen formulation and dose does not cause adverse side effects. The product's value would be maximized if it offered multi-year efficacy in a single dose, while also minimizing injection site reactions.

Further study and refinement of GonaCon formulations for both dogs and cats is required before this product can be considered for wider use. At present, injection-site reactions, particularly with dogs, are the major concern with GonaCon formulations. There have been no trials for cats since those conducted at the Maddie's Shelter Medicine Program at the University of Florida's College of Veterinary Medicine (see Levy 2006, 2011; Levy et al. 2004, 2011). Further work, particularly in a field context, is needed to advance this potential contraceptive option for cats. ACC&D is very interested in gaining a better understanding of the practicality of field use of GonaCon for free-roaming cats, as well its use to complement or supplement free-roaming cat population control programs that currently rely solely on surgical spay/neuter.

ACC&D believes that multiple questions and concerns must be addressed prior to the development, regulatory approval, and marketing of GonaCon formulations for cats and dogs in order to protect the health and welfare of the animals receiving treatment. The risks and benefits of using a GonaCon formulation for free-roaming dogs and cats must also be balanced against the welfare implications of no intervention, uncontrolled breeding, euthanasia, and/or inhumane culling

[2] A team convened by ACC&D is currently using *VORTEX* stochastic demographic simulation modeling as a tool to evaluate different methods of reducing abundance of free-roaming cat populations. Population size changes following use of two forms of contraception (a 3-year temporary contraceptive and a contraceptive modeled on return-to-fertility results reported in Levy [2006] and Levy et al. [2004, 2011]) are compared to population size changes following trapping/removal and permanent sterilization interventions across different time steps and geographic/demographic contexts. To learn more, please visit ACC&D's Population Modeling webpage at https://www.acc-d.org/Population_modeling.

in communities where surgical sterilization options are not available at levels required to have a significant impact on population size.

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